

IAEA

Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Animal Production Unit

Activities Report 2006



FAO/IAEA Agriculture & Biotechnology Laboratory
Agency's Laboratories, Seibersdorf
International Atomic Energy Agency
Austria



Executive Summary

In 2006, the genetic activities in the Animal Production Unit (APU) were focused on the development of techniques to identify genetic markers for three interesting traits: resistance to gastrointestinal parasite infestation, resistance to scrapie and increased prolificacy. The sheep was the model animal for all three traits. The aim was to develop molecular techniques for animal breeding programmes. Research strategies have focussed on the use of a simple and reproducible technology, the TaqMan PCR technology, to identify single nucleotide polymorphism (SNPs). Standardisation of microsatellites for identification of sheep resistant to helminths was also made. These techniques now need validation on more DNA samples before their transfer to FAO and IAEA Member States for the implementation of their animal breeding programmes and plans for evaluation and conservation of livestock genetic diversity.

Foot and mouth disease (FMD) is one of the most feared animal diseases and also one of the most important constraints to the development of markets for animals and animal products. To facilitate the control/surveillance of this disease, many laboratories are developing serological tests as valuable tools to monitor FMD infection/circulation in a country irrespective of the vaccination status of animals. Information on the status of the national herd is part of the dossier to be submitted to the World Organisation for Animal Health (OIE, Office International des Epizooties) by countries applying for FMD freedom status. In 2000, the Animal Production and Health Subprogramme (APH) embarked on the development and validation of FMD diagnosis tests that would enable the differentiation between infected and vaccinated animals. After having developed in 2005 an indirect ELISA for such a purpose, the Unit worked in 2006 on the development of the c-ELISA format of the test based on the use of a monoclonal antibody anti-FMD virus non-structural protein (NSP) 3ABC. The advantage of the new test is that only one conjugate for testing sera from different species is needed. With the previous test, the indirect ELISA format, a conjugate specific to each animal species was needed. Preliminary results of this new test indicate that it has nearly same performance as the previous one: 80% sensitivity and 93 to 100% specificity.

Another important disease on which the Unit put a lot of effort in 2006 was the peste des petits ruminants (PPR). It is considered as one of the main sheep and goat diseases which need to be addressed in poverty alleviation policies in many countries in Africa and Asia. Currently it is in expansion in those two continents. For the better control of PPR, the APU is collaborating with many other institutions to develop marker vaccine and a companion test that would enable differentiation of vaccinated animals from infected animals. In that project, the APU is in charge of mapping the viral nucleoprotein for the identification of sites non indispensable to the virus life-cycle and where the marker can be introduced. The work that was carried out in 2006 confirmed the zones of the viral nucleoprotein identified previously as involved in its self association, a process essential for the encapsidation of the viral genomic RNA and therefore for the viability of the virus. Zones should not be deleted for developing the marker vaccine. The interaction of the nucleoprotein with the viral matrix protein, which leads to the formation of the virus particle, was also studied. Four peptides were identified on the nucleoprotein as potential sites for the binding of the N to the matrix protein. Although each of them can be deleted without affecting too much the internalization of the nucleoprotein into the envelope, two peptides seem to constitute major binding sites.

Finally, as part of its contribution to the transfer of technology to FAO and IAEA Member States, staff of APU took part in the organisation of two training courses: training on the diagnosis of highly pathogenic avian influenza and on the diagnosis of PPR and contagious bovine pleuropneumonia (CBPP).

TABLE OF CONTENTS

Executive Summary

1. Introduction	1
1.1. Sub-Programme and Unit Objective	1
1.2. Organizational Chart and Unit Staff	2
2. Research and Development	4
2.1. Animal Genetics	4
2.1.1. Resistance to Gastrointestinal Parasitic Infestation.....	4
2.1.2. Resistance to Scrapie	10
2.1.3. Increasing prolificacy in small ruminants: The FecB gene	13
2.2. Animal Health	14
2.2.1. Development of c-ELISA for the diagnosis of Foot and Mouth Disease: Use of Non-Structural-Protein (NSP) of Foot and Mouth Disease Virus to Differentiate between Vaccinated and Infected Animals (DIVA)	14
2.2.1.1. <i>Monoclonal Antibodies (Mabs) production</i>	15
2.2.1.2. <i>Studies to optimize assay conditions in c-ELISA</i>	16
2.2.1.3. <i>Specificity studies of the c-ELISA</i>	17
2.2.2. CRP D3.20.23: Veterinary Surveillance of Rift Valley Fever	19
2.2.3. Reverse ELISA	20
2.2.4. Development of peste des petits ruminants (PPR) marker vaccine and companion diagnostic tests with improvement of epidemiological knowledge to facilitate control of the disease	21
2.2.4.1. <i>Mapping the protein-protein interaction sites on PPRV nucleoprotein</i>	21
A) <i>Mapping the protein-protein interaction sites on PPRV nucleoprotein</i>	21
B) <i>Mapping domains of PPRV nucleoprotein interacting with the matrix protein</i> ...	22
2.2.4.2. <i>Analysis of antigenic structure of PPRV nucleoprotein</i>	26
3. Training	28
4. Fellows and Interns	28
5. Appendices	29
5.1. Publications	29
5.2. Duty Travels	30

1. INTRODUCTION

1.1. Sub-Programme and Unit Objective

The vision and goal of the Animal Production and Health (APH) Subprogramme 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 in FAO and IAEA Member States. 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 their 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, in particular nuclear and nuclear-related techniques to developing countries, promoting and implementing applied research projects for the development of 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.

To implement its activities, the transfer of new technologies to developing countries, the Subprogramme usually acts as an interface between developed and developing countries. In that line, in 2006 it organised two training courses on molecular techniques for the diagnosis of diseases, one for highly pathogenic avian influenza at Seibersdorf, and the second for contagious bovine pleuropneumonia and peste des petits ruminants in Mali. The Subprogramme held also a consultant meeting for identifying new technologies to be transferred to developing countries for transboundary animal diseases and for the validation of animal diseases diagnostic tests.

The Animal Production Unit has worked very closely with the Section for all these activities.

1.2. Organizational Chart and Unit Staff

Agency's Laboratories

Name	Title	E-Mail Address	Extension
Gabriele Voigt	Director	G.Voigt@iaea.org	28200

FAO/IAEA Agriculture & Biotechnology Laboratory

Name	Title	E-Mail Address	Extension
Erik Busch-Petersen	Head, FAO/IAEA Agriculture & Biotechnology Laboratory	E.Busch.Petersen@iaea.org	28267

Animal Production Unit

Name	Title	E-Mail Address	Extension
Adama Diallo	Unit Head, Molecular Biologist	A.Diallo@iaea.org	28355
Massoud Malek	Molecular Geneticist	M.Malek@iaea.org	28358
Eva Maria Winger	Senior Laboratory Technician	E.Winger@iaea.org	28302
Mamadou Lelenta	Laboratory Technician	M.Lelenta@iaea.org	28321
Ericka Adelina Pestana Delgado	Laboratory Technician	E.A.Pestana.Delgado@iaea.org	28378
Lincy Thevalakara	Clerk	L.Thevalakara@iaea.org	28362
Sanne-Charles Bodjo	Consultant	S.Bodjo@iaea.org	28356
Caroline Adombi	Consultant	C.M.adombi@iaea.org	28380
Charles Euloge Lamien	Consultant	C.Lamien@iaea.org	28314
Maria Butschety	Contract staff		

Unit Staff

Animal Production Unit



Massoud MALEK



Adama DIALLO



Mamadou LELENTA



Ericka PESTANA
DELGADO



Eva-Maria WINGER



Charles Euloge LAMIEN



Caroline Melanie ADOMBI



Sanne Charles BODJO



Lincy THEVALAKARA



Gabriele VOIGT
Director
Agency's Laboratories



Erik BUSCH-PETERSEN
Head
FAO/IAEA Agriculture & Biotechnology Laboratory

2. RESEARCH & DEVELOPMENT

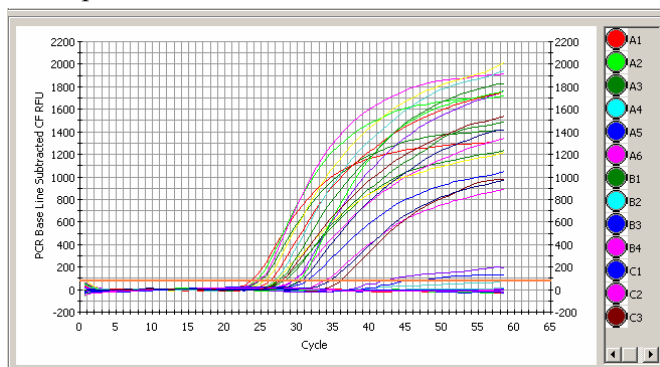
2.1. Animal Genetics

Genetic markers are small sequences of DNA located close to or in protein genes to which they have remained linked during the evolution. Therefore, their study provides valuable information about a population and individuals can be selected on the basis of the presence of a particular marker based on result of a simple DNA test (blood sample). Animal genetics research in APU in 2006 was



focused on the search of genetic markers for three economically important traits: resistance to gastrointestinal parasite infestation, susceptibility to scrapie and increased prolificacy. The aim was to provide molecular techniques for animal breeding programmes. Research strategies have been purposeful by the use of a simple and reproducible technology such as the TaqMan PCR technology that can be transferred easily to Member States.

TaqMan Single Nucleotide Polymorphisms (SNPs) genotyping Assay (TaqMan SNP) is a highly flexible technology for detection of SNPs. It can provide results in a fast, accurate, economic and high throughput manner. With the simplest workflow available, the TaqMan assay allows generating genotypic data very quickly. It should be added that straight forward screening from a population can be done on pooled DNA for allele frequency studies, a possibility making the study less costly and less laborious. More importantly, the technology's simplicity and reproducibility allow it to be easily transferred to IAEA and FAO Member States.



2.1.1. Resistance to Gastrointestinal Parasitic Infestation

Gastrointestinal nematode parasite infestations of livestock are among diseases that have high negative impact upon animal health and productivity. It had been known for years that some breeds of sheep have natural genetic ability to cope with internal parasite infestations. Selection of sheep, which are more “tolerant” to helminth infestations, can be achieved via identification of molecular markers known to be associated with the parasite resistance trait. Using available information on the gene bank and data from other on-going research projects, the interferon gamma (INFG) gene has been selected for the identification of putative SNP sites related to parasite resistance. For that, the INFG gene sequences covering exons 2 and 4 and intron 1 of sheep were obtained from the GenBank database and aligned using the programme ClustalW (see figures 2 and 3). Analysis of these sequence alignments allowed identifying potential zones of interest for SNPs. Additionally,

new microsatellite primers known to be linked to parasite resistance in cattle were also subject to study and standardization in order to be applied to a reference mapping population of Dorper x Red Massai sheep developed by the International Livestock Research Institute in Kenya (ILRI).



Primer pairs were designed, based on the genes sequences of IFNG extracted from the gene bank, (see fig. 2, and 3), to amplify two sections of the IFNG gene (see figure 1). The first primer pair (named Allele-IFGN) was designed to amplify a section of the gene between exons 2 and 4. The second primer pair was designed to amplify the region comprising the IFNG intron-1, which has a microsatellite marker previously shown to be associated with resistance against parasites.

In order to verify the validity of these data and to find SNPs that can be used as markers to select resistant sheep breeds, 4 Dorper (helminth susceptible sheep breed) and 5 Red Massai (RM) (helminth resistance sheep breed) parental DNA samples were subjected to PCR amplification and sequencing. Four SNP sites and a point deletion in the RM samples were found in the gene sequence amplified between exons 2 and 4. Out of these 4 sites, 3 can be used as potential markers, as these 3 sites were found to be homozygotic in all the RM samples while they were found to be heterozygotic in the case of the Dorper samples, the susceptible sheep breed (see table 1). The presence of these markers in homozygosity in the Red Massai samples could suggest that the heterozygosity stage in the Dorper samples (mixture of the two alleles) could somehow influence the susceptibility in Dorper, and thus making those 3 SNPs sites attractive for further studies.



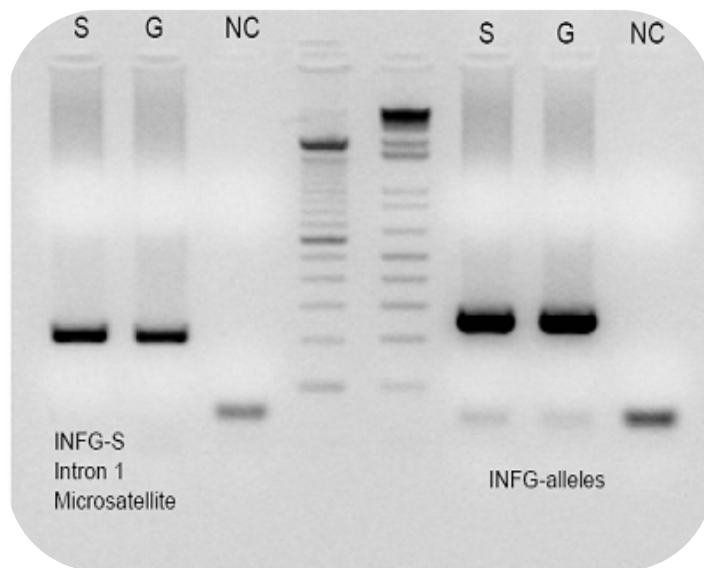


Figure 1: The picture shows the results of the PCR amplification from genomic DNA of sheep (S) and goat (G) using the primer pairs designed to amplify the two sections of the IFNG gene.

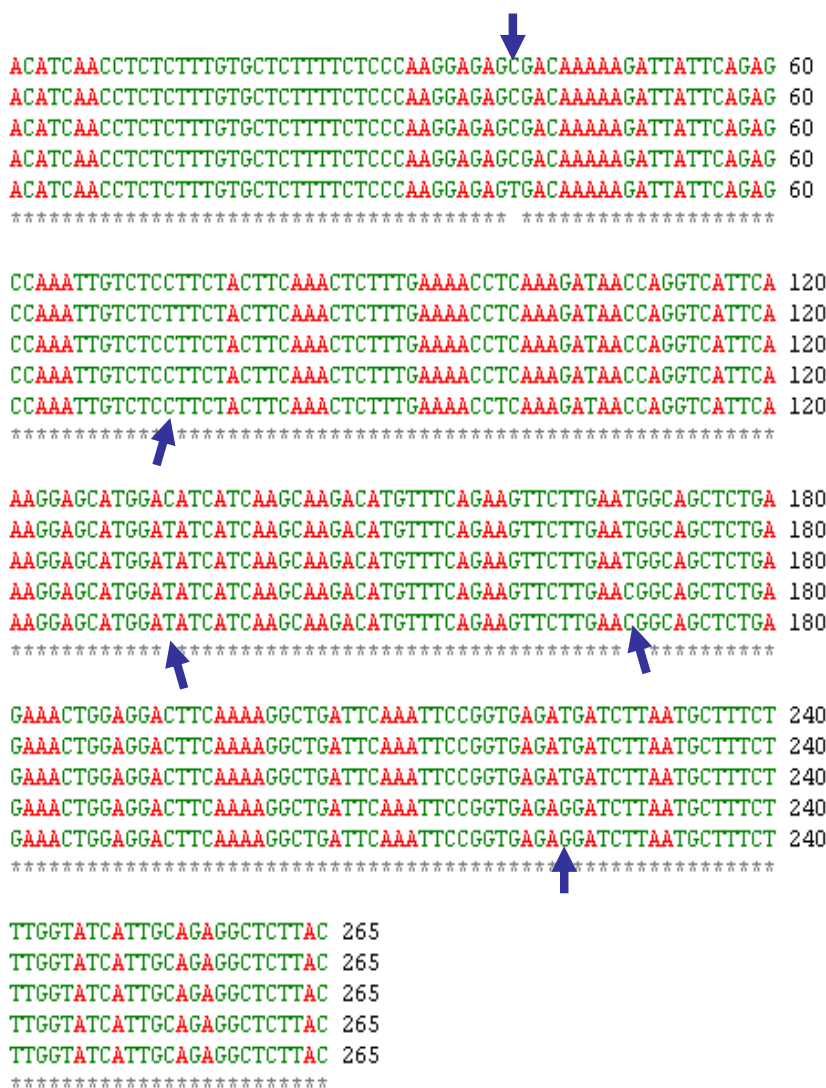


Figure 2: Multiple sequence alignment covering the Exon 2-4 of the IFNG gene. Five gene sequences, belonging to the IFNG from sheep were extracted from the gene bank and aligned for in-silico SNP discovery, using the programme ClustalW. The figure is showing the 5 SNPs found during sequence alignment. The sequence helps to elucidate were to design the primers for amplification using the genomic DNA from Dorper and Red Mas-sai.

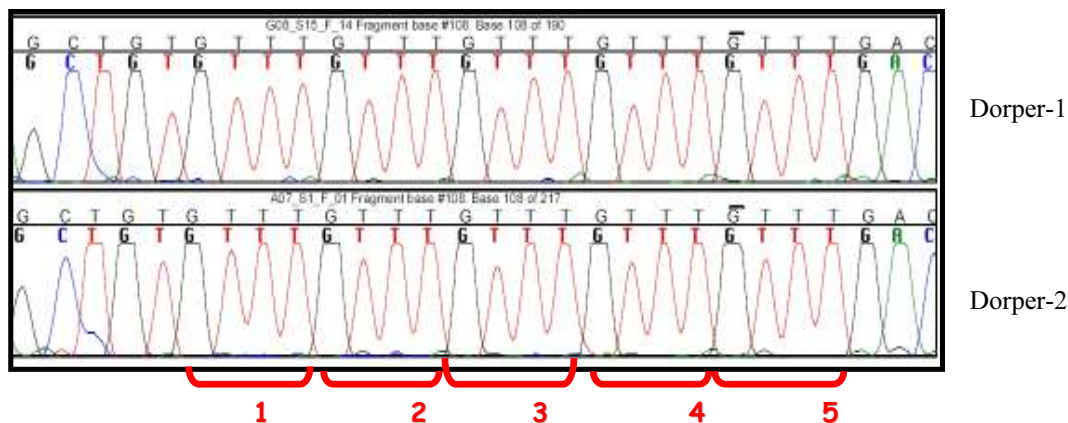


Figure 4. Chromatogram showing the IFNG intron 1 samples from 3 Dorper sequences. As seen from the picture, Dorper samples have a prevalence of short microsatellite (GTTT)₅.

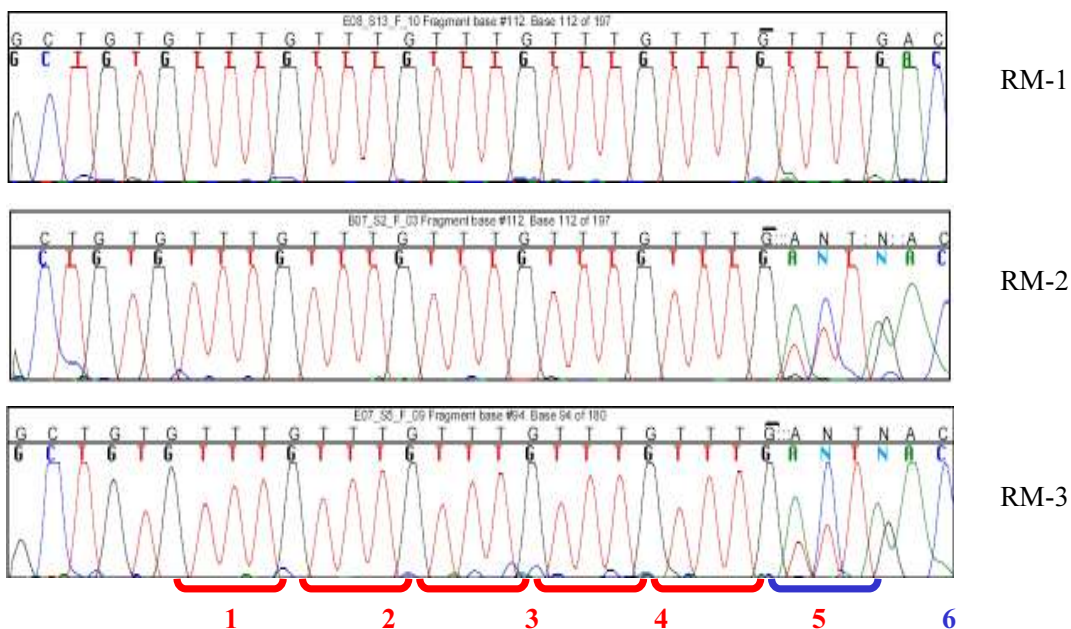


Figure 5: Chromatogram of 3 Red Massai samples sequenced using the primers to amplify the Intron 1 of the IFNG gene. Red Massai has a prevalence of a long microsatellite (GTTT)₆. The first sequencing trace shows a homozygous state of the microsatellite. The second and third RM samples were heterozygous (mixture of the short and long microsatellite).

Standardization of microsatellite primers

In addition to the microsatellite identified above, primers used for the amplification of 9 microsatellites from cattle were standardised for use in sheep samples (see figure 6 and table 2). After standardization the protocol and primers were shipped to the Brazil Collaborative Centre to be applied in sheep population samples.

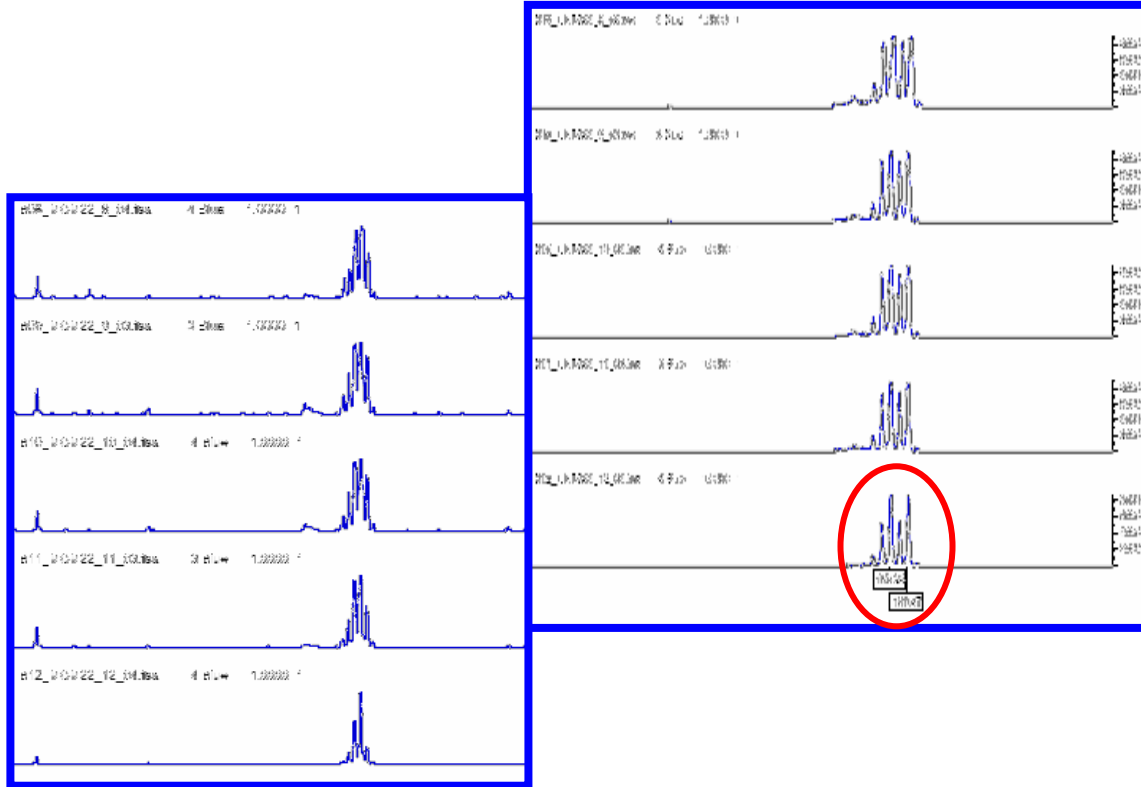


Figure 6: Standardization of microsatellite primers. Primers were subjected to gradient PCR in order to elucidate the best annealing temperature for amplification and the best separation of the microsatellite peaks. Samples were then diluted 1/5 and loaded into the AB-3100 for electrophoresis. After that the correct temperature according to the best differentiation of the peak formation was selected for further experiments (as seen in the red circle).

Table 2: The standardised conditions for the 9 microsatellite primers (annealing temperatures, size of the microsatellites in sheep as well as information of the primers)

Primer name	Primer Reverse	Primer Forward	Labelling	Tm F	Tm R	Optimization	Status	range size
ILSTS44-R	5' ACATGTTGTATTCCAAGTGC 3'	FAM- AGTCACCCAAAAGTAACTGG 3'	FAM	45.8	45	Tm=60C; dil=1/5	Ready and tested	160-182
MCM130-R	5' CTCACCTCTGCTCTTCTATCTCTCT 3'	HEX-AAACTTTTGTCTGTTGGGTATC 3'	HEX	54	54	Tm=57C; dil=1/10	Ready and tested	132-158
MCM22-R	5' GATGGAAATAGGGAGAATTGG 3'	FAM-CAGGGCCATAGCTGTAAAGC 3'	FAM	52	51	Tm=60C; dil=1/5	Ready and tested	237-257
ERCDV10-R	5' CTGACTTGCTCGAACTTGA 3'	FAM- GCGCACTGCTCTGTCTCC 3'	FAM	51.3	47	Tm=68C; dil=1/5	Ready and tested	118-150
MCM0214-R	5' AATGCTTGCAATTTATCAAAAGCC 3'	HEX-AAGCGACTCAGGAGCAGCAG 3'	HEX	54.7	54.5	Tm=60C; dil=1/20	Ready and tested	74-102
CP73-R	5' TAAACGTCCATCAACAGAGGAAGGG 3'	HEX-AAAACTGAGAAATATTCAGATGCAAC 3'	HEX	52.3	59.3	Tm=59C; dil=1/10	Ready and tested	165-175
MCM357-R	5' CCTGAGAAAACATTGAGTGTCCG 3'	FAM- ATCTCTTTGCTCACCATTAAAGCA 3'	FAM	54	56	Tm=53C; dil=1/10	Ready and tested	to optimise. Charge less
BM51617-R	5' TCTGTGTCGGAATACCCTCC 3'	HEX-GCCTGCATGTGCTGTGG 3'	HEX	50	51	Tm=65C; dil=1/10	Ready and tested	160-164
BM1815-R	5' CAAGGAGACAAGTCAAGTCCC 3'	FAM-AGAGGATGATGGCCTCTG 3'	FAM	52	52	Tm=58; dil=1/10	Ready and tested	137-157

2.1.2. Resistance to Scrapie

Scrapie is one of the transmissible spongiform encephalopathies (TSE), fatal degenerative disorders of the central nervous system that occur naturally in man and animals. Scrapie is specific to sheep. A characteristic which is common to all TSEs is the accumulation of an abnormal form of the normal prion protein (PrP^C), primarily in the brain and spinal cord. In its abnormal configuration the PrP (PrP^{Sc}) is infectious and extremely resistant to proteolytic enzymes. The normal PrP protein (PrP^C) is expressed in most tissues of the body, the higher level of expression being in the nervous tissues. Scrapie in sheep appears to be entirely an infectious disease with genetic susceptibility playing an important role.

Table 3: Polymorphisms on the PrP gene

Codon No.	Amino Acid	Sequence
136	Valin (V)	gCt
136	Alanin (A)	gTt
154	Arginin (R)	cAt
154	Histidin (H)	cGt
171*	Arginin (R)	cGg
171*	Glutamin (Q)	caG
171*	Histidin (H)	caT

* Consist of two adjacent SNPs

This susceptibility seems to be determined largely by genotypes of the PrP gene. In sheep, polymorphisms of codons 136, 154 and 171 (see table 3) are the most important parameters. This polymorphism might influence the conversion of PrP^C into PrP^{Sc}. Results from many studies have indicated that the V(136)R(154)Q(171) allele is associated to the susceptibility of sheep to scrapie while animals with the A(136)R(154)R(171) or the A(136)H(154)Q(171) alleles are resistant to developing the clinical signs of the disease. However it is recognized that the study of scrapie susceptibility is complicated by different PrP genotypes, about fifteen (see table 4), found in the different breeds and due to the possibility that several infectious scrapie strains may exist, each with a different affinity to host genotypes. Nevertheless, for the eradication of scrapie, many sheep selection programmes are being implemented to eliminate susceptible scrapie animals based on genotyping test results.

In 2006, APU started developing a cheap and easy to use technology to be transferred to counterparts for genotyping and selecting animals potentially resistant to scrapie.

Table 4: Known Prion PrP Genotypes

PrP Genotype	Risk Group/Genotype Group
ARR/ARR	R1/G1 Resistant
ARR/AHQ	R2/G2
AHQ/AHQ	R2/G3 Tolerant Group
ARR/ARQ	R3/G2 Group
ARR/ARH	R3/G2
AHQ/AHR	R3/G3
AHQ/ARQ	R3/G3 Moderate Risk
ARH/ARH	R4/G3 Risk
ARH/ARQ	R4/G3 group
ARQ/ARQ	R4/G3
ARR/VRQ	R4/G4
AHQ/VRQ	R4/G5 High Risk
ARH/VRQ	R5/G5 group
ARQ/VRQ	R5/G5
VRQ/VRQ	R5/G5 Susceptible

Table 5: Estimates of the number of reported cases of scrapie per million of sheep of each genotype in the UK

Genotype	Cases per year (n)	Percentage of sheep	Cases per year per million (n)	95 % CI (lower-upper)
ARR/ARR	0	21.3	0	0.0-0.3
ARR/AHQ	0.3	5.6	0.3	0.0-1.6
ARR/ARQ	2.0	28.0	0.4	0.2-0.8
ARR/ARH	0	2.1	0	0.0-2.9
AHQ/AHQ	2.0	1.9	5.0	2.3-10.9
ARQ/AHQ	11.0	6.3	8.7	6.2-12.1
AHQ/ARH	0	0.3	0	0.0-20.6
ARH/ARH	0.7	1.6	2.0	0.5-7.3
ARQ/ARH	1.6	1.6	5.2	2.2-12.1
ARQ/ARQ	89.6	12.2	36.9	33.1-41.0
ARR/VRQ	12.0	9.6	6.3	4.5-8.6
AHQ/VRQ	0.3	2.5	0.7	0.0-3.7
ARQ/VRQ	264.0	5.9	225.4	214.7-236.0
ARH/VRQ	22.7	0.3	405.0	321.9-508.2
VRQ/VRQ	94.8	0.9	544.5	490.5-602.9

The Amplified Refractory Mutation System (ARMS) approach was used to design allele specific primers for the detection of the alleles contained in the codons 136 (C/T), 154 (A/G), and 171 (A/G, and G/T), located in exon 3 of the PrP^C gene. Destabilizing mismatches were introduced at the first or second penultimate base in addition to the allele-specific base at the 3' termini of the primers (ARMS principle).

One ARMS primer is designed for each allele of a SNP. Each of the two primers shares a common primer on the opposite strand. To detect the assay in a Real Time manner a TaqMan probe is designed between these two primers. The allele specificity of each set of primers was first tested separately using DNA samples of known Scrapie genotype. This method required only one probe per assay, while in the traditional TaqMan approach for detection of each allele site one probe has to be synthesised (two probes per assay). All possible genotypes could be distinguished when we validated the method with samples from known genotype. All primers were synthesised with the same annealing temperature in order to run at the same time in a single PCR instrument. Multiplexing of the primers is also possible by utilizing different dyes attached to the probe (FAM and HEX dyes, for instance). This ARMS-TaqMan method for PrP^C genotyping for sheep is rapid, and could differentiate all genotypes. Reproducibility of this technique is achieved through the use of the quantitative method. It is quick because no post-PCR steps are required. Also costs are lower than the normal TaqMan assay because only one probe is needed to resolve the two alleles. The method established in this study is universally suited for a broad range of genotyping projects with different requirements. It provides an efficient and inexpensive diagnostic mutation analysis that will improve the quality of PrP^C genotyping.

In addition to the above SNP technology to screen sheep for resistance to scrapie, which requires Realtime PCR equipment, the Unit has developed another scrapie genotyping technique based on the classical PCR and digestion of the amplified product by a DNA restriction enzyme: the Restriction Fragment Length Polymorphism (RFLP) technique. Primers were designed to introduce a mutation on the gene that allows recognition of the marker of interest via a simple restriction enzyme reaction. This method allows the recognition of two of the three important SNP sites, positions 136 and 154,

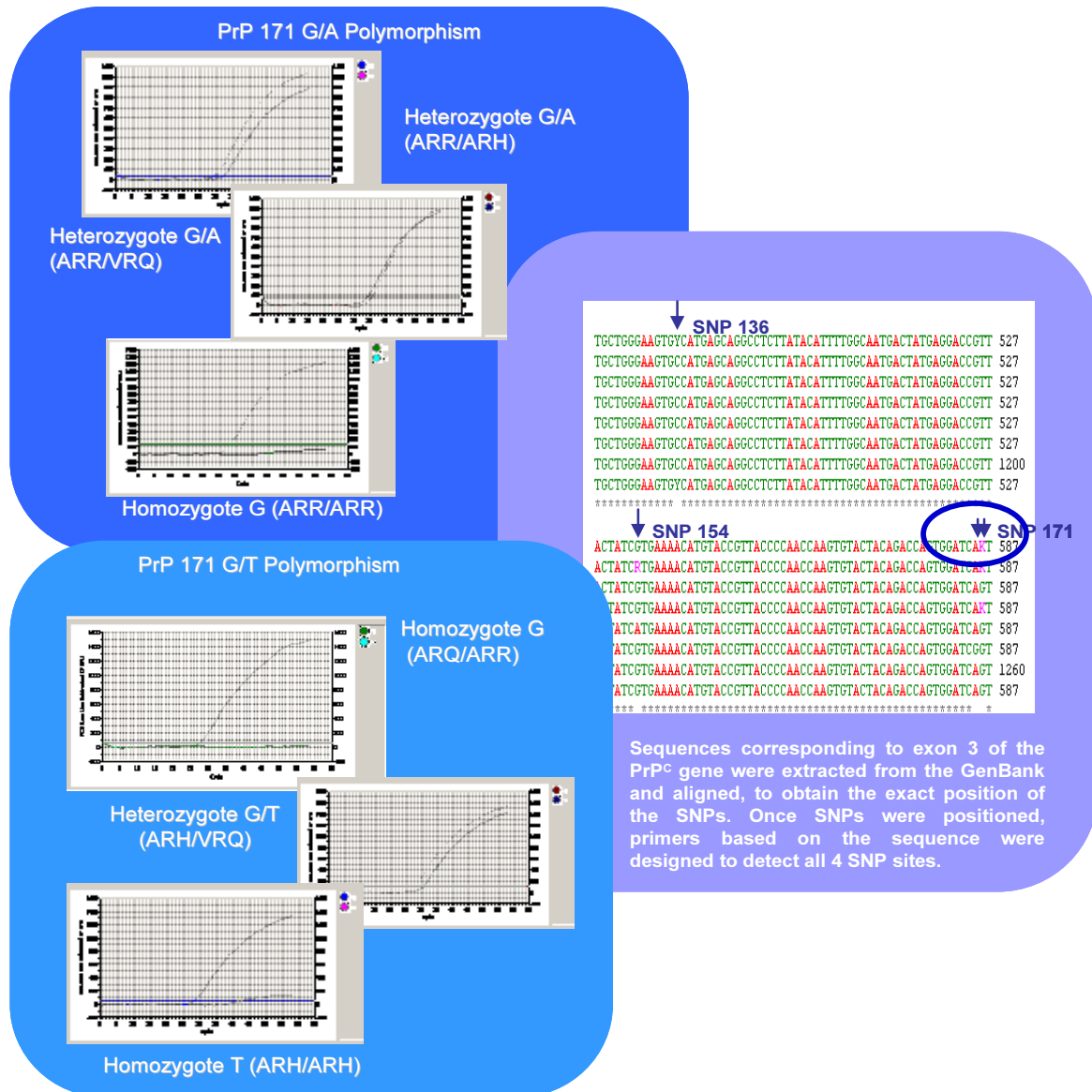


Figure 7: Showing the results obtained with the primers designed to detect the polymorphisms on the 171 site (circled in blue) of the PrPC gene.

by a single amplification and digestion with a restriction enzyme. The study aims at finding an approximate indication of the PrP allele frequency of individual breeds and eventually identifying scrapie susceptible or resistant animals. The gene fragment of interest is amplified by the following pair of primers:

PrP-RFLP-F: 5'GTCAAGGTGGTAGCCACAGTCAGTGG3'

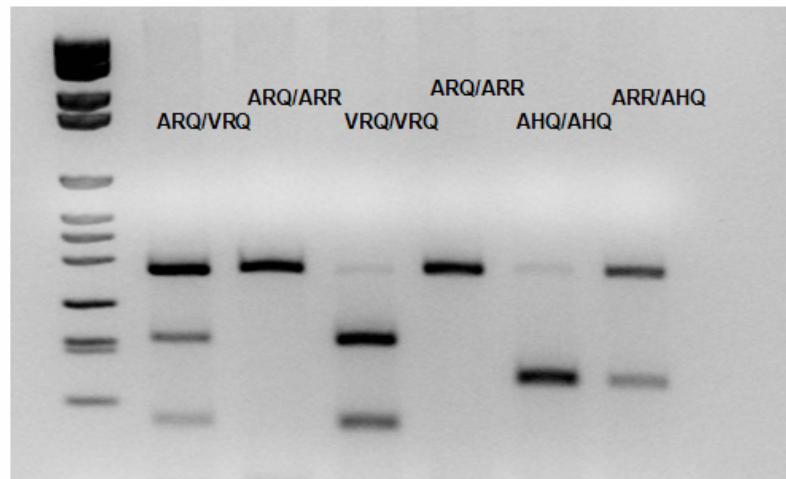
PrP-RFLP-R: 5'GCTCCACCACTCGCTCCATTATCTTG3'

After the PCR reaction, the amplified DNA of 380 bp long is submitted to digestion with the *BspHI* DNA restriction enzyme. As shown in figure 8, analysis of the digested products by electrophoresis on agarose gel allows differentiating the genotypes which differ from each other by their amino acid residues at positions 136 and 154 of the prion protein sequence.

Digestion with BspHI:
Positions 136, 154

Pos. 136: Enzyme **cuts**
when valine (V) is
present,
no cut when Alanine (A)

Pos. 154: Enzyme **cuts**
When Histidine (H) is
present,
No cut when R
(Arginine) is present



136 154



Expected: one higher band for 136 non-cut A/A allele plus 154 (H allele), therefore the band here looks stronger (combination of two alleles)
Lower band is corresponding to 154 allele H.

136 154



No cut at pos. 154
Cut at pos. 136 (1 of the alleles (V))
Therefore two bands are expected

136 154



One higher band for non-cut 136 (A/A) allele.
Expected one low band for 154 both alleles are cut (H/H)

136 154



No cut, expected just 1 band

136 154



No cut, expected just 1 band

136 154



Expected one low band for 136 both alleles are cut (V/V). One higher band for non-cut 154 allele.

Figure 8: PCR RFLP technique for detection of alleles at positions 136 and 154 of the PrP gene.

2.1.3. Increasing prolificacy in small ruminants: The FecB gene

The autosomal Booroola fecundity gene (FecB) mutation in sheep increases ovulation rate and litter size. Booroola genotypes carry a point mutation in the kinase domain of *the receptor*, at nucleotide n° 746 of the coding region (A to G transition) which results in a change from a glutamine in the wild type to an arginine residue in the Booroola animals (see table 6).

Table 6: Booroola mutation is a A→G transition that increases the ovulation rate in sheep carriers. The mutation is located at position 746 in the FecB gene. Genotypes: FecB⁺ when A is present and FecB^B when G is present.

	<u>FecB⁺</u>	<u>FecB^B</u>
Pos. 746	CAG	CGG
Pos. 249	Glutamina (Q)	Arginina (R)

This mutation is a marker which offers a possibility for selecting sheep with high levels of prolificacy by genetic introgression of the desire trait. By using a simple detection system such as the TaqMan technology, it is possible to select certain breeds containing the Boorola mutation. Specific primers were designed to enable the detection of the Boorola single mutation by TaqMan technology. The test is performing well.

Table 7: Genotype ovulation rate according to the genotype observed in carriers, when both alleles for G (FecBB/ FecBB), sheep offspring are or the order of more than 5.

FecB ^B / FecB ^B	≥ 5
FecB ^B / FecB ⁺	3 - 4
FecB ⁺ / FecB ⁺	1 - 2

2.2. Animal Health

2.2.1. Development of c-ELISA for the diagnosis of Foot and Mouth Disease: Use of Non-Structural-Protein (NSP) of Foot and Mouth Disease Virus for Differentiation between Vaccinated and Infected Animals (DIVA)

Currently, foot and mouth disease (FMD) is one of the most important constraints to international trade of animals and animal products. The causal agent is a virus which is highly infectious and can spread easily between wild and domestic ruminants and pigs as well. In developed countries, this disease is controlled through the implementation of the stamping out policy, a measure by which all susceptible animals in the outbreak area, infected or not, are culled because of the current inability to differentiate infected animals from vaccinated ones by traditional serological diagnostic techniques. This policy is not only expensive but is less and less accepted by the public. Currently efforts are being undertaken to develop tests that are able to distinguish truly infected from vaccinated animals (*DIVA: Differentiation between Infected and Vaccinated Animals*). This is possible by detecting antibodies produced by infected animals against the FMD virus Non-Structural-Proteins (NSP) which are synthesized in virus infected cells but are not part of the virion particles. Therefore with the current FMD vaccine, which is a purified and killed virus, vaccinated animals will not produce antibodies against NSP while infected animals in which the virus multiplication has taken place will develop anti-NSP antibodies. The use of these proteins as antigen in serological tests is the basis for the development of FMD DIVA tests. In 2005, an indirect ELISA format was developed by the APU. This test needs the use of a conjugate anti-bovine or anti-pig immunoglobulin if sera from cattle or pig, respectively, have to be analysed. With a competitive format, a single conjugate will be used for any serum. Because of this advantage, it was decided to develop a FMD NSP-based c-ELISA. As for the indirect ELISA, the FMD recombinant NSP antigen produced *in vitro* in bacterial lysate extract was used as antigen for the development of the c-ELISA.

2.2.1.1. Monoclonal Antibodies (mAbs)

Monoclonal antibodies against FMDV 3ABC NSP were produced in 2004 and 2005. In 2006, they were sub-cloned and characterized in peptide ELISA. For that characterisation, 68 synthetic overlapping peptides covering the 3ABC NSP were used. Among these monoclonal antibodies, three were binding to the 3B1, 3B3, 3B5 regions of the NSP. The sequence they recognize is GPYAGP, a sequence identified by other scientists as a B-epitope (see figure 9 and 10 as an example of peptide mapping the binding site of the mAb 1E6-11). Those three mAbs were considered as potential reagents for the development of a c-ELISA.

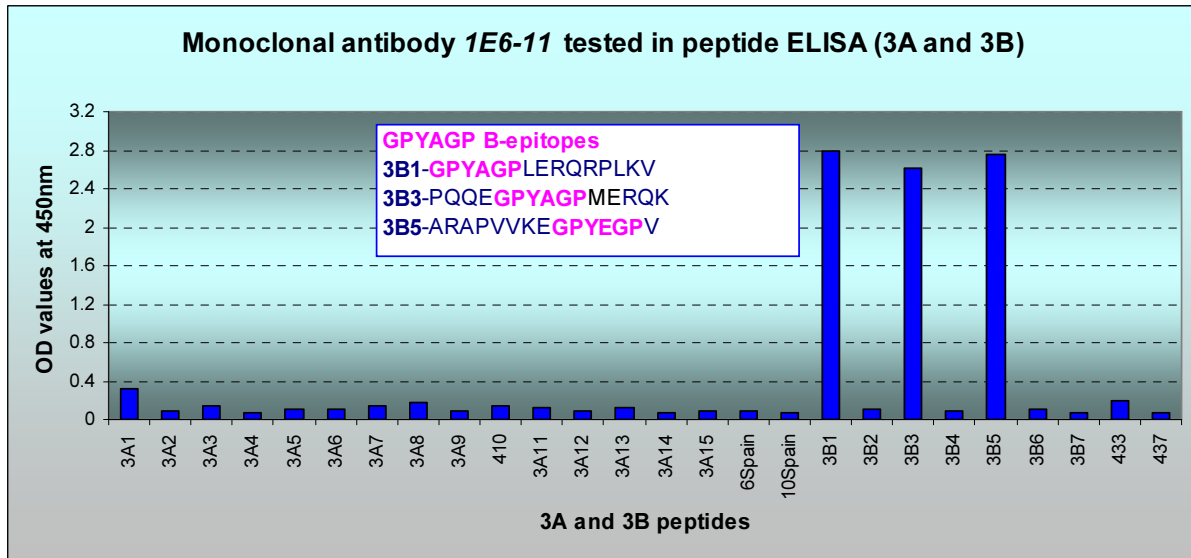


Figure 9: Peptide mapping of the binding site of mAb 1E6-11 on peptides covering the 3AB region of the NSP 3ABC (26 overlapping peptides covering the entire 3AB region). The sequence GPYAGP is common to all 3 peptides recognized by the mAb.

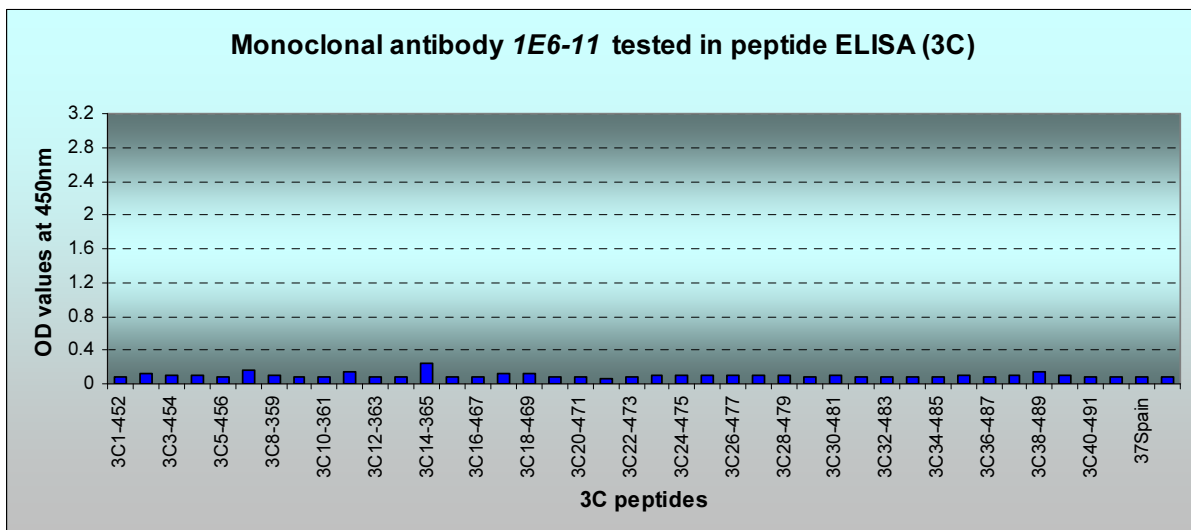


Figure 10: Peptide mapping of the binding site of mAb 1E6-11 on peptides covering the 3C region of the NSP 3ABC (42 overlapping peptides covering the entire 3C region).

2.2.1.2. Studies to optimize assay conditions in c-ELISA

Initially the **Percent Inhibition (PI)** of the negative control serum was relatively high. To improve assay conditions and reduce unspecific signal, extended background studies were performed by testing different brands of microtitre plates, comparing conjugates from different suppliers and using different concentrations of blocking substances in the dilution buffers.

Nine brands of microtitre plates were tested and the Immulon 1B plates gave the best results. Similarly, 5 conjugates of anti-mouse immunoglobulin (Ig) were tested. The results have shown that the HRP A-0412 from Sigma performed best (see results in figures 11 and 12).

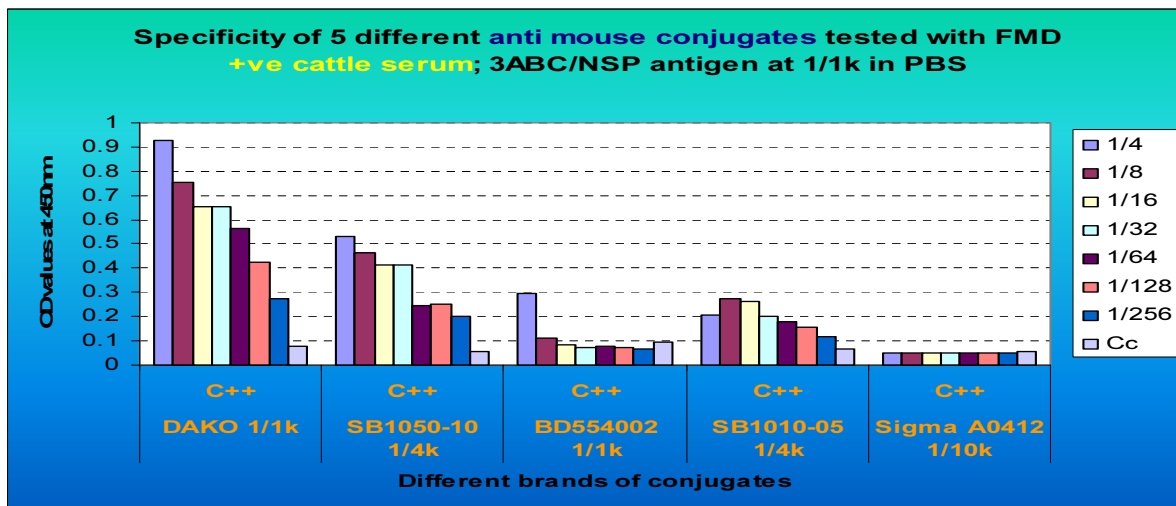


Figure 11: Comparison of different anti mouse-conjugates tested on a positive cattle serum.

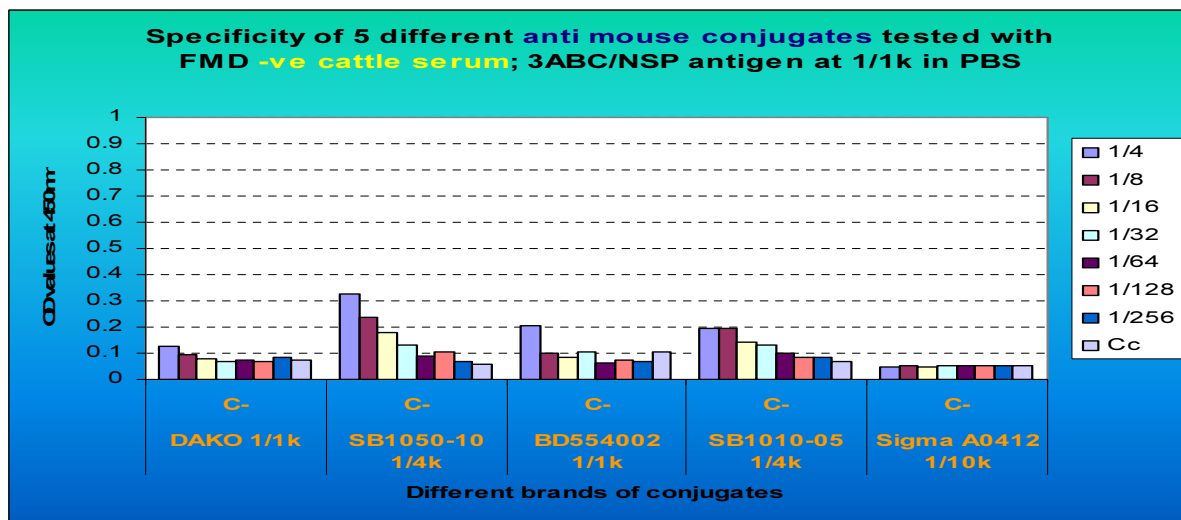


Figure 12: Comparison of different anti mouse conjugates tested on a negative cattle serum.

Following all preliminary studies the subsequent assay parameters were fixed: Coating of the antigen (NSP 3ABC) at 1/8000 in carbonate-bicarbonate buffer onto Immulon 1B microtitre plates, final serum dilution 1/5 in PBS-T-F (10%), mAb 1E6-11 as competitor at 1/2000 final dilution in PBS-T-F (10%), HRP A-0412 at 1/10000 in PBS-T-F (10%), substrate TMB/H₂O₂. The working volume at all working steps is 100µl. All incubations of test plate are carried out at 37°C with agitation for one hour but 15 min after addition of the substrate into wells. The plate is washed 3 times

between each incubation step. The optical density is determined at 450nm on an automated ELISA plate reader.

Results are expressed as percent inhibition (PI) and calculated on the mean optical density of a duplicate sample. The results were calculated by the following formula: $100 - (\text{sample OD}/\text{mAb OD}) * 100$.

2.2.1.3. Specificity studies of the c-ELISA

To check the specificity of the test a panel of negative sera from cattle, sheep, goats and pigs were tested. Sera from animals with different diseases other than FMD, like PPR, trypanosomosis, babesiosis, helminthosis, were also included in the study. Some of the results that were achieved are illustrated in figures 13 to 16.

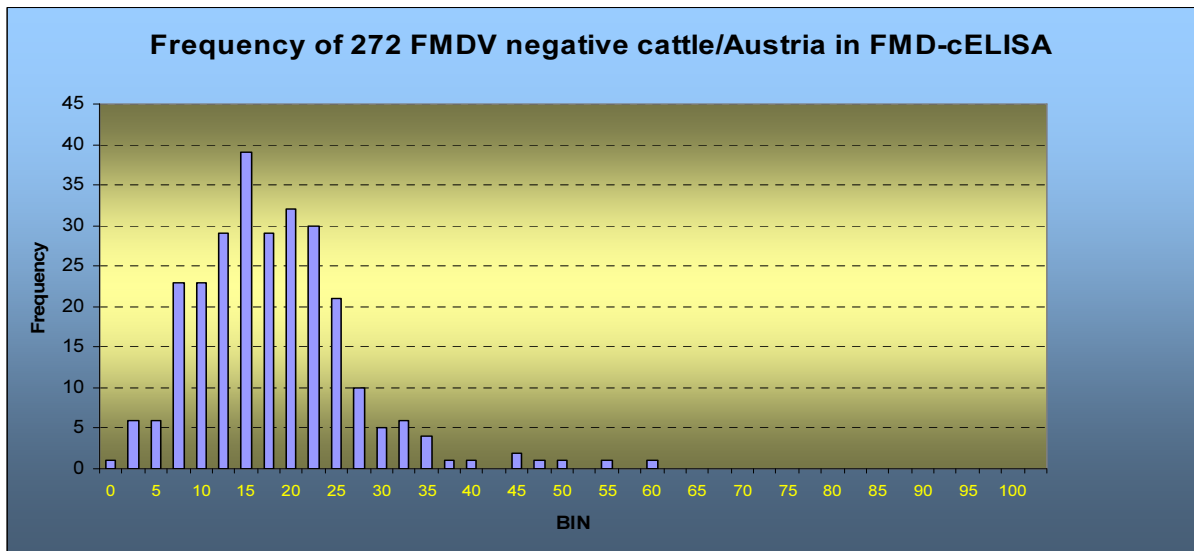


Figure 13: Frequency distribution of FMD negative cattle.

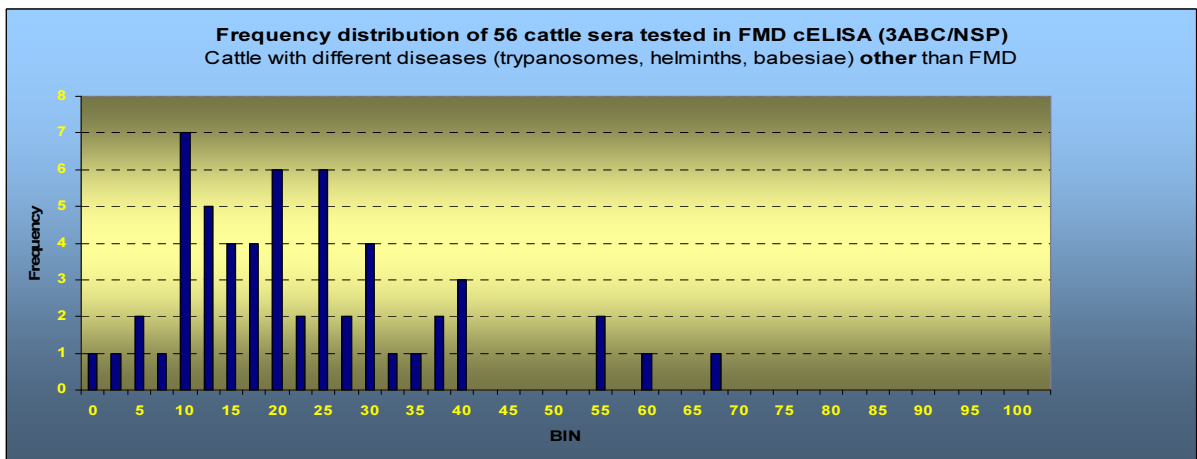


Figure 14: Frequency distribution of FMD negative cattle, but with other animal diseases.

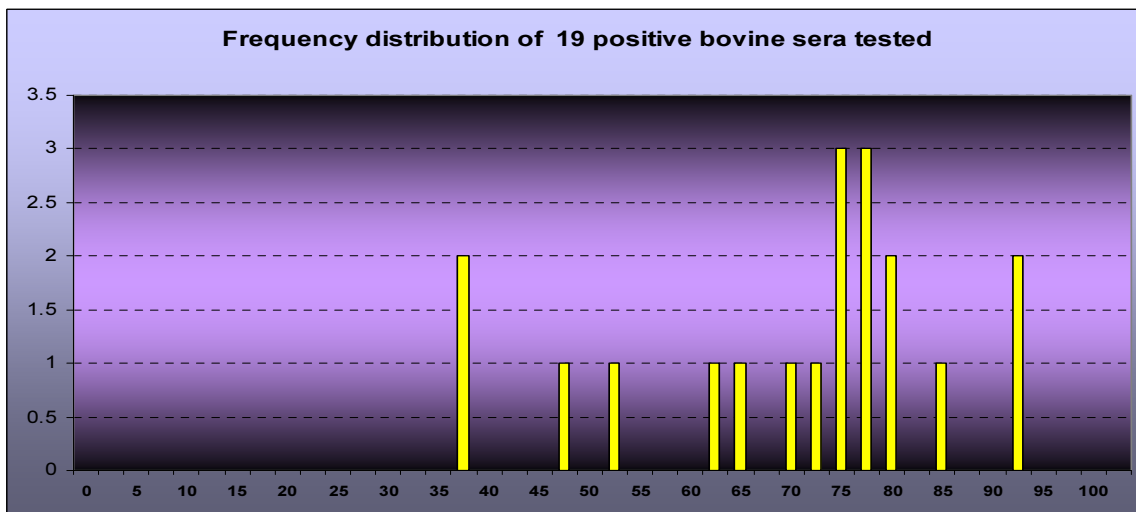


Figure 15: Frequency distribution of FMD positive cattle.

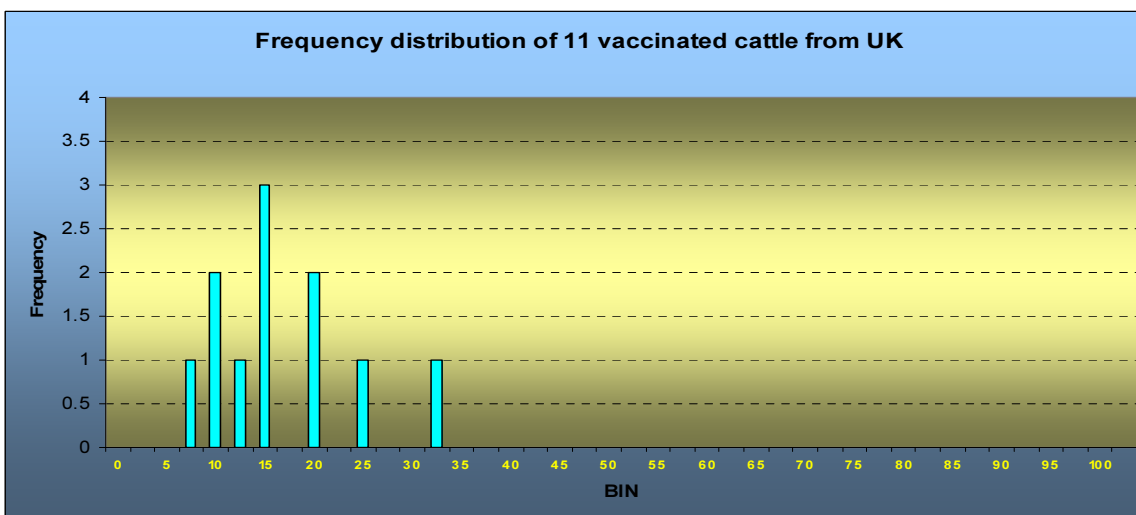


Figure 16: Frequency distribution of FMD vaccinated cattle.

The sensitivity was evaluated by analysing positive sera obtained from 19 cattle. The results are presented in table 8: they indicate that the test is highly specific, 93 to 100%, with sensitivity at around 84%, results which are similar to the performance of the indirect ELISA developed a year ago.

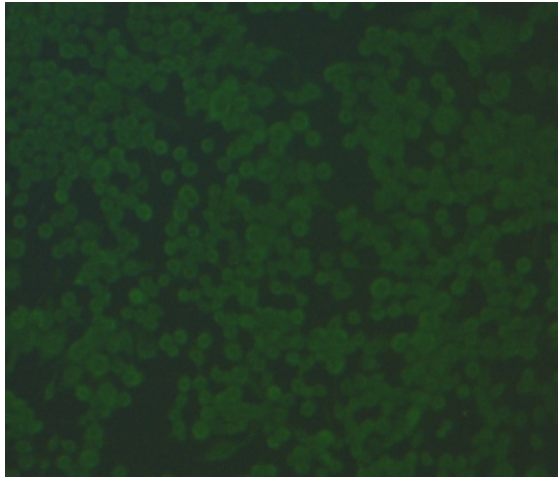
Table 8: The specificity/sensitivity calculated at cut off point 50.

Species/no tested	Health status	Specificity %	Sensitivity %
Cattle/272	FMD negative	98.9	
Cattle/56	Diseases other than FMD	92.9	
Cattle/11	FMD vaccination	100	
Cattle/19	FMD positive		84.3
Buffalo/35	FMD vaccination	97.2	
Sheep/80	FMD negative	100	
Goat/80	FMD negative	100	
Goat/34	Diseases other than MD	94.12	
Pig/80	FMD negative	98.75	
Pigs/95	FMD vaccination	100	

In 2007, activities will continue with the objectives to improve the quality of the test and to validate it by testing a high number of sera from different animal species. First of all, a new antigen will be used, more stable than the current one. Indeed, the FMDV NSP 3ABC has a protease activity in its C fragments, activity responsible for the auto-processing of the FMDV polyprotein synthesized from the viral genomic RNA. It induces also the auto cleavage of the 3ABC into 3A, 3B1, 3B2, 3B3 and C fragment. This auto cleavage may affect the sensitivity of the test. In order to obtain a stable 3ABC protein, a DNA corresponding to its gene has been synthesized chemically with some point mutations to inactivate the protease active site and the cleavage sites. Codons of the gene have been optimized for the protein production in insect cells. The gene was cloned in the baculovirus vector. The recombinant virus that was obtained expressed well the 3ABC protein in insect cells as shown in figure 17. With this new antigen, further evaluation of the test with a high number of sera at Seibersdorf and also in other institutes will be carried out.

Negative control:

Non-infected insect cells



Positive result:

Infected insect cells

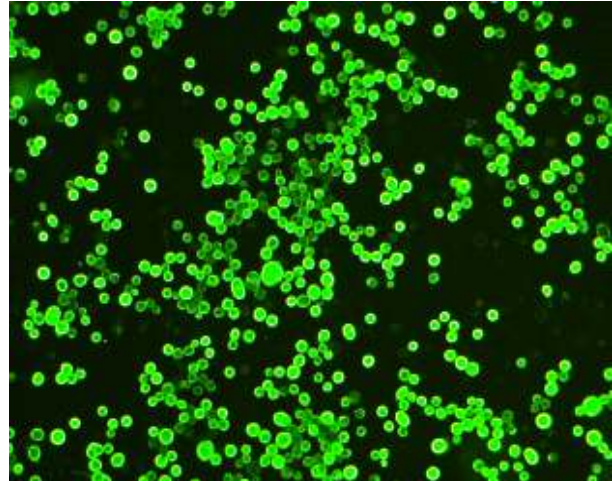


Figure 17: Insect cells infected with a recombinant baculovirus expressing a protease negative FMDV 3ABC protein. The protein is detected by immunofluorescence (IFT) with a monoclonal anti FMDV 3ABC antibody.

2.2.2. Validation of ELISA kits for Rift Valley Fever diagnosis (CRP D3.20.23)

Rift valley fever (RVF) is an acute disease caused by a virus, which is transmitted by blood-feeding arthropods, including more than 30 species of mosquitoes. The transmission of the virus can occur also by contact with infected patients, animals, blood or other pathological samples. This disease affects many wild and domestic animals and humans. It is an important zoonose found in Sub-Saharan Africa. From east Africa, it has now spread into some countries in the Middle East. For a long time, the diagnosis of RVF was done by heamagglutination-inhibition, complement fixation, immunofluorescence and virus neutralization tests. Each of these tests have drawbacks. To improve the diagnosis of this disease in the laboratory, ELISA kits have been developed in the recent years. One of the objectives of the CRP initiated by the APH Subprogramme is to validate those new kits. APU is involved in that validation. The APU received 2 mini kits from the two suppliers:

- 1) Anti-RVF IgG iELISA from the Onderstepoort Institute (OVI) in South Africa (SA);

Three run studies were carried out with this kit. In only one out of the 3 runs all three criteria of acceptance could be met. Twelve out of 20 samples gave identical results in all the 3 runs. 7 out

of the 20 samples gave “suspect” results, because the data fall within the “grey-zone”. The background in the antigen-negative part of the plate was very good and the binding ratio (B/B0) remained constant in all 3 runs. Binding ratio for the acceptance or rejection of the plate might be taken into consideration. The CV value criteria should be reconsidered.

2) RVF Multi purpose mini ELISA kit from the *National Institute for Contagious Diseases* (NICD) from SA. It contains 3 different ELISA formats:

- The RVF IgG-sandwich ELISA for the detection of anti-RVFPV IgG antibody in sheep and goat sera,
- a RVF IgM-capture ELISA for the detection of anti-RVFPV IgM antibody in sheep, goat and cattle sera
- a RVF inhibition ELISA for the detection of antibody to RVFPV in humans, domestic and wild ruminants. Some of the reagents are used in all 3 test systems.

The proficiency testing was started with the IgM-capture ELISA. The ABTS substrate for all 3 ELISA formats turned out to be destroyed upon arrival and was replaced at a later stage.

2.2.3. Reverse ELISA

The reverse ELISA is a system by which a serum in a plate can be screened for about 5 diseases at the same time. The system seems easily applicable in the field and the test result is readable visually. Therefore it could be useful in remote areas as a first line test in disease diagnosis. It has been developed by the Università degli Studi del Molise, Italy. In collaboration with that institute, APU made in 2006 a first trial of the Reverse ELISA. In this initial study two different antigens were used: a purified Foot and Mouth Disease Non-Structural Protein (NSP) produced in E.coli (RTSystem) and a non-purified PPV N protein produced in cells by a recombinant baculovirus-PPRV N.

With the purified NSP/FMD antigen, the results achieved were promising. It gave similar results as with the classical FMD iELISA by testing the same sera. However, with the non-purified PPRV N antigen there was an unacceptable background. Therefore any further work on reverse ELISA will be based on purified proteins only.

Cattle blood only /1/50 2-fold
 Cattle blood spiked with C-
 Cattle blood spiked with C++
 Cattle blood spiked with C+

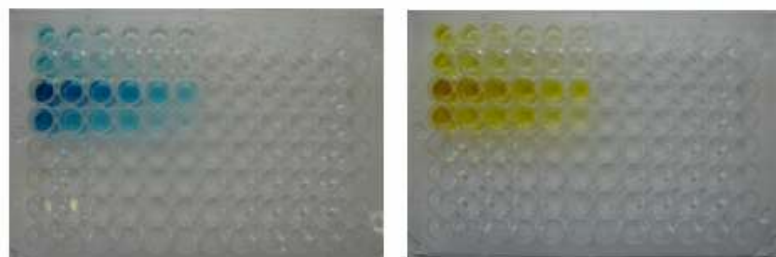


Figure 18: Reverse ELISA with whole cattle blood spiked with FMD C-, C++ and C+ sera. The test plate is shown before (left photo) and after adding the stopping solution (right photo).

2.2.4. Development of peste des petits ruminants (PPR) marker vaccine and companion diagnostic tests to facilitate control of the disease

The objective of this project is to develop tools which would enable the differentiation between infected and vaccinated animals, thereby facilitating the epidemiosurveillance and control of the disease. It involves the collaboration between many partners in Africa and Europe. The Joint FAO/IAEA programme, on behalf of the FAO, is one of the partners. It is responsible for:

- ▶ mapping the nucleoprotein of the peste des petits ruminants virus (PPRV) to identify B-epitopes which can be useful in test development and also to identify potential site of deletion for the development of a negative marker of the vaccine,
- ▶ developing PPR specific diagnostic test,
- ▶ developing a marker vaccine companion test.

Activities on this project have recently been initiated and will be reported in 2007 activities report.

2.2.4.1. Mapping the protein-protein interaction sites on PPRV nucleoprotein

The nucleoprotein (Np) of PPRV, as for all morbilliviruses, is the most immunogenic and major viral protein. Therefore it is the most interesting viral protein to be used as antigen in a diagnostic test. For all negative sense genome viruses, Np plays a central role in the biology: it protects the genomic RNA against nucleases and it is essential for RNA polymerase complex formed by the viral large protein (L) associated to the phosphoprotein (P), to function. Np molecules 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) interactions which are essential for the RNA polymerase complex activity,
- Np-matrix protein (M) interactions which are essential in the formation of the viral particles.

A) Study of the Np-Np interactions for the formation of the nucleocapsid.

During the first year of the project, seven deleted mutants and a double point mutant of the PPRV nucleocapsid gene were constructed into the baculovirus vector and expressed in insect cells. These mutants were used in co-expression study, mutant and wild-type proteins, and monoclonal antibodies reactions to identify the sequences involved in the Np self-association to form the nucleocapsid. Following the results that were obtained, it was concluded that Np-Np self-association sites are localised in two regions of the protein: the fragments corresponding to amino acids number 1-120 and 145-242 of the Np sequence. As for 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 the position 228-229 in the protein sequence. It was decided to confirm those results by electron microscopy study of purified N proteins, both mutants and wild type. This study was carried out in 2006. The different deleted N protein mutants were purified by ultracentrifugation on CsCl gradient and examined by electron microscopy (EM). This EM observation indicates that the full length N and the mutant Np Δ 420-525 proteins present herringbone-like structures which resemble the authentic viral nucleocapsid of Paramyxoviruses. The N-terminus and central deletions mutants (Np Δ 1-145, Np Δ 120-145, Np Δ 120-241 and Np Δ 120-278) form aggregate crystalline structures and some mutants

show inconstant helical structures (see figure 19). These helical structures observed with Np Δ 120-241 and Np Δ 120-278 mutants seem to be different from those of full length N. These data indicate that the N-terminus domain of N protein, amino-acid (aa) 1-241, is essential and critical for stable helical nucleocapsid formation, while the c-terminal fragment aa 421-525 is dispensable for this structure.

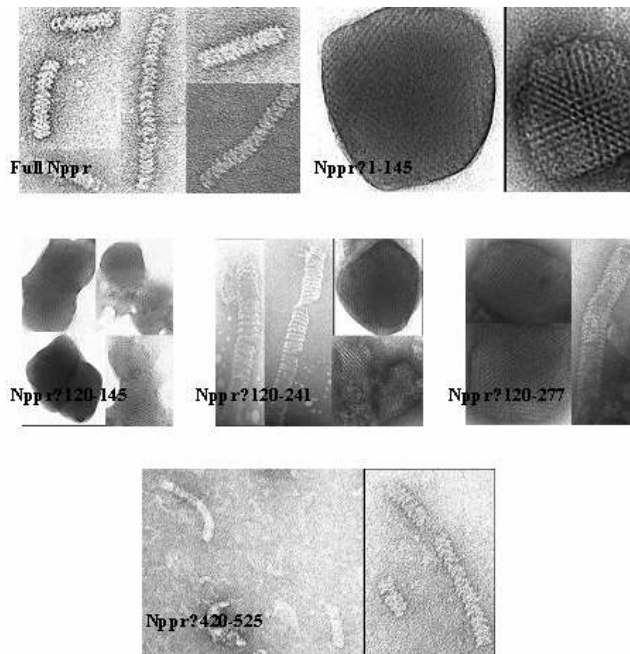


Figure 19: Full length and deleted mutant PPRV N proteins expressed in insect cells and purified. The purified proteins were observed in electron microscopy (EM) after having been coated on grids. The grid was examined in a Jeol 100cX11 electron microscope at 80 kV at a magnification of 72.000.

B) Mapping domains of PPRV nucleoprotein interacting with the matrix protein.

As for all negative single-stranded RNA viruses, M protein of PPRV is coating the internal face of the viral envelope. By this position, M plays a central role during the virus assembly by acting as a bridge between the ribonucleoprotein complex, N-RNA-P-L, via the nucleoprotein (N) and the two viral glycoproteins, the haemagglutinin (H) and the fusion protein (F). For this project, the N and M interactions were studied in order to map the N zones interacting with M and that should be conserved in the live PPRV marker vaccine for the viability of the virus. In 2005, the PPRV M protein was expressed in insect cells as recombinant protein by a recombinant baculovirus which has this protein gene. This recombinant virus was used in 2006 in N-M interactions study to map on N the binding site of M. This study was done by ELISA, co-immunoprecipitation and confocal microscopy observation.

B-1) Detection interaction N-M by ELISA.

ELISA plates were coated by the recombinant full length or the deleted mutant PPRV N protein produced in insect cells. Then the recombinant PPRV M protein is added into the well of the plate which is then incubated at +37° C for one hour to allow N-M reaction. The attached M protein is detected by a monoclonal anti PPRV M protein antibody. By that technique, there was no important difference between the full length N and the deleted mutant in their capacity to retain M onto the plate. This result indicates that N has probably several domains which interact with the M protein. These domains are localised in both N-terminal and C-terminal regions.

To better define the N residues which bind to the M, plates were coated with peptides instead of full N or deleted mutants. For that strategy, 62 N overlapping peptides were used as M capture ligand. The result of that study is presented in fig. 20. It shows that five N peptides interact with M. These peptides correspond to four discontinuous regions on N which bind M protein. According to the optical density value obtained for the peptides which reacted with M protein, the peptides 24 (RRFMVSLILDIKRTP) and 36 (GGLNFGRSYFDPAYF) seem to be the main binding site for M. The alignment of N amino acid sequence of *Morbillivirus* shows that the peptides interacting with M are well conserved. Three of their peptides are localised in the central region and one in the terminal of N.

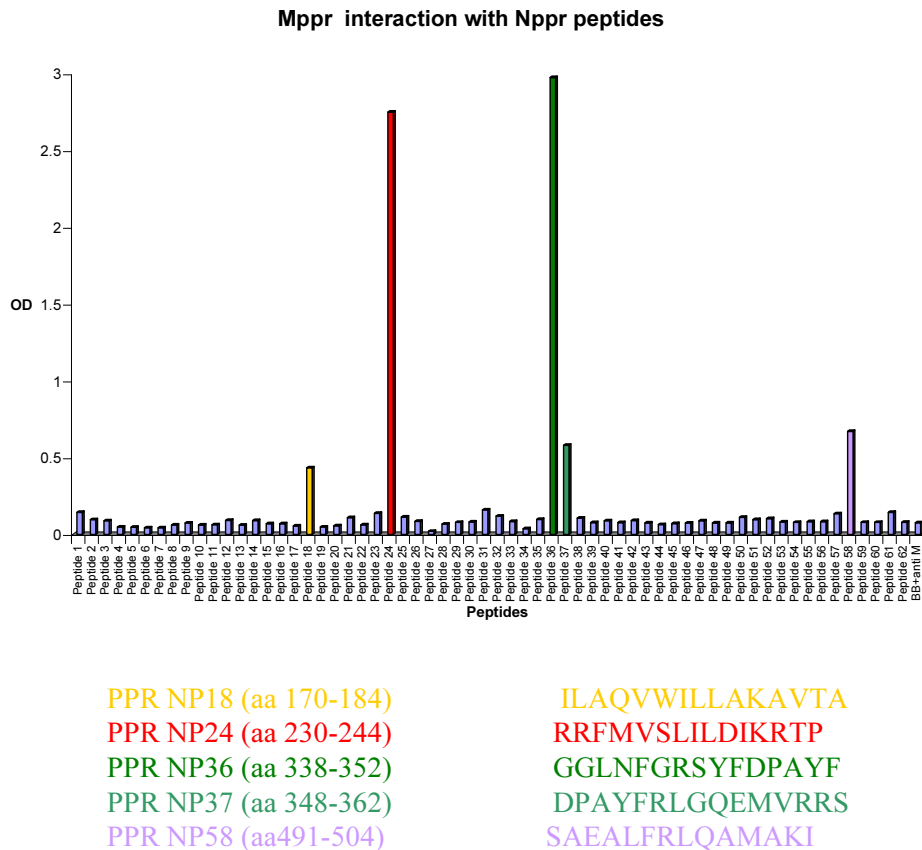


Figure 20: N peptides were used for coating in microplates and the binding of M protein was detected by mAb 19-6 which is directed against PPRV M. Peptides 18, 24, 36, 37 and 58 interact with M.

B-2) Detection N-M interaction by immunoprecipitation.

Insect cells were co-infected with the recombinant baculoviruses expressing the PPRV M and one of the PPRV N, full length or deleted mutant. The interaction between M and N molecules was detected by immunoprecipitating the complex with the monoclonal antibody (mAb) 19.6 which binds only to the M. The precipitates were separated by electrophoresis on polyacrylamide gel and transferred onto filter membrane (Western blot analysis). The proteins on the membrane were detected by chemiluminescence after probing the membrane with rabbit hyperimmune anti-rinderpest (RHS), a serum cross-reacting with PPRV N and M. In figure 21 is presented the western blot photo of total M-N co-infected cell lysates and probed with the RHS showing that both M and N, full length N and its deleted mutants are well expressed in the co-infected cells. Figure 22 represents the western blot photo of the proteins that were pulled down with the mAb anti-PPRV M. It shows that the full length and the different deleted mutant N proteins analysed in this study co-precipitate with M although the

amount of Np Δ 120-277 mutant is very low, probably because of the weak link with M, an important binding site of M on N being deleted. These data confirm that M protein has several interaction sites on N protein.



Figure 21: Total lysates of cells co-infected with recombinant baculovirus PPRV N and M virus were analysed by Western blot. The cell lysates were separated by SDS-PAGE and blotted onto a membrane and the M and N proteins were detected by the rabbit rinderpest hyperimmune serum (gift from J. Anderson, IAH, UK). Lines 1: SF21 cells, 2: Mppr, 3: Mppr+Nppr, 4: Mppr+Np Δ 1-145, 5: Mppr+Np Δ 120-145, 6: Mppr+Np Δ 120-241, 7: Mppr+Np Δ 120-277, 8: Mppr+Np Δ 420-490, 9: Mppr+Np Δ 420-525.

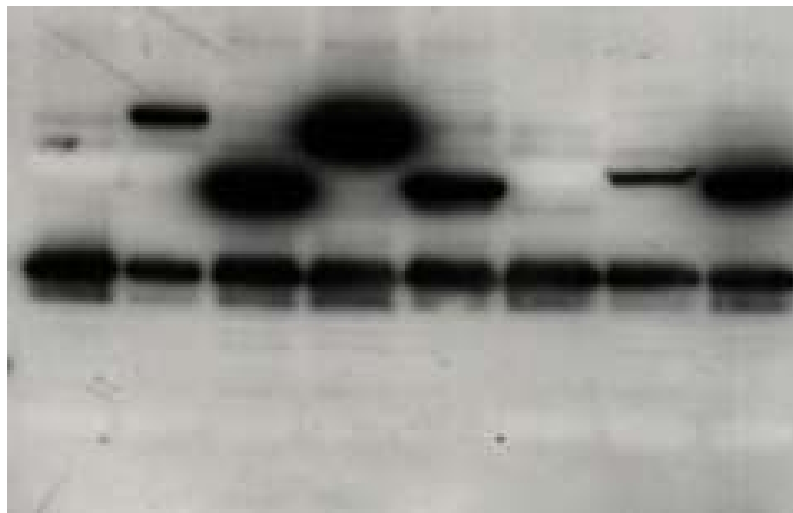


Figure 22: Analysis of interaction between the PPRV M protein and full N protein and various N deleted mutants. The lysates of cells co-infected with recombinant baculovirus PPRV N and M virus were immunoprecipitated with the mAb anti-M. The precipitates were electrophoresed and blotted onto a membrane. M and N proteins were detected by the rabbit rinderpest hyperimmune serum (gift from J. Anderson, IAH, UK). Lines: 1: Mppr, 2: Mppr+Nppr, 3: Mppr+Np Δ 1-145; 4: Mppr+Np Δ 120-145; 5: Mppr+Np Δ 120-241; 6: Mppr+Np Δ 120-277; 7: Mppr+Np Δ 420-490; 8: Mppr+Np Δ 420-525.

B-3) N-M proteins interaction study by confocal microscopy observation of cells co-infected with baculovirus expressing M and different N proteins.

For this study, two anti PPRV N monoclonal antibodies, mAb 38-4 and mAb P4G5, were labelled with the rhodamine dye while the anti PPRV M mAb, mAb 19-6, was labelled with fluorescein. They were used in the confocal microscopy observation of cells infected either with recombinant baculovirus-PPRV M, or with recombinant baculovirus-PPRV N (wild type or deleted mutant), or co-infected with recombinant virus expressing PPRV M or N proteins. The confocal microscopy observation showed that PPRV M protein is always associated with the cell membrane (fluorescein associated with cell membrane). The PPRV N protein is detected only in the cytoplasm of cells infected with the baculovirus-PPRV N alone but not on membrane of cells co-infected with recombinant viruses expressing the two PPRV proteins (see figure 23). This observation was made with all recombinant baculoviruses expressing the full length or the mutated N proteins used in this study. This result indicates that PPRV N-M interactions may involve more than one site on the N protein.

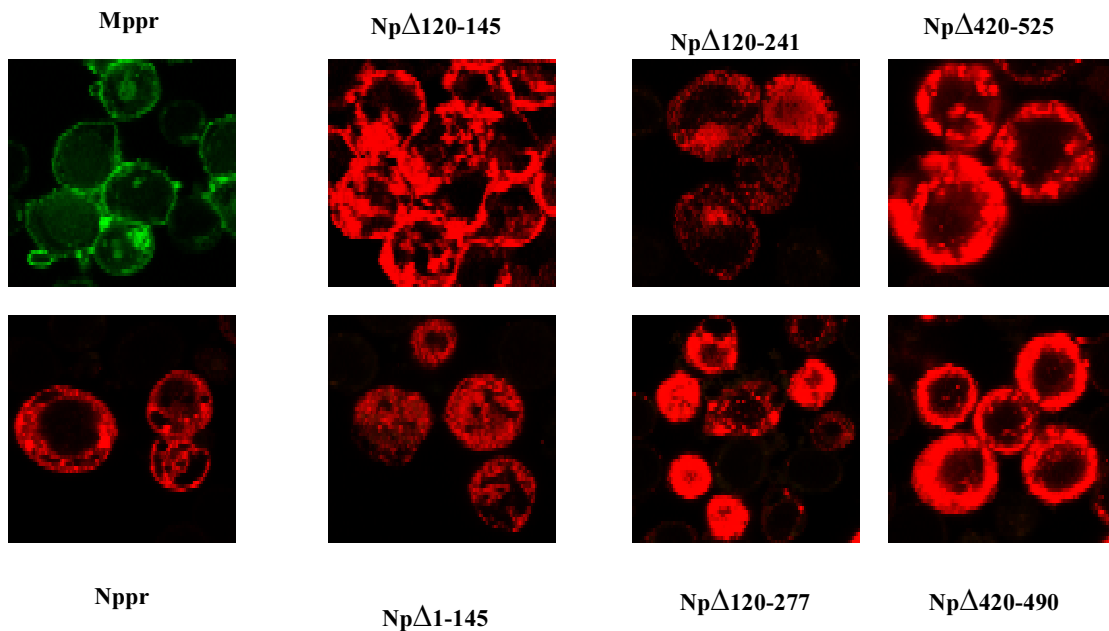


Figure 23: Individual expressed of M, N and his mutants. 72 hours post infection, was determined by immunofluorescence with specific mAbs: anti-M 19-6 labelled fluorescein and anti-N (P4G5 and 38-4) labelled rhodamine. mAb P4G5 was used for mutants NpΔ1-145, NpΔ120-145, NpΔ120-241, NpΔ120-277 and 38-4 NpΔ420-490 and NpΔ420-525. After labelling the cells were observed by confocal microscopy. The vesicle due to M contain nucleocapsid-like structures.

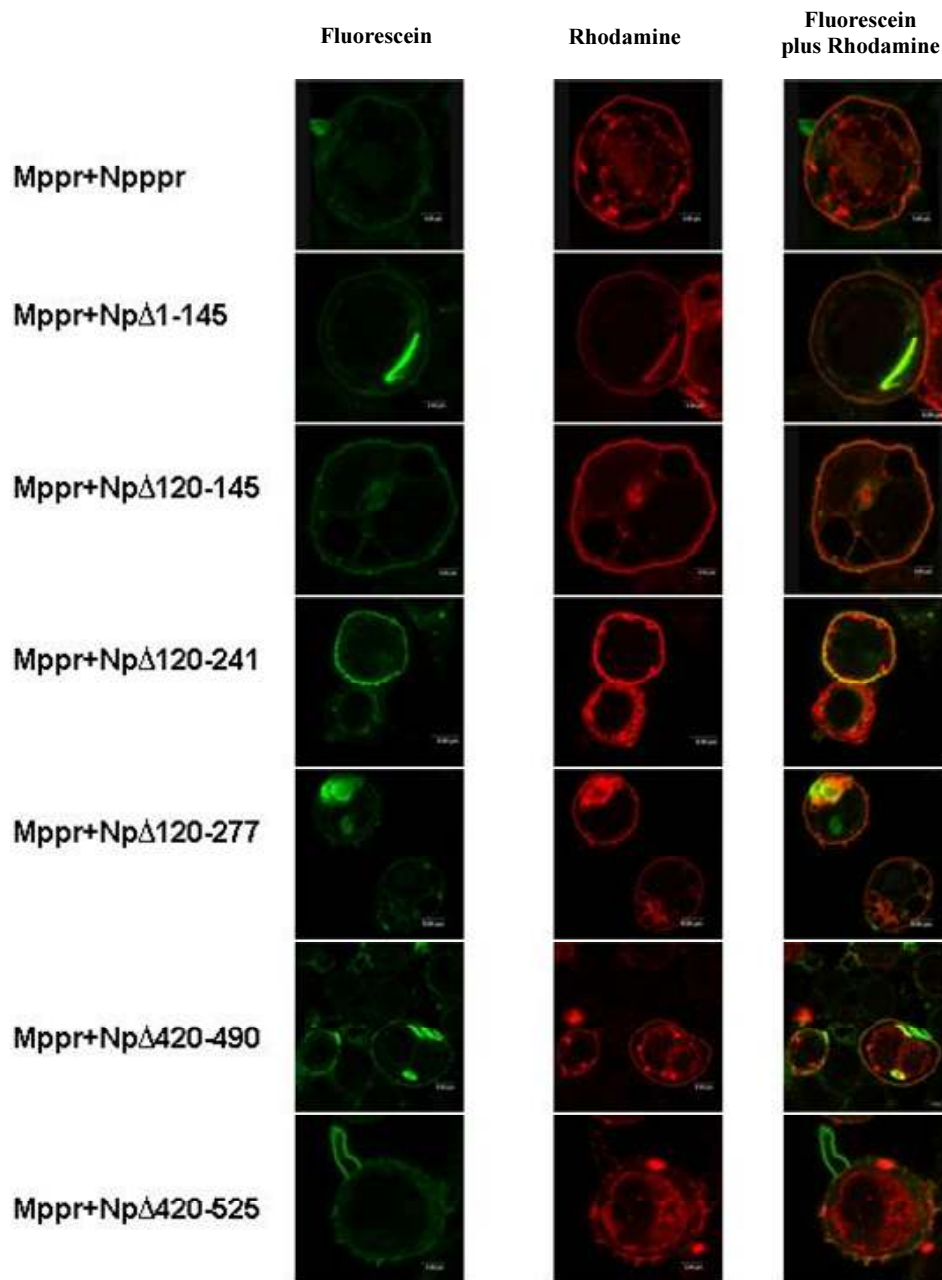


Figure 24: Co-expression of M and N or deleted mutants. Localisation of the proteins was done as described above. Np or the mutants are mainly localized in the cell plasma membrane like M protein.

2.2.4.2. Analysis of antigenic structure of PPRV nucleoprotein

In order to characterize the antigenic structure of the PPRV N protein and in particular to determine the most antigenic regions, the full length recombinant N protein, the recombinant deleted mutants and the overlapping N peptides were used in indirect ELISA test (iELISA) with PPR positive and negative sera obtained from goats and sheep. Compared to negative sera, the positive sera reacted well with N and to some extent with all deleted N mutants except the deletion mutant NpΔ120-277 (see figure 24). These results might indicate the importance of the region aa 120-277, and particularly aa 241-277 in the serological response of sheep and goat against PPRV N. The denatured N protein is partially recognized by the positive sera. From all these results taken together, it was concluded that anti-N antibodies developed by small ruminants are recognizing mainly conformational epitopes.

Attempts to identify linear epitopes by the use of overlapping N peptides as antigens in the indirect ELISA were unsuccessful because of high background. Analysis of monoclonal antibody (mAb) reaction on the different proteins (Figure 25) show that mAb 38-4 has conformational epitope since this mAb failed to detect denaturated N protein and all mutants which have deletion from aa 1 to 277. The mAb P4G5 which was produced against the c-terminal peptide 421-525 recognized all proteins except the deletion mutants 420-490 and 420-525.

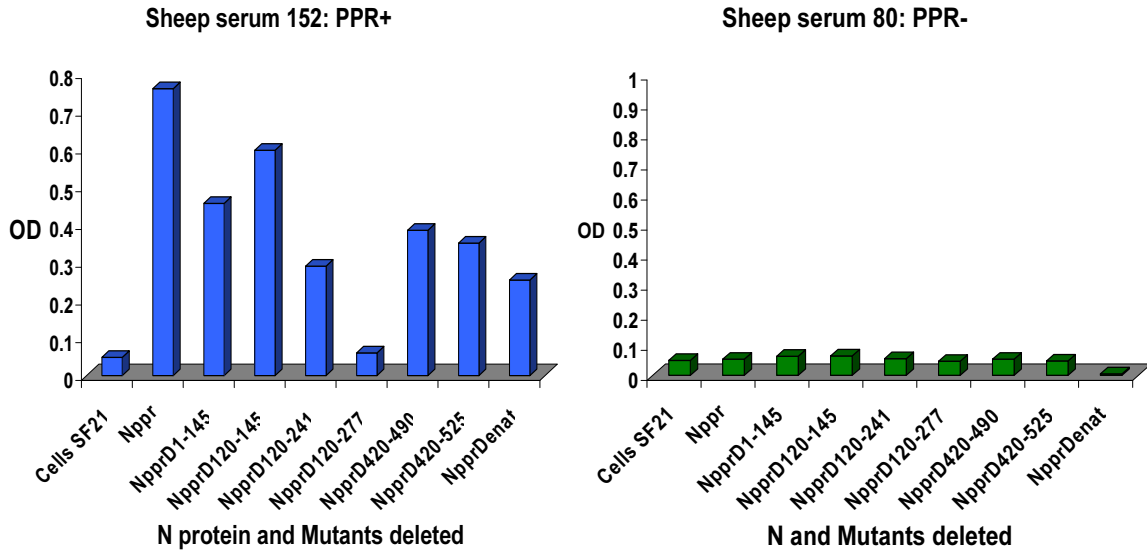


Figure 24: Evaluation of the reaction antibodies anti PPRV on different PPRV N proteins (full length and deleted mutants). Test carried by indirect ELISA.

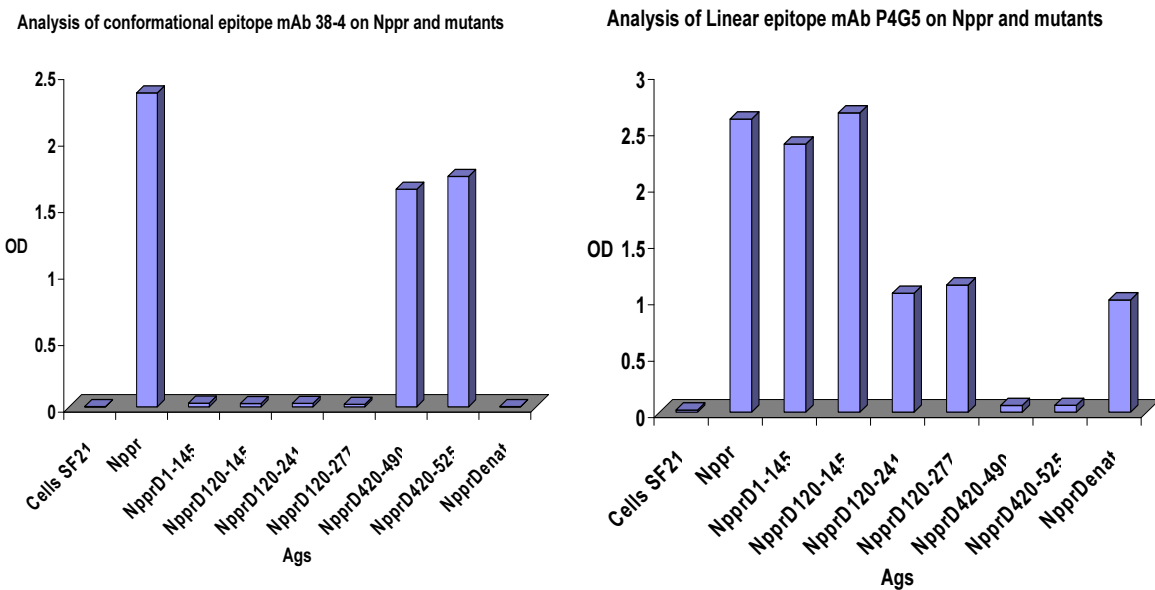


Figure 25: Evaluation of the reaction of mAb 38-4 and P4G5 (antibodies anti PPRV N) on different PPRV N proteins (full length and deleted mutants). Test carried out by indirect ELISA.

3. TRAINING

3.1. The APU provided assistance in a two week Inter-Regional Training course on the “Rapid Diagnosis of Avian Influenza”. This course, which was co-funded by the FAO and the IAEA TC-Asia, was attended by 28 trainees from Asia, Africa and Latin America. All participants appreciated very much this training course.

3.2. The APU also participated in a IAEA TC-Africa sponsored training course on the application of PCR to peste des petits ruminants (PPR) and contagious bovine pleuropneumonia (CBPP) diagnosis. It was organised for French speaking scientists. This course was also well appreciated by the attendants, who wrote a motion of congratulation to IAEA TC.

3.3. The APU transferred the I-TAB ELISA method for the detection of anti trypanosomal antibodies to the Onderstepoort Veterinary Institute, South Africa. Mr Olivier Matthee from that institute, who will be in charge of production of kits for this test, was trained at APU for four weeks. This training was sponsored by IAEA TC.

3.4. Two staff members from APU attended training courses organised by the TATAA BIOCENTER on the Quantitative PCR (qPCR). These training courses involved practical applications with hands-on experience as well as theoretically oriented seminars.

4. FELLOWS and INTERNS

Ms. Mette Ely Munch from the Royal Veterinary and Agriculture University, Denmark was an intern in APU from July 10 to September 10, 2006.

5. APPENDICES

5.1. Publications

- 1) 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 PP and WP Taylor (eds) Elsevier, Academic Press, 2006.
- 2) Kerdiles YM, Cherif B, Marie JC, Tremillon N, Blanquier B, Libeau G, Diallo A, Wild TF, Villiers MB and Horvat B. Immunomodulatory properties of morbillivirus nucleoproteins. *Viral Immunol.*, 2006, 19: 324-34
- 3) Diallo A. Control of Peste des Petits Ruminants and Poverty Alleviation? *J. Vet. Med.*, 2006, 53: 11-13
- 4) Couacy-Hymann E, Bodjo SC and Danho T. Interference in the vaccination of cattle against rinderpest virus by antibodies against peste des petits ruminants (PPR) virus. *Vaccine*, 2006, 24: 5679-83.
- 5) Diallo A and Viljoen G. Genus Capripoxvirus. In Poxviruses, Birkhäuser Advances in Infectious Diseases, Mercer A, Schmidt A and Weber O (eds), *in press*.
- 6) Couacy-Hymann E, Bodjo SC, Koffi MY, Danho T. Observations on rinderpest and rinderpest-like diseases throughout West and Central African countries during rinderpest eradication projects. *Res Vet Sci.*, *in press*,
- 7) Diallo A, Minet C, Le Goff C, Berhe G, Albina E, Libeau G and Barrett T. The Threat of Peste des Petits Ruminants: Progress in Vaccine Development for Disease Control. *Vaccine*, *accepted for publication*
- 8) Couacy-Hymann E, Bodjo S.C, Danho T, Libeau G, Diallo A. Evaluation of the virulence of some strains of peste-des-petits-ruminants virus (PPRV) in experimentally infected West African dwarf goats. *Vet J.*, *in press*.
- 9) Bodjo SC, Kwiatek O, Diallo A, Albina E and Libeau G. Mapping and structural analysis of B-cell epitopes on *Morbillivirus* nucleoprotein N-terminus. *J. Gen. Virol.*, *accepted for publication*
- 10) Couacy-Hymann E, Bodjo SC, Danho T, Koffi MY, Libeau G, Diallo A. Early detection of viral excretion from experimentally infected goats with peste-des-petits-ruminants virus. *Preventive Veterinary Medicine*, *in press*.
- 11) Kwiatek O, Minet C, Grillet C, Hurard C, Carlsson E, Karimov B, Albina E, Diallo A, Libeau G. Peste des Petits Ruminants (PPR) Outbreak in Tajikistan. *J. Comp Path*, *in press*

5.2. Duty Travel

Adama Diallo

- OIE Meeting on the diagnosis of Foot and Mouth Disease by ELISA based on the non-structural protein, Paris, France, 23-24 January 2006.
- OIE Biological Standards Commission meeting, Paris, France, 25-27 January 2006.
- Meeting on "Control of Transboundary Animal diseases and Poverty Alleviation" (EU project). Istanbul, Turkey, 22 - 26 February 2006.
- PACE Advisory Committee Meeting. Bamako, Mali, 26 April-4 May 2006.
- International Veterinary Vaccines and Diagnostics Conference (invited speaker), Oslo, Norway, 25-30 June 2006.
- OIE Biological Standards Commission meeting, Paris, France, 12-16 September 2006.
- PACE Advisory Committee meeting, Nairobi, Kenya, 26-29 October 2006.
- Meeting with Ethiopian partner on the Project Reinforcing African Laboratories, Addis Ababa, Ethiopia, 29-30 October 2006,
- Meeting with partners of the MARVAC project, Montpellier, France, 9-10 November 2006.

Massoud Malek

- Intergovernmental Technical Working Group for Animal Genetic Resources (ITWG-AnGR) meeting, hosted by FAO in Rome, 13-15 December 2006.

Ericka Adelina Pestana Delgado

- "Quantitative PCR Course" in Florence, Italy, 18-21 September 2006.

Charles Bodjo

- Visit to Counterpart CIRAD-EMVT to work on electronic and confocal microscopy for analysis of interaction between Peste des Petits Ruminants virus (PPRV) nucleoprotein and Matrix protein (MARKVAC EU project). Montpellier, France, 16-20 October 2006.
- Visit to CIRAD-EMVT to work with the counterpart on the analysis of the PPRV N protein by confocal microscopy (MARKVAC EU project). Montpellier, France, 13-15 February 2006.

Mamadou Lelenta

- Visit to evaluate (ELISA test) the PPR serum bank prepared by LANADA for the Joint FAO/IAEA Programme. Abidjan, Côte d'Ivoire, 16-28 January 2006.
- Training on Real-Time Quantitative PCR applied to the diagnosis of animal diseases. Göteborg, Sweden, 9-27 October 2006.



Visit our Website!

<http://www-naweb.iaea.org/nafa/aph/index.html>