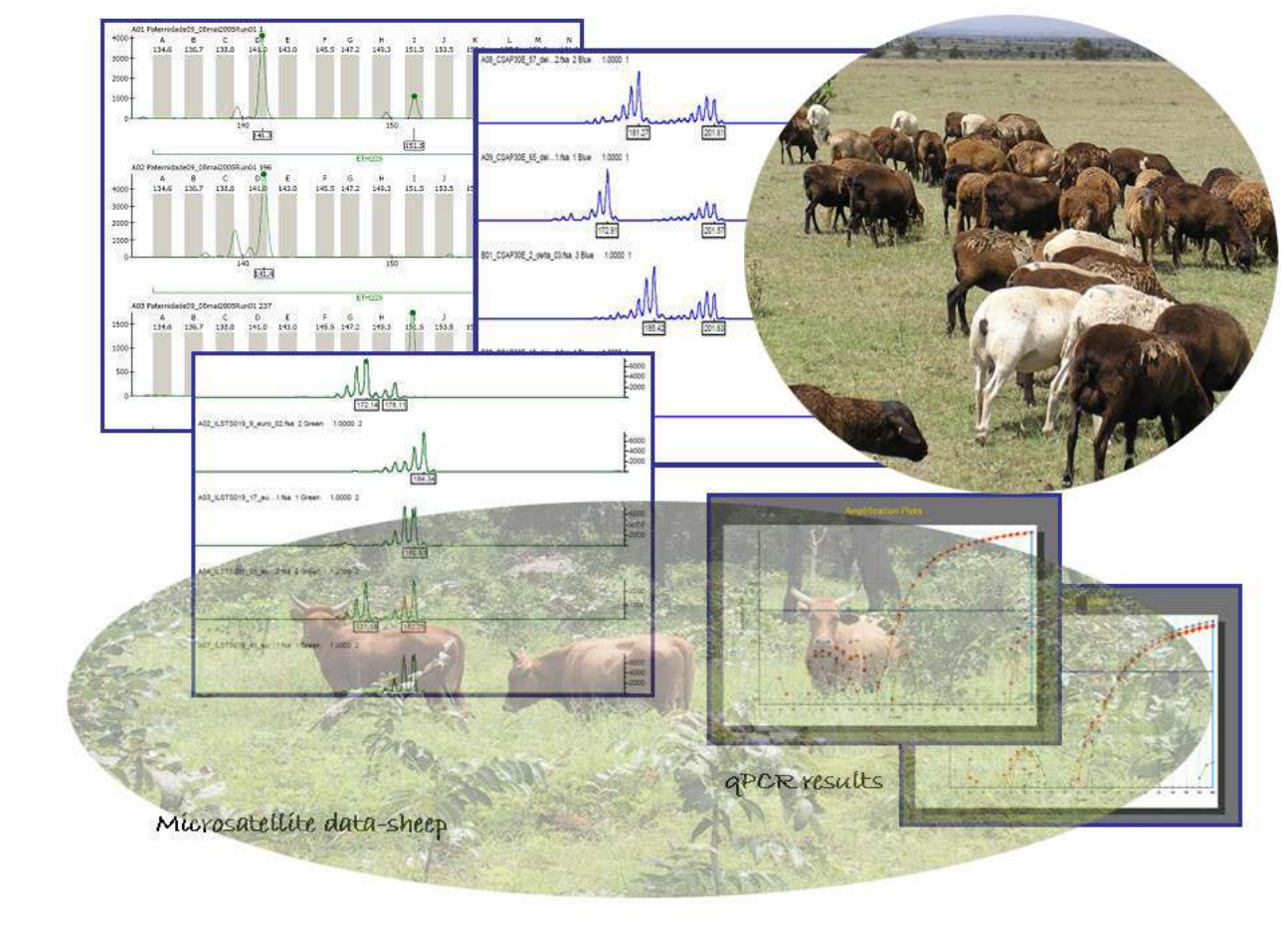
Animal Production Unit

ANNUAL REPORT 2005



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Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

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Executive Summary

Foot and Mouth Disease (FMD) is one of the most feared animal diseases because of the economic losses it may cause in animal productivity. As an example of the economic importance of this disease, the cost of the control of the FMD epidemic that occurred in the UK in 2001 has been estimated at about US$ 14 billion and involved culling about 6.5 million animals. To facilitate the control/surveillance of this disease, many laboratories are developing serological tests based on a FMD virus-specific protein, which is not part of the virus particle itself but is found only in infected cells, the 3ABC protein. Therefore the current FMD vaccines which are made of purified and killed virus particles do not contain normally this non-structural protein (NSP). Animals vaccinated with current FMD vaccines, contrary to those infected with live viruses, do not develop antibodies against 3ABC. With serological tests based on the use of this protein as antigen it is possible to differentiate vaccinated from infected animals. Such tests constitute valuable tools to monitor FMD infection/circulation in a country irrespective of the vaccination status of animals. The results they provide will constitute part of dossiers to be submitted to the World Organisation for Animal Health (OIE, Office International des Epizooties) by countries applying for FMD freedom status. Since 2002, the Animal Production Unit has been involved in the development of ELISA tests based on the FMD virus 3ABC protein. In 2005 validation studies of an indirect kit produced by the APU were conducted at the World Reference Laboratory for Foot and Mouth Disease (UK). Preliminary results gave 82% sensitivity and 100% specificity for the assay, suggesting this kit could be a strong candidate test for confirming the FMD status of an animal because a high proportion of infected animals would be detected (high sensitivity rate) and the chance of getting false reactors would be minimal, (high specificity rate). However, these results need to be confirmed on a larger number of sera and further studies need to be performed on sera from sheep and pigs.

For four years, APU has been working on another important animal disease: the peste des petits ruminants (PPR). Like FMD, PPR is a contagious viral disease. It is considered in the FAO EMPRES Programme and is on the list of animal diseases to be reported to the OIE. Its importance has been highlighted recently by an international study that ranked PPR among the top 10 diseases affecting sheep and goats, the control of which should be considered in policies for poverty alleviation in Africa and West Asia. Based on the epidemiological information available, it is estimated that some one billion sheep and goats are currently at risk of contracting PPR. In 2005, the Animal Production Unit, in collaboration with other institutions in Europe and Africa, embarked on the development of a marker vaccine and its companion test for better control of PPR. Within this collaborative project, supported financially by the European Commission and the Wellcome Trust Foundation Funds, APU is responsible for the development of a diagnostic test and for the mapping of the virus nucleoprotein with a view to identifying a non-essential zone to be deleted as a potential negative marker for the vaccine. The nucleoprotein (Np), the most abundant and the most immunogenic of PPR virus proteins, is central to the replication process of the virus. Indeed it envelops the viral genomic RNA and protects it against nuclease degradation. The nucleocapsid is the structure composed of the genomic RNA, the nucleoprotein and the viral RNA polymerase complex. During the virus particle formation this nucleocapsid is pulled into the vesicle formed by the viral matrix protein (M) and the host cell membrane through the interaction between Np and M. Deletion of Np for the

development of a negative marker vaccine should be made in a region of Np not essential for its interaction with other virus components. Preliminary results of the Np mapping studies have led to the identification of two zones in the N-terminus of the protein, which might be involved in the Np-Np self-association. For PPR serological diagnosis, APU has produced monoclonal antibodies to the PPRV Np and one of them seems to be suitable for the development of a specific competitive PPR ELISA platform.

For the past two years, APU has been involved in an international collaboration led by the International Livestock Research Institute (ILRI) and the United States Department of Agriculture (USDA). This project entails chromosome mapping to facilitate the search for genes related to gastro-intestinal helminth parasite resistance in sheep. Molecular data generated by all partners are presently under combined analysis. It is expected that very soon useful variations in candidate gene linked to helminth resistance will be identified and exploited in marker introgression programmes in Member States, to aid genetic selection for helminth resistance.

1. Introduction

1.1. Sub-Programme and Unit Objective

As poverty indicators, it has been estimated that at the beginning of the current century some 2 billion people are living on less than US$ 2/day. Out of these some 800 million are living at starvation level of less than US$ 1/day. For poor people living in developing countries, livestock account about 70% of their livelihoods. Sometimes the animal breeds they are rearing are not high producers but they have some other advantages that can be exploited, for example adaptation to harsh conditions, resistance to diseases, etc. These breeds are at risk of extinction because of misuse in crossbreeding with exotic breeds. Infectious animal diseases, and particularly those of transboundary nature, constitute important and continual risks to livestock and the livelihoods of the farmers. The vision and goal of the Animal Production and Health Sub-programme are to minimise risks to livestock in FAO and IAEA Member States in order to increase food security, to fight hunger and to improve the livelihoods of the poor. To achieve this objective, two strategies guide the activities of the Subprogramme:

1) Capacity building within regions and countries. Success in the control of highly infectious diseases relies on the capacity of early warning and early reaction, a capacity that is missing in many developing countries because of a lack of financial resources and also human and physical resources. Training of scientists is important for helping developing countries to manage different risks that are threatening livestock production.

2) Promotion of applied research targeting areas that help alleviate risks for livestock in developing FAO and IAEA member states. This involves promoting transfer of technologies to developing countries, promoting and implementing applied research projects for improved diagnostic tests and vaccines, for better breeding strategies, for improved farm management to optimize use of animal feed resources and to protect the environment.

The Subprogramme activities are conducted through the implementation of three projects:

- E.2.01. Entitled “Technologies for integrated management of natural resources in small scale dairy production systems”. The objective of this project is to promote livestock development in peri-urban and mixed crop-livestock system by using isotopic methods to identify and reduce nutritional and reproductive constraints;
- E.2.02. Entitled “Technologies for reducing risk from transboundary livestock diseases and those of veterinary public health importance”. The aim of this project is to contribute to control and prevention of livestock and zoonotic diseases through improved diagnosis and surveillance using immunoassay and molecular methods;
- E.2.03. Entitled “Molecular techniques for improving productivity in small-holder livestock systems”. The objective of this project is to improve productivity in small-holder livestock systems using gene-based and related technologies.

All activities conducted in the Animal Production Unit and by its staff members in 2005 were in support of the projects E202 and E203.

1.2. Organizational Chart and Unit Staff

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2. Research & Development

2.1. Animal Health:

2.1.1. Development of PPR marker vaccine with companion diagnostic test.

Peste des petits ruminants is a serious emerging plague of small ruminants. Although its morbidity and mortality rates may vary considerably from 0 to 80-90% depending on animal husbandry, breed and age, among other factors, PPR is considered to be the disease which constitutes the main constraint to the increased production of sheep and goats in countries where it is endemic. It is one of a group of animal diseases whose outbreaks must be reported to the World Organisation for Animal Health (Office International des Epizooties: OIE). It is a transboundary disease whose effects on the local and rural economies of affected countries can be devastating. Since small ruminants are a major resource for the less wealthy livestock farmers, the poor are more seriously affected by PPR epidemics. The use of a vaccine that was developed nearly 20 years ago to prevent this disease is helping to control outbreaks in affected zones, Africa and Asia, but reaching all the animals that require vaccination is a very difficult and expensive task. As a result the disease can remain in a less visible form since high death rates are prevented by the resulting partial immunity of herds or flocks. It is therefore difficult to be sure where the main problem areas are located because the current vaccine doesn't allow serologically vaccinated animals to be differentiated from naturally infected animals. An improvement in the situation could be achieved by the use of a "marker vaccine" so that vaccinated animals could be identified and distinguished from those which had recovered from the natural disease. This would allow detecting the wild virus circulating among partially immune animal population. One of the aims of the project is to make such a marker vaccine for PPR using recombinant DNA technology to determine the level of circulating wild virus in different regions. The other aims are to improve the diagnostic tests needed to identify the virus in infected animals and those that are needed to carry out serological surveys in the endemic areas. Studies to compare different virus strains and their effects on local African breeds of sheep and goats will indicate whether or not some may suffer only a mild form of the disease, which would help the silent spread of the virus to more susceptible breeds.

This project benefits from the financial support of the European Commission and the Wellcome Trust. It involves the collaboration between many partners in Africa and Europe. The Animal Production Unit is responsible for:

- mapping the virus nucleoprotein to identify sequences essential for the function of the protein and which should not be deleted for the development of a negative marker vaccine;
- developing a companion test for the PPRV marker vaccine.

The nucleoprotein (Np) molecules of morbilliviruses, a group which includes the peste des petits ruminants virus (PPRV), are involved in four types of interactions:

- Np-Np self-association to form the nucleocapsid;
- Np-RNA interaction; the nucleocapsid enwraps and protects the viral genomic RNA against nucleases;

- Np-phosphoprotein (P) interaction, which is essential for the RNA polymerase activity of the complex formed by the phosphoprotein (P) and the large protein (L);
- Np-matrix protein (M) interaction by which the nucleocapsid is pulled into new vesicles for the virus particle formation.

In 2005, studies were initiated to identify sequences in the Np molecule involved in its self-association and also its interaction with the virus matrix protein (M).

2.1.1.1. Study of the Np-Np interactions for the formation of the nucleocapsid.

Different deleted mutants of the PPRV nucleocapsid gene were constructed into the baculovirus vector and expressed in insect cells (see Table 1). These mutants were then used to identify the sequences involved in the Np self-association to form the nucleocapsid. Insect cells were co-infected with two recombinant baculoviruses: one carrying cDNA corresponding to the full Np gene and the other being a virus with a deleted Np gene. These co-infections resulted in the random association of different types of Np molecules. The interaction between Np molecules was detected by immunoprecipitating the complex with a monoclonal antibody (mAb) that binds only to the wild type Np but not to the truncated mutant protein. In such cases, a deleted mutant molecule will be precipitated only if it is associated with a wild type molecule. The mAbs 38-4 and 33-4, which recognize epitopes in the N-terminal domain of Np, were used in the immunoprecipitation analysis of proteins of the cells co-infected with the full Np recombinant and the following deleted mutants: Nppr▲1-145, Nppr▲1-242, Nppr▲120-145, Nppr▲120-242, Nppr▲120-278. For proteins of cells co-infected with full Np and C-terminal domain truncated Np (Nppr▲420-490, Nppr▲420-525) recombinant viruses, the mAbs P4A3 and P4G5 were used. These mAb recognize epitopes that are located in the C-terminal of Np (see section 2.1.2.2). The precipitates were separated by electrophoresis on polyacrylamide gel and transferred onto filter

Name of mutants	Protein sequence deleted (number of amino acids)
Nppr▲1-145	1-145
Nppr▲1-242	1-242
Nppr▲120-145	121-144
Nppr▲120-242	121-241
Nppr▲120-278	121-277
Nppr▲420-490	421-489
Nppr▲420-525	421-525

Table 1: List of the recombinant baculoviruses expressing different deleted PPRV Np and used in the Np mapping study

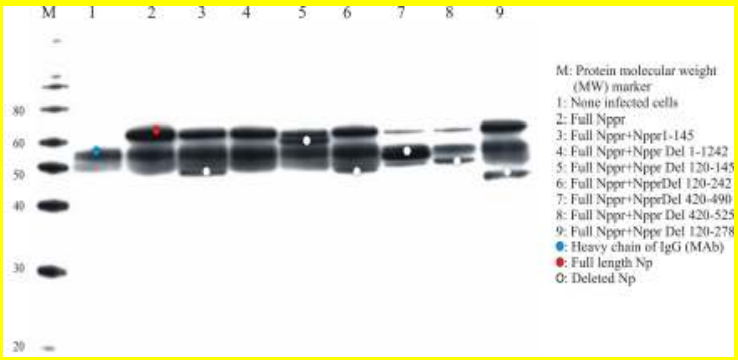


Figure 1: Analysis of interactions between the PPRV wild type Np and the various Np mutants by Western Blot

membrane (western blot analysis). The proteins on the membrane were detected by chemiluminescence after probing the membrane with a mixture of mAbs 33-4, P4A3 and P4G5 followed by incubation with the peroxidase conjugate anti mouse IgG.
The full length of the PPRV Np is composed of 525 amino acids (aa) and has an apparent molecular weight of about 60 kilo Daltons (kD). As seen in Figure 1, it co-precipitates with all Np mutant proteins except Nppr▲1-242 (see lane 4, Figure 1). These data indicate that sequences involved in the Np-Np molecules interactions are located in region aa 1-242 and probably in at least two different areas, aa 1-120 and aa 120-242, since mutants lacking either of these regions still associate with the full length Np molecule.

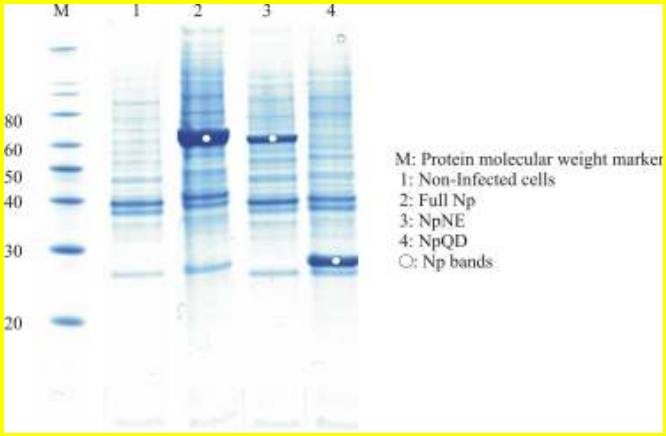


Figure 2: Expression of Np, NpNE and NpQD in insect cells infected with recombinant baculoviruses carrying cDNA corresponding to the wild type Np and the mutated protein genes

In another morbillivirus, the measles virus (MV), it has been demonstrated that the serine (S) and leucine (L) residues at positions 228-229 compose a site involved in the Np-Np interactions. Their mutation into glutamine (Q) and aspartic acid (D) residues impairs the formation of the nucleocapsid structure. The same mutations were introduced in the PPRV Np gene cloned into the baculovirus. The mutant produced a nucleoprotein in infected cells that is very susceptible to cellular protease degradations (see lane 4, figure 2). However, a protein in which the amino acids S-L at positions 228-229 are mutated into asparagine (N) and glutamic acid (E) is not degraded (see lane 3, figure 2).

Cells	Control conjugate	38-4	33-4	P4G5	P4A3
Non-infected insect Cells	-	-	-	-	-
Np	-	+	+	+	+
NpNE	-	-	+	+	+
NpQD	-	-	+	+	+
Nppr▲1-145	-	-	-	+	+
Nppr▲1-242	-	-	-	+	+
Nppr▲120-145	-	-	-	+	+
Nppr▲120-242	-	-	-	+	+
Nppr▲120-278	-	-	-	+	+
Nppr▲420-490	-	+	+	-	-
Nppr▲420-525	-	+	+	-	-

Table 2. Results of Immunofluorescence test for the detection of PPRV Np and mutants expressed in insect cells by the recombinant baculoviruses.

The reactivity of the different recombinant nucleoprotein mutants against 4 monoclonal antibodies anti Np were evaluated by immunofluorescence (IF) in view of detecting eventual changes in epitopes exposed on the protein surface. The following monoclonal antibodies were used: mAb 38-4, 33-4, P4A3 and P4G5.
The results from preliminary studies indicate that while mAb 33-4, P4A3 and P4G5 recognize linear epitopes on Np, the binding site of the mAb 38-4 is conformational since it does not recognize denaturised PPRV Np. The IF test results presented in table 2 indicate that mutants NpNE, NpQD, Nppr▲1-145, Nppr▲1-242, Nppr▲120-145, Nppr▲120-242 and Nppr▲120-278 do not associate into the normal nucleocapsid structure because they are not recognized by the mAb 38-4. Indeed, while the native Np forms aggregates in solution, all

those mutants do not and they yield clear solutions. The mutants 420-490 and 420-525 which still self-associate into nucleocapsid are recognized by the mAb 38-4.

In summary, by mutation study, Np-Np self-association sites were mapped to two regions of the protein: fragments corresponding to amino acids 1-120 and 145 to 242 of the Np sequence. As in the case of the measles virus, the amino acids involved in the Np-Np interaction site in the region 145-242 are probably the serine and the leucine residues at positions 228-229 in the protein sequence. The amino acids in the aa 1-120 region responsible for the Np self-association are yet to be determined. To complete the Np mapping analysis, the different mutants are being purified and analysed by electron microscopy observation.

2.1.1.2. Identification of domains of Np involved in N and M interaction.

This study has just been initiated. A DNA corresponding to the PPRV matrix protein gene was synthesized to optimize the codons for better expression in mammalian and insect cells. This DNA was inserted into the baculovirus virus genome in fusion with a histidine tag sequence. Insect cells infected with this recombinant virus expressed the PPRV M protein well (see figure 3). It will be used in the N-M interaction study.

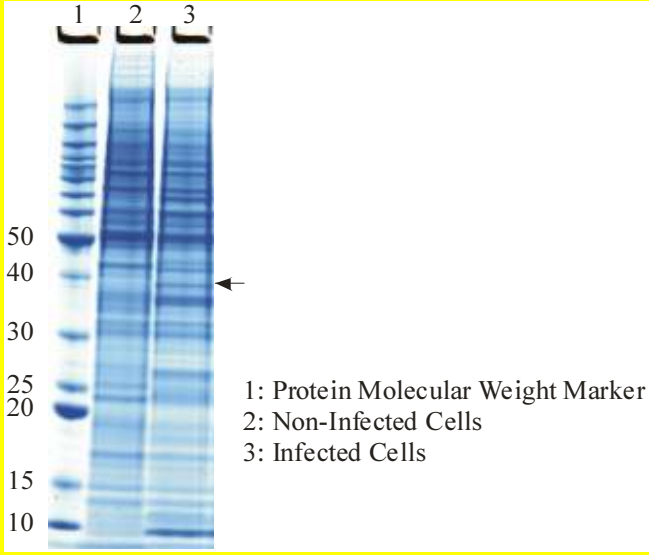


Figure 3: Analysis of proteins of cells infected by recombinant baculovirus M PPRV (the position of the PPRV M is indicated by the arrow)

2.1.2. Development of a PPR specific diagnostic ELISA test.

Currently, there are two competitive ELISA tests for the serological diagnosis of PPR. They are based on the use of monoclonal antibodies (mAbs) against the haemagglutinin or nucleocapsid proteins. Although these mAbs are specific to PPRV, both tests give cross-reactions with rinderpest sera. This project was initiated to develop new ELISA test (s) specific to PPR (Research Project of the **P**an **A**frican programme for the **C**ontrol **E**pizootics, PACE).

2.1.2.1. Indirect ELISA (iELISA):

The nucleoprotein, the most immunogenic protein of morbillivirus, is also one of the most conserved proteins within the group. However, it has a fragment of 70 amino acids (aa) that is specific to each virus. In 2003 and 2004, this fragment was expressed both *in vitro* and in insect cells. Using a number of anti-PPR sera received from Côte d'Ivoire and irradiated at

Seibersdorf in March 2005, this peptide of 70 aa was evaluated as an antigen for the development of specific PPR indirect ELISA.

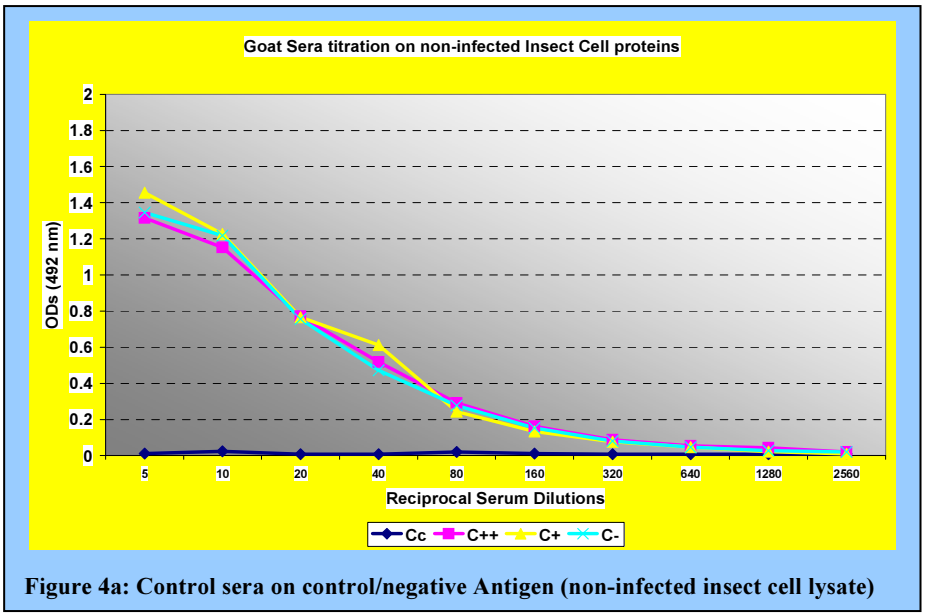


Figure 4a: Control sera on control/negative Antigen (non-infected insect cell lysate)

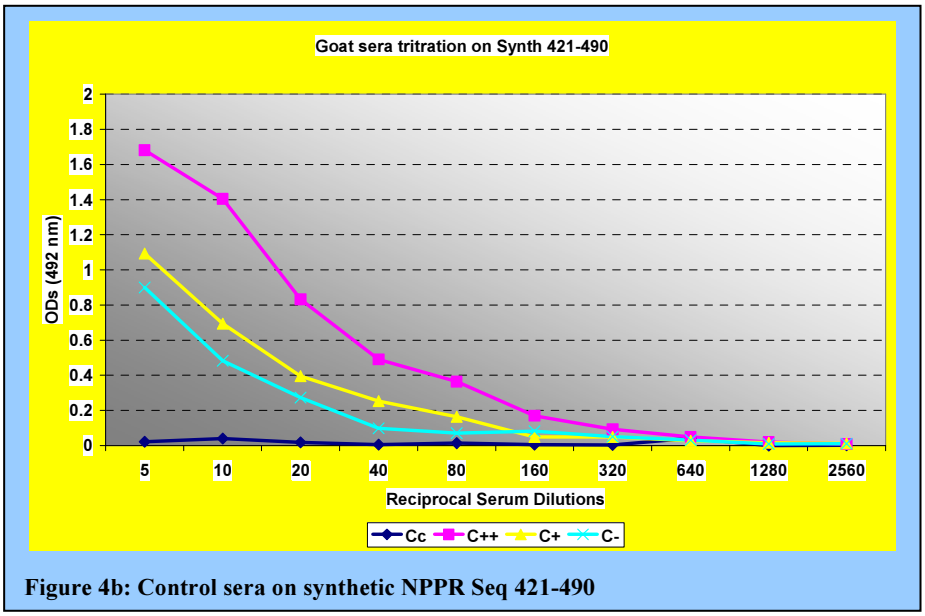


Figure 4b: Control sera on synthetic NPPR Seq 421-490

The results that were obtained did not allow clear differentiation between negative and positive sera because of high background: the sera reacted highly with non-infected cell proteins (figures 4a and 4b). Use of different ELISA plates, blocking buffers and semi-purified antigen could not improve the test. To rule out the possible effect of the irradiation on the background, studies were carried out in Côte d'Ivoire with non-irradiated sera: but the results were not improved.

2.1.2.2. Competitive ELISA (cELISA):

Spleen cells from a mouse immunized with the PPRV Np c-terminal peptide (70 aa) were fused with myeloma cells. Four hybridomas secreting monoclonal antibodies (mAb) against the PPRV Np were selected. The figures 5a and 5b summarize their characteristics, isotypes and binding sites (mapping by using overlapping synthetic peptides).

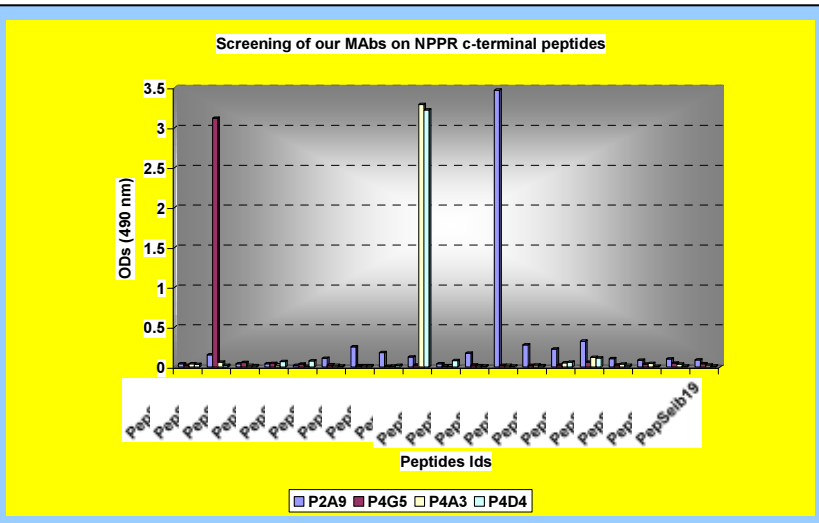


Figure 5a: Peptide epitope mapping of 4 APU MAbs anti-NPPRV (C-terminal)

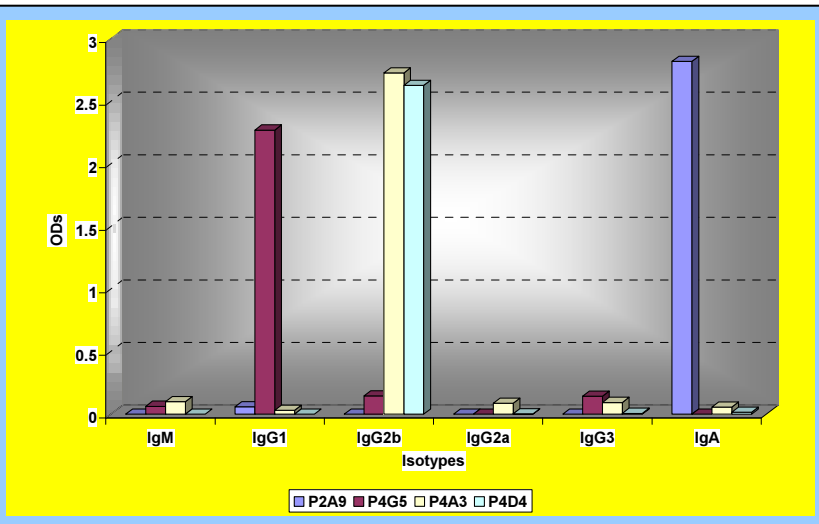


Figure 5b: Isotyping of APU MAbs

Two of these mAbs, P4A3 and P4D4, are of the same isotype, IgG2b, and recognize the same epitope: they are probably from the same cell clone. Therefore, only the mAb P4A3 was considered for further studies. The two other mAbs do not belong to the same isotypes and they recognize different epitopes.

The potential capability of the three mAbs to compete with PPRV antibodies was evaluated. Another mAb, the mAb 38-4, used in the current Np-based cELISA and whose binding epitope is located in the N-terminal region of the protein, was included in the study as a positive control. First trial of competitive ELISA test was done with those Mab in comparison of the current N-based cELISA which the mAb 38-4, test developed in France. The mAb P4G5 competes with PPR positive serum in nearly the same manner as the mAb 38-4, and it was selected for the development of a cELISA for PPR serodiagnosis.

2.1.3. Development of an ELISA for the serodiagnosis of Capripox.

Capripox and sheeppox infections are viral transboundary diseases of sheep and goats. They are endemic in many parts of Asia, the Middle and the Near East, and regions of Africa extending from North to the Equator. The only test currently used for the detection of antibodies anti-capripox in the serum samples of infected animals is the virus neutralisation test. It is neither easy to implement nor convenient for testing large numbers of samples. One of the objectives of the project funded by the French Ministry of Foreign Affairs to support some veterinary laboratories in Africa, is to develop an ELISA test for the diagnosis of capripox infection.

In some publications, it is claimed that the viral protein named p32 is one of the major antigens of the virus. It is a potential antigen for the development of an ELISA to detect antibodies anti-capripox. A DNA oligomer corresponding to its gene sequence was synthesised *in vitro*. The codons were changed to adapt them for optimum protein synthesis in insect cells. It was introduced into the baculovirus genome.

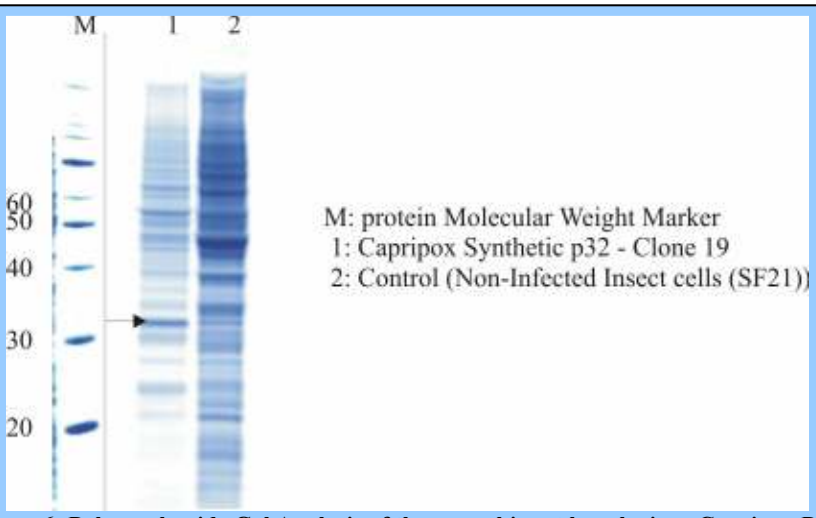


Figure 6: Polyacrylamide Gel Analysis of the recombinant baculovirus-Capripox P32 infected cells (the position of the capripox p32 protein is indicated by the arrow)

The recombinant virus which was selected expressed the recombinant protein in infected insect cells well (Figure 6).

Immunological studies are being carried out with this protein at Cirad-emvt, the coordinator of the project. If they confirm that small ruminants infected or vaccinated with capripox virus develop good serological responses against p32, the protein produced in insect cells by the recombinant baculovirus will be used to develop an ELISA test.

2.1.4. Development of an ELISA for the diagnosis of Foot and Mouth Disease (FMD) and to differentiate vaccinated from infected animals (CRP D3.20.20).

The objective of this work is the development of an ELISA (indirect ELISA and competitive ELISA) based on the of FMD virus 3ABC protein to discriminate vaccinated from infected animals. Currently, the different FMD vaccines in use consist of purified, killed virus. If highly purified, the vaccines are devoid of viral proteins which do not enter into the composition of the virus particles but are found in virus-infected cells, the viral non-structural proteins (NSP) such as the so-called 3ABC protein. Thus, and contrary to infected animals, sera from vaccinated animals would not be expected to contain antibodies against NSP 3ABC.

2.1.4.1. Indirect ELISA (iELISA)

A few years ago, the FMDV non-structural protein 3ABC was produced in APU as a recombinant protein in two ways: a) in-vitro by the “Rapid Translation System (RTS)” using

bacterial cell lysate and b) in insect cells by a recombinant baculovirus carrying the cDNA encoding 3ABC. Following preliminary studies, it was necessary to semi purify the antigen to reduce the background in the iELISA. The graphic in Figure 7 illustrates an example of results obtained in testing 3 sera of known status with the 3ABC antigen produced *in vitro* by RTS and semi-purified: the serum from an infected animal can be distinguished clearly from vaccinated or non-infected animals (result obtained in 2004).

Based on these results, a prototype of iELISA kit was produced and sent to the World Reference Laboratory (WRL) for FMD at the Institute for Animal Health (IAH) in the UK for evaluation. Samples used in that evaluation were also tested by using other assays that detect antibodies developed by animals against the FMDV structural proteins: the SPCE (Solid Phase cELISA), the LPBE (Liquid Phase Blocking Elisa) and the VNT (Virus Neutralisation Test). These tests detect animals that are infected with live FMDV or vaccinated. The results obtained in testing 86 serum samples from animals inoculated with vaccines of different serotypes (O, A and Asia 1) are summarized in table 3. While at 21 days post vaccination all animals seroconverted against the FMD structural proteins as revealed by SPCE, LPBE and VNT test results, they all remain negative with the IAEA NSP-based ELISA (100% of specificity).

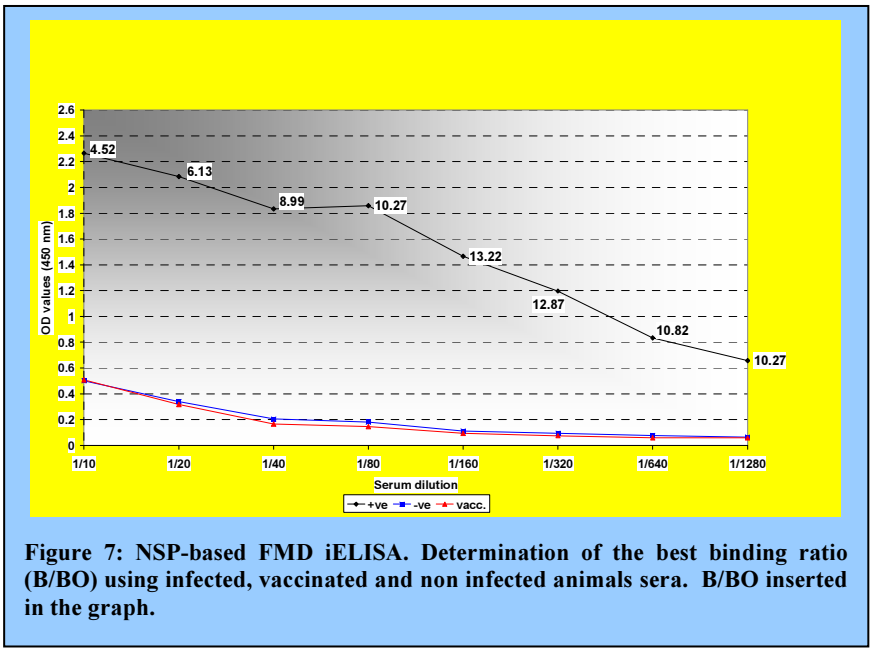


Figure 7: NSP-based FMD iELISA. Determination of the best binding ratio (B/BO) using infected, vaccinated and non infected animals sera. B/BO inserted in the graph.

Averages of test plates done with IAEA kit at the WRL:

AVG Cc	**0.04**
AVG C++	**1.39**
AVG vaccinated animals	**0.17**
AVG C-	**0.14**
cut off	**>0.30**

Sera from animals inoculated with different FMDV vaccines	SPCE No. of positive sera /No. of tested	LPBE No. of positive sera /No. of tested	VNT No. of positive sera /No. of tested	IAEA NSP No. of positive sera /No. of tested
O1 Manisa/Day 0	0/25	**1**/25	25/25	0/25
O1Manisa/Day 21	25/25	25/25	25/25	0/25
A iran'96/day 0	0/10	**2**/10	0/10	0/10
A iran'96/day 21	10/10	10/10	10/10	0/10
Asia 1 shamir/day 0	0/10	0/10	0/10	0/10
Asia 1 shamir/day 21	6/6	6/6	6/6	0/6

Table 3: Results of testing serum samples from animals at day 0 and day 21 after vaccination.

Further results were obtained by testing 65 field serum samples by both SPCE and the IAEA NSP-based iELISA. Compared to the SPCE results, the IAEA NSP-based ELISA has a sensitivity of 82% (41 versus 50 positive samples out the 65 sera tested).
Studies were conducted to improve this sensitivity rate (improvement of the binding ratio to better discriminate between positive and negative sera) involving a comparison of the capacity of two ELISA plates to allow better discrimination between positive and negative sera.

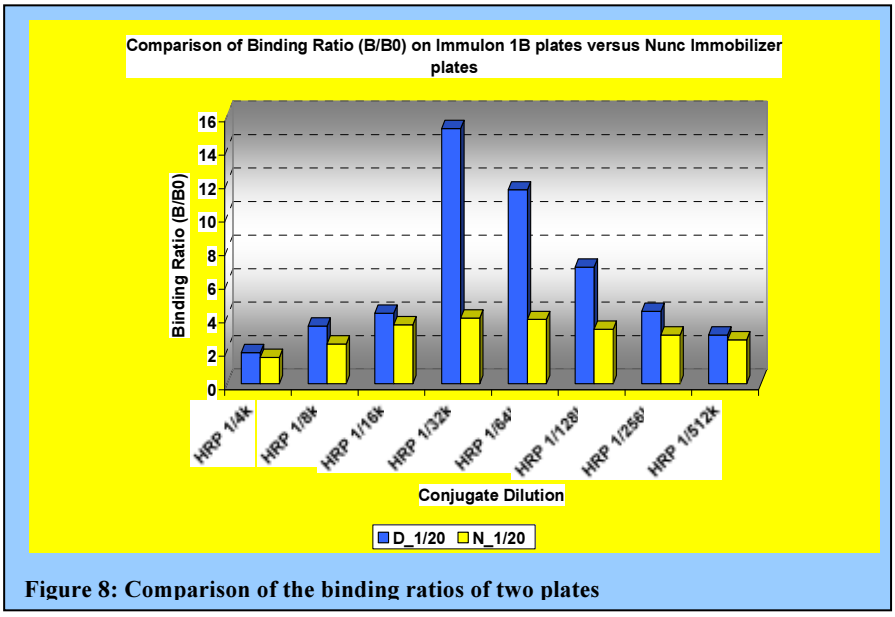


Figure 8: Comparison of the binding ratios of two plates

In Figure 8, the binding ratios obtained with the two ELISA plates Nunc Immobilizer and Dynex Immulon 1B are summarized. The latter has given the best binding ratio (B/BO).

Two conjugates anti-bovine immunoglobulin were also compared: HRP Sigma Reference A7414, the conjugate that was used in a kit sent to the WRL, and HRP Sigma Reference A8917. The latter has given the best results.
Based on new parameters, another prototype of the NSP-based iELISA was produced and sent to the WRL for evaluation, including sheep sera.

2.1.4.2. Competitive ELISA (cELISA):

For the indirect ELISA, testing sera from different animal species requires the use of a conjugate specific to each species. The advantage of the cELISA over the indirect ELISA is that a single conjugate can be used for all sera from different animal origins; the conjugate binding to the antibody used as the competitor. In addition, it is not necessary to purify the 3ABC antigen, which is a step required for the reduction of the

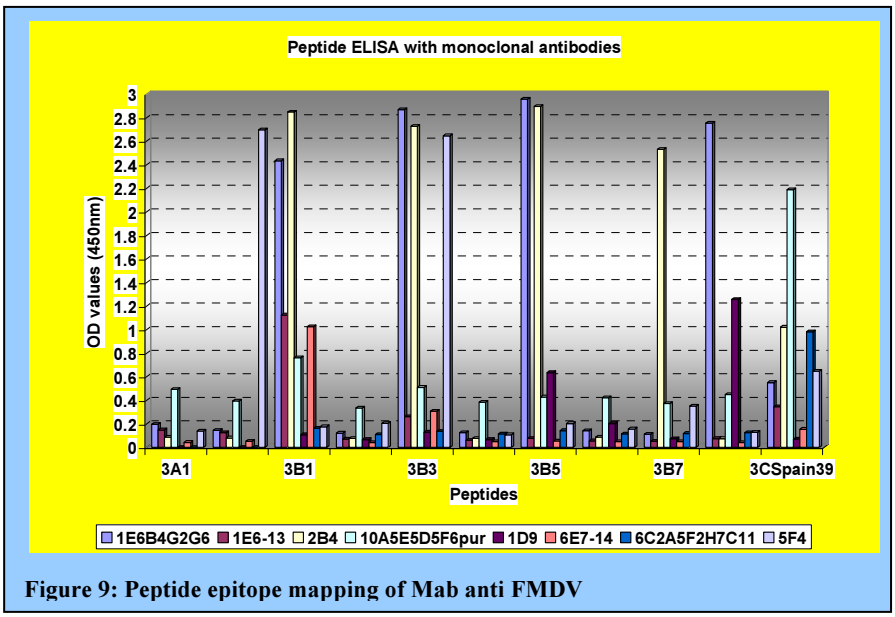


Figure 9: Peptide epitope mapping of Mab anti FMDV

background in the iELISA. For the development of the cELISA, studies were conducted to select a monoclonal antibody that could be used as a competitor.
In 2003, many monoclonal antibodies (mAb) anti-FMD 3ABC protein were produced. In 2005, they were further characterized by determining their isotypes and their binding sites (see the results of the peptide mapping in Figure 9).
The capability of the 8 mAb to compete with an FMDV positive serum was evaluated in a blocking ELISA experiment. No competitive activity was observed. New mAbs will be produced and tested for their capability to compete with sera from infected animals.

2.2. Animal Genetics

2.2.1. Genetic mapping for helminth resistance in sheep

One of the major constraints in small ruminant production systems is the helminth infestation that affects productivity by altering nutrients absorption in the gastro-intestinal system. Depending on the degree of infestation, this can lead to severe anaemia and eventually death (mainly in newborns). To exploit molecular genetics information accumulated over the past decade on naturally observed helminth resistance in sheep breeds, APU has completed the laboratory work of an international collaboration led by the International Livestock Research Institute (ILRI) and the United States Department of Agriculture (USDA). The laboratory work entailed a large chromosome mapping exercise to search for genes related to gastro-intestinal parasite resistance in sheep. Twenty-one microsatellite DNA markers were analysed in a sheep population consisting of 400 crossbred animals (Red Maasai and Dorper breeds), covering 4 chromosomes (OAR21, OAR23, OAR25 and OAR26).
The animals were selected based on the natural manifestation of helminth resistance phenotype, in the case of the Red Maasai breed (figure 10), and helminth susceptibility, in the case of Dorper breed (figure 11). A backcrossed population of these two breeds was raised at ILRI in Nairobi – Kenya over the past 10 years from which both phenotypic and genotypic data have been recorded in detail. This represents a powerful resource in the search for suitable markers for breeding and selection for parasite resistance. Molecular data related to these markers and chromosomes were produced at APU. They are under conjunct analysis by international partners (ILRI and USDA) with their own data. Figure 12 illustrates the chromosomes and DNA microsatellite markers accessed by APU during 2005, from which 21 were selected, showing good coverage of the region of sheep genome that was analyzed and molecular data that has added strong statistical power to the search for quantitative trait loci (QTL).

Figure 10: Red Maasai (Photo ILRI): helminth resistant sheep breed

The major expectation is to identify candidate gene variations that could be exploited in marker introgression programmes in Member States to enhance helminth resistance. The analysis of these specific markers in selected sheep and goat populations around the world has the potential to strengthen this livestock sector by adding value to breeds and/or individuals and promoting higher productivity rates.

Figure 11: Dorper (Photo ILRI): helminth susceptible sheep breed

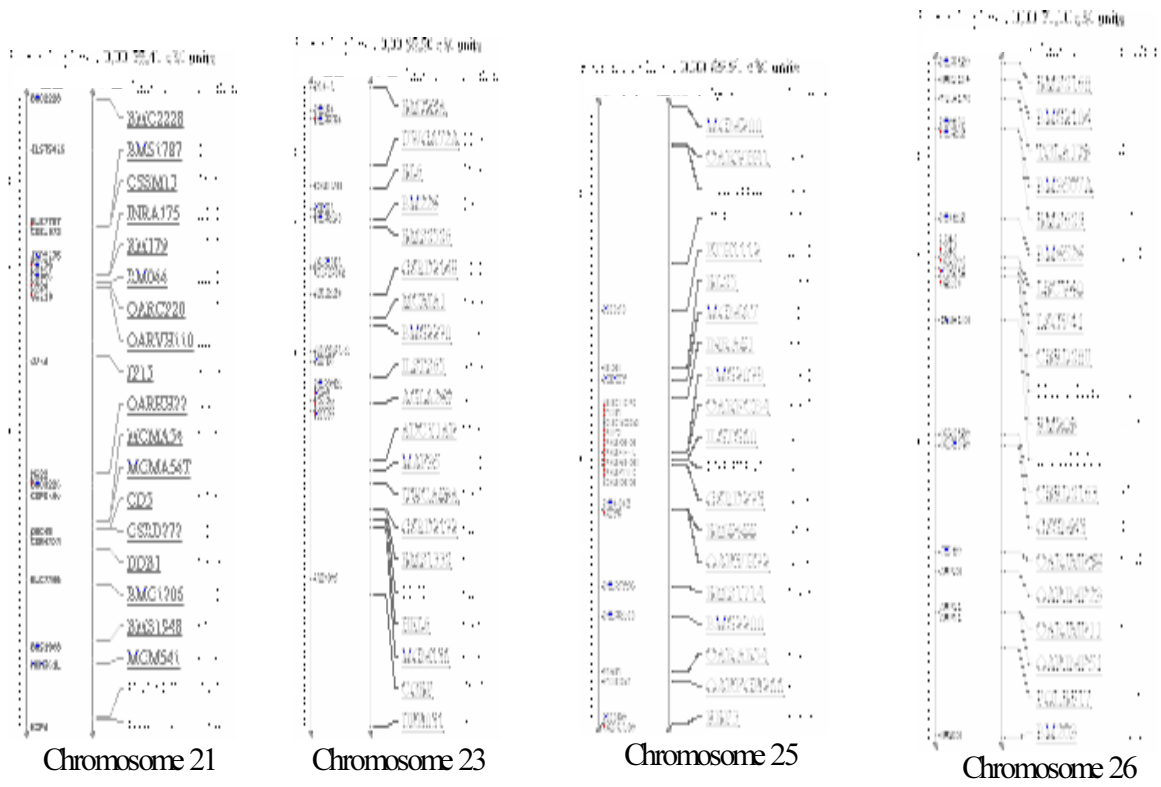


Figure 12: APU was involved in the genotyping of 4 sheep chromosomes (OAR21, OAR23, OAR25 and OAR26). The figures correspond to the genetic linkage maps of the 4 chromosomes covered in the study. Markers employed and genetic distances are shown for each chromosome under study (underlined markers were evaluated for heterozigozity).

2.2.2. Single Nucleotide Polymorphism (SNP)

After the completion of several genome initiatives in animals and human, the amount of data deposited in public databanks is enormous. Exploitation of this freely available information is considered to be a platform for identifying new molecular markers and applying them in livestock production.

In this context, APU is undertaking a second research programme in the field of livestock genetics, which involves the detection of single nucleotide polymorphism (SNP) using PCR-based tests targeting fecundity, muscle growth and disease susceptibility genes in sheep. During 2005, *in silico* DNA sequence analysis was performed in order to design optimal primers and probes, which were then synthesized and applied in field experiments. These tests will be used in the future on sheep and goat samples collected during CRP-D3.10.25.

This work started in 2005 and the first SNP detection using real-time based technology (involving Taq Man probes) has been achieved. Figure 13 illustrates the typical results of real-time based SNP detection in the prion protein in sheep DNA. Other SNP tests for fecundity, muscle growth and disease resistance are under development with the aim of applying these within the CRP during 2006. The major objective is to develop a panel of SNP markers for small ruminants that could be validated in different sheep and goat breeding schemes around the world.

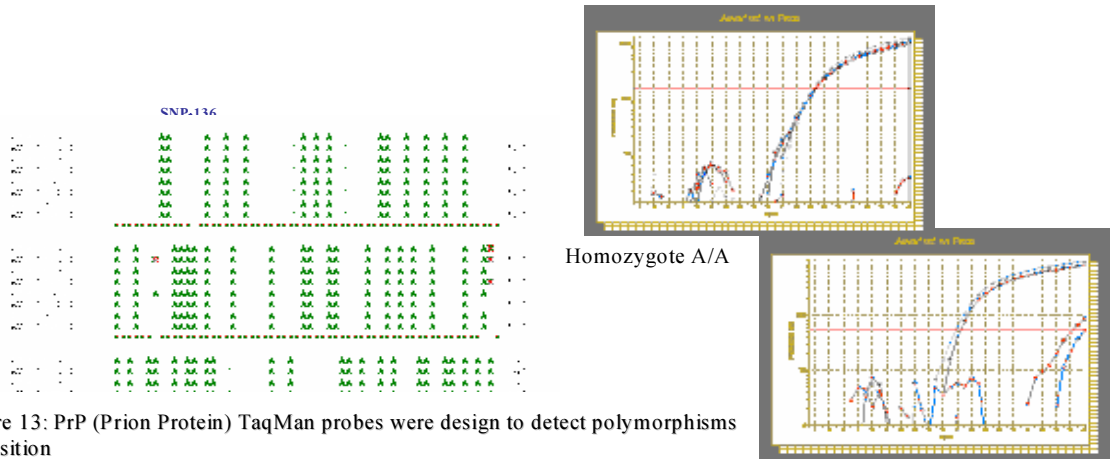
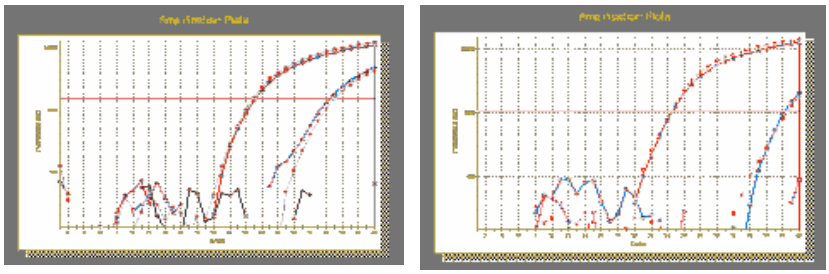


Figure 13: PrP (Prion Protein) TaqMan probes were design to detect polymorphisms in position
171 (A/G; G/T polymorphisms) (see figure above) of the Prion Protein gene in sheep. Probes were tested in different sheep DNA samples to prove their utility.
The pictures show real-time PCR graphics for PrP-171 A/G, G/T polymorphism detection.



Homozygote G/G

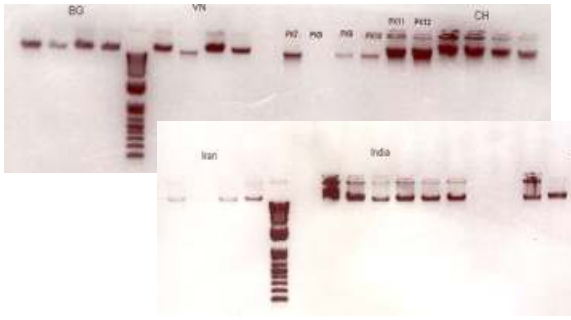
Heterozygote G/T

2.2.3. Coordinated Research Project (CRP) on genetic characterization of small ruminants in Asia

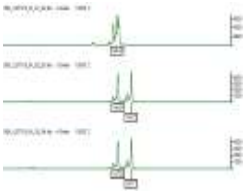
An IAEA Coordinated Research Project (D3.10.25) was started in December 2004 with the overall objective to utilize gene-based technologies to characterize small ruminant genetic resources in Asia. The specific objective is to use an FAO panel of DNA microsatellite markers (followed by other relevant DNA markers), to better understand the genetic structure of sheep and goat breeds from Asia and to allow the National Agricultural Research Systems (NARS) to formulate policies and action plans to preserve and exploit indigenous breed potentials.

APU has been involved from the formulation to the implementation of the technical component of this project (in close collaboration with ILRI). For this purpose, the APU Scientific Officer participated in a training course and workplan development meeting in Nairobi – Kenya (December 2004), which defined the tasks to be performed during 2005 by the group of counterpart scientists from Iran, Pakistan, Bangladesh, Indonesia, Sri Lanka, Vietnam, Saudi Arabia and China, with the consultancy of an IAEA contracted experts (Dr. Paolo Ajmone-Marsan from Italy, specialist on sheep and goat diversity analysis, Dr. Han Jianlin and Dr. Olivier Hanotte from ILRI). In September 2005 the first Research Coordination Meeting took place in Bogor, Indonesia, where the first scientific/technical results were presented. During this meeting, participants shared their results and defined new tasks. The strong input of APU to the technical programme of the project was obvious from the excellent results of the blood sampling and DNA purification protocol developed at APU during 2005 (see the description of this activity below). By using this certified approach, the counterparts from all over Asia were able to generate DNA samples of high purity, suitable for sensitive DNA microsatellite analysis. The DNA quality and DNA microsatellite results from samples obtained using APU suggested protocols are provided in Figure 14.

Figure 14: Goat and Sheep samples from 7 counterpart countries in Asia: Testing of the blood samples collected from livestock in special preservatives



Genomic DNA samples from counterpart countries were tested for quality in IAEA/APU facilities.
DNA samples from each country were tested for quality in agarose gel. Subsequently, samples were subject
of a PCR amplification using DNA microsatellite specific primers. As shown in pictures, efficiency of the blood sampling and DNA purification protocols were proved.



Goat DNA samples tested with DNA microsatellite primers, in order to prove efficiency of the blood sampling and DNA purification protocols.

Sheep DNA samples tested with the same DNA microsatellite primers used with the goat DNA samples.

Also during this meeting, a new task was proposed by the APU Scientific Officer and accepted by the counterparts. The task was to develop, at APU Seibersdorf and the IAEA Collaborating Centre on Animal Genomics and Bioinformatics in Brazil, an SNP panel that could be used on the biological samples collected under the framework of the CRP to improve the genetic characterization of small ruminants. For this purpose, CRP counterparts were instructed by the APU Scientific Officer to send sheep and goat DNA samples to Seibersdorf during 2006 to be used for validating the SNP tests. Austrian government clearances are being acquired and these samples should provide a useful resource at APU for optimising tests throughout the timeframe of the CRP.

2.3. Methods Development

2.3.1. *A DNA purification protocol using blood samples collected from livestock in special preservatives was tested in 8 Asian countries.*

The objective of using special preservatives for whole blood is to allow researchers to collect blood samples in regions where environmental conditions pose difficulties (hot, dry, far distances, lack of cooling systems) and maintain their suitability for DNA purification. This methodology could be particularly valuable in the current CRP (D3.10.25) where approximately 4000 sheep and goat samples are to be collected from geographical regions covering the territories from Saudi Arabia to Vietnam. Reagents and protocols were provided to counterparts in different Asian countries who then shipped to APU for molecular analysis the DNA they had extracted. It was of a enough high quality to perform molecular analyses, demonstrating the feasibility and appropriateness of using this protocol for fieldwork in livestock genetics. The development of the protocols for blood preservation has benefited the CRP and will be used in future research activities of the FAO/IAEA Joint Programme in livestock genetics.

2.3.2. Microsatellite markers detection.

Routine assay conditions for fluorescent detection of microsatellite markers on the automated DNA fragment analyser ABI3100 were established in conjunction with Plant Breeding Unit staff. At present we are able to perform DNA microsatellite analysis using a fluorescent labelling approach with high accuracy and fidelity. An intermediate step was introduced in the routine protocol that consisted of drying DNA solutions in 96 well format plastic plates (coating the internal surface of the wells) allowing the manipulation of several plates in a short time span and the easy interchange of DNA in ready-to-use plates between collaborating laboratories.
The establishment of the microsatellite analysis was a landmark for livestock genetics at APU since, from this point on, the cooperation with Member State laboratories in order to assist them in performing IAEA-related activities in this area became possible.

2.4. External Collaborations and Partnerships

APU has maintained international research collaboration with the International Livestock Research Institute (ILRI) and the United States Department of Agriculture (USDA) using genome-wide mapping to search for genes related with gastro-intestinal parasite resistance in sheep.
APU has collaborated with partners involved in three extra-budgetary funded projects: Cirad-emvt (France), Institute for Animal Health (UK), Royal Veterinary College (UK), Laboratoire Central Vétérinaire (Mali), National Veterinary Institute (Ethiopia), LANAVET (Cameroon), KARI-Muguga (Kenya), LANADA (Côte d'Ivoire), IBET (Portugal).
The Head of the Unit represented the IAEA in two meetings of the PACE Advisory Committee. He also attended two meetings of the Biologicals Standards Commission of the World Organisation for Animal Health (OIE).

3. Guidelines and Standards

The Joint FAO/IAEA Programme has been the initiator of the current OIE guidelines for the validation of animal disease diagnostic assays. For that it convened 2 consultants meetings which made a number of recommendations. One of these was that assays should be validated according to fitness for purpose with the expectation that such assays will give more confidence in animal disease management in general and also in animal and animal product trade specifically. Another recommendation was that reference material collections be established and used for test validation. The Joint FAO/IAEA Animal Production and Health Subprogramme, operating the OIE Collaborating Centre for the Application of ELISA and Molecular Techniques in Animal Disease Diagnosis, is highly involved in animal disease diagnostic tests, mainly those of transboundary nature. For its test development activity, the Subprogramme has started establishing reference sera to be used in the assay validation process. A set of field sera (PPR and RPV experimental infection/vaccination) consisting of 116 bovine sera, 116 goat sera and 140 sheep sera was received from Côte d'Ivoire. These sera were irradiated, distributed in aliquots and stored in our serum bank at -20 °C.

4. Training Activities

4.1. Fellows and Scientific Visitors

Name	Country or Institute	From	To
Fellows			
Mr Wijaya Kumara Hirimburegama	Sri Lanka	05 October 2005	05 December 2005
Scientific visitors			
Mr. Tad Sonstegard	USDA	04 May 2005	07 May 2005
Mr. Curt Van Tassel	USDA	04 May 2005	07 May 2005
Mr. Fuad Iraqi	ILRI (Kenya)	04 May 2005	07 May 2005
Mr. Mulumba	Democratic Republic of Congo	11 April 2005	15 April 2005

4.2. Interns

Ms. Carolina Luque, a Veterinary Medicine student, spent 2 months at APU working on the analysis of microsatellite DNA markers.
Ms. Patricia Stevens, a Biomedicine student, spent 2 months at APU working on the preparation of DNA purification protocols.

5. Appendices

5.1. Publications

Diallo (A). Control of Peste des Petits ruminants: vaccination for the control of Peste des Petits Ruminants. *Dev Biol. Basel*, 2005, **119**: 93-98

Barrett T., Banyard A. and **Diallo A.** Molecular biology of the morbilliviruses. In Rinderpest and Peste des Petits Ruminants. –Biology of Animal Infections. Barrett T., Pastoret P.P. and W.P. Taylor (eds) Elsevier, Academic Press, 2005

Couacy-Hymann E., **Bodjo C**,. Danho T, Libeau G, **Diallo A.** Evaluation of the virulence of some strains of peste des petitsruminants virus (PPRV) in experimentally infected West African dwarf goats. *Veterinary Journal*, 2005, **23,**

Couacy-Hymann E., **C. Bodjo**, T. Danho, G. Libeau, **A. Diallo**. Surveillance of wildlife as a tool for rinderpest and peste des petits ruminants monitoring in West Africa. *Rev. sci. tech. Off. int. Epiz.*, 2005, **24** (3), 869-877

Kerdiles Y. M., B. Cherif, J. C. Marie1, N. Tremillon, B. Blanquier, G. Libeau, **A. Diallo**, T. F. Wild, M.-B. Villiers and B. Horvat. Immunological properties of Morbillivirus nucleoproteins. *Viral Immunology*, in press (2006)

Diallo A. and Viljoen G. Genus Capripoxvirus. In Poxviruses, Birkhäuser Advances in Infectious Diseases, A. Mercer/A. Schmidt/O. Weber (eds), Submitted for publication..

5.2 Travel

Staff Member	Destination	Period	Purpose of Travel
A. Diallo	Paris, France	29-31 January	To attend the OIE Biological Standard Commission meeting
	Bamako, Mali	14-16 April	To attend the PACE Advisory Committee meeting
	Montpellier, France	14–19 April	To attend the first meeting of the EC funded MARKVAC project meeting
	Montpellier, France	2-3 June	To attend the first meeting of the LABOVET project
	Pirbright, U.K.	18-19 July	To visit and discuss with the coordinator of the project funded by the Wellcome Trust
	Paris, France	21-23 September	To attend the OIE Biological Standard Commission meeting
	Nairobi, Kenya	17-20 October	To attend the PACE Advisory Committee meeting
F. Garcia	Uppsala, Sweden	5-8 June	Participation in a symposium on genetics
	Jakarta, Indonesia	19-23 September	To attend, as the technical officer, the RCM related to the CRP D3.10.25

5.3. TCPs and CRPs

a) CRP D3.10.25 – Gene-based technologies in livestock breeding: Characterization of small ruminant genetic resources.

b) TCP PER/5027 – Use of nuclear techniques to improve alpaca productive and reproductive methods.

c) TCP NER-5011 – Modernisation des Services de Laboratoire pour le diagnostic des maladies animales