

First Annual Meeting EPIZONE

“EPIZONE INSIDE OUT”

May 30th – June 1st 2007

Lublin/Pulawy

Poland



Network of Excellence for Epizootic Disease Diagnosis and Control

PREFACE **9**

PROGRAMME **11**

ABSTRACTS INVITED/KEY NOTE SPEAKERS **21**

WORLD ORGANISATION FOR ANIMAL HEALTH (OIE): PRESENTATION ON ITS ACTIVITIES WITH SPECIAL ATTENTION TO EPIZON-RELATED TOPICS	23
E. ERLACHER VINDEL, G. BRÜCKNER	23
AVIAN INFLUENZA IN WILD WATER FOWL AT THE LAKE OF CONSTANCE; A MULTI NATIONAL STUDY	24
A. BAUMER ¹ , B. THÜR ¹ , M. HOFMANN ¹ , I. BRUNHART ² , M. FALK ² , K STÄRK ³ , C. GRIOT ¹	24
RISK ANALYSIS IN A DYNAMIC ENVIRONMENT – MISSION IMPOSSIBLE?	25
KATHARINA D.C. STÄRK, DIRK U. PFEIFFER	25
PATHOGENESIS, PREVENTION AND DIAGNOSIS OF NIPAH VIRUS	26
H. WEINGARTL, Y. BERHANE, B. SAWATSKY, C. RANADHEERA, M. CZUB	26
TRANSBOUNDARY ANIMAL DISEASES AND THEIR CONTROL STRATEGIES IN CENTRAL ASIAN COUNTRIES	27
M. HUSSAIN ¹ , G. FERRARI ¹ , S. ALEXANDERSEN ² , J. KLEIN ² , M. AHMAD ¹	27
BLUETONGUE: VECTORS, EPIDEMIOLOGY AND CLIMATE-CHANGE	28
PHILIP S. MELLOR	28

ABSTRACTS WORK PACKAGE PRESENTATIONS **29**

PROGRESS OF THE WP 4.1-REAL TIME PCR IN THE LAST YEAR	31
B. HOFFMANN, K. UTKE, M. BEER	31
WP 4.3 DIVA DIAGNOSTICS	32
Å. UTTENTHAL,	32
IMPACT OF ENVIRONMENTAL EFFECTS ON THE RISK OF THE OCCURRENCE OF EPIZOOTIC DISEASES IN EUROPE: IDENTIFICATION AND PRIORITISATION	33
E. L. SNARY ¹ , P. GALE ¹ & EPIZONE WP7.4 PARTICIPANTS	33

ABSTRACTS FREE COMMUNICATIONS: THEME 4 DIAGNOSTICS **35**

HIGH THROUGHPUT RRT-PCR DIAGNOSTICS FOR BLUETONGUE BASED ON GENOME SEGMENT 10	37
P. VAN RIJN, R. HEUTINK, R. VAN GENNIP	37
A TAQMAN-MGB REAL-TIME RT-PCR ASSAY FOR THE UNIVERSAL DETECTION OF AFRICAN HORSE SICKNESS VIRUS (AHSV)	38
J. FERNÁNDEZ ¹ , E. SOTELO ¹ , P. FERNÁNDEZ-PACHECO ¹ , B. RODRÍGUEZ ² , A. ROBLES ¹ , M. ARIAS ¹ , J.M. SÁNCHEZ-VIZCAÍNO ²	38
DEVELOPMENT OF A MICROARRAY FOR CHARACTERISATION OF VETERINARY VIRUSES	39
J. DUKES ¹ , A. ABU-MEDIAN ² , M. WATSON ² , R. CARD ³ , M. BANKS ³ , P. BRITTON ² , D. KING ¹	39
TOWARDS MICROARRAY TECHNOLOGY FOR INFLUENZAVIRUS DIAGNOSTICS	40
A. GALL, B. HOFFMANN, M. BEER	40
WORKSHOP ON THE DESIGN AND INTERPRETATION OF POST FOOT-AND-MOUTH DISEASE (FMD)-VACCINATION SEROSURVEILLANCE BY NSP TESTS; PART I (DENSE CATTLE-PIG COUNTRIES)	41
K. DE CLERCQ ¹ , K. LUYTEN ¹ , J. WESTERGAARD ²	41
EVALUATION OF LATERAL FLOW DEVICES FOR RAPID PENSIDE DIAGNOSIS OF AVIAN INFLUENZA: A NEW STRATEGY FROM FLUAID	42
M. SLOMKA, J. BANKS, L. KERR, T. PAVLIDIS, V. COWARD, I. BROWN	42

ABSTRACTS FREE COMMUNICATIONS: THEME 5 INTERVENTION STRATEGIES **43**

ANALYSIS OF IMMUNE RESPONSES IN SHEEP FOLLOWING VACCINATION WITH DNA PLASMIDS ENCODING STRUCTURAL RIFT VALLEY FEVER VIRUS PROTEINS	45
G. LORENZO ¹ , R. MARTÍN ¹ , B. BORREGO ¹ , F. RODRÍGUEZ ² , A. BRUN ¹	45
RECOMBINATION VECTORS FOR GENE TRANSFER INTO VERTEBRATE CELLS BY BACULOVIRUSES.	46
G. KEIL, C. HÖHLE, K. VOGT AND K.GIESOW	46

CLASSICAL SWINE FEVER VIRUS INDUCES ACTIVATION OF PLASMACYTOID AND CONVENTIONAL DENDRITIC CELLS IN BLOOD AND SECONDARY LYMPHOID ORGANS OF INFECTED PIGS	47
A. JAMIN ¹ , S. GORIN ¹ , R. CARIOLET ² , M. LE POTIER ¹ , G. KUNTZ-SIMON ¹	47
INTERACTION OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV) WITH DENDRITIC CELLS	49
T. RODRÍGUEZ-CALVO ¹ , M. SANZ-RAMOS ^{1,2} , F. DÍAZ-SAN SEGUNDO ³ , AND N. SEVILLA ¹	49
ADJUVANT EFFECTS OF RECOMBINANT PLASMIDS OF CPG AND PORCINE CYTOKINES TO FMD VACCINE IN VIVO	50
Z. JING, Y. DOU, X. MENG, G. CHEN, X. CAI	50
POTENT AND SELECTIVE INHIBITORS OF THE REPLICATION OF FOOT-AND-MOUTH DISEASE AND SWINE VESICULAR DISEASE VIRUS	51
N. GORIS ¹ , A. DE PALMA ² , J. NEYTS ² , K. DE CLERCQ ¹	51

ABSTRACTS FREE COMMUNICATIONS: THEME 6 SURVEILLANCE AND EPIDEMIOLOGY **53**

STUDY ON BIOLOGICAL CHARACTERISTIC OF FOOD-AND-MOUTH DISEASE TYPE ASIA 1 VISUSES ISOLATED FROM OUTBREAKS IN THE MAINLAND OF CHINA DURING RECENT TWO YEARS	55
Y. SHANG, X. LIU, Z. LIU, H. YIN, J. MA, H. CHANG, Y. WANG, Q. ZHANG, D. LI, Z. LU, H. BAO, J. GUO, Q. XIE	55
GENETIC TYPING OF CLASSICAL SWINE FEVER VIRUS ISOLATES FROM CHINA	56
S-Q. SUN, X-T. LIU, S-H. YIN, Y-J. SHANG, H. TIAN, Y. JIN, H-X. ZHENG, Z-X. LIU, X-Q. HAN, H. YIN, X-P. CAI, Q-G. XIE	56
QUANTIFICATION OF TRANSMISSION PARAMETERS OF FMDV STRAIN O TAIWAN AMONG NON-VACCINATED AND VACCINATED PIGS	57
P. EBLÉ ¹ , A. DE KOEIJER ² , M. DE JONG ² , B. ENGEL ² , A. DEKKER ¹	57
MOLECULAR EPIDEMIOLOGY OF SWINE VESICULAR DISEASE VIRUSES ISOLATED IN ITALY OVER 15 YEARS (1992-2006)	58
E. BROCCHI ¹ , S. GRAZIOLI ¹ , I. BARBIERI ¹ , M. BUGNETTI ¹ , G. WILSDEN ² , N.J. KNOWLES ²	58
MOLECULAR EPIDEMIOLOGY OF AFRICAN SWINE FEVER VIRUS (ASFV) ISOLATES RECOVERED FROM SAMPLES COLLECTED IN KENYA DURING 2006-2007 SUSPECTED OUTBREAKS	59
C. GALLARDO ^{1,3} , D. MWAENGO ² , V. PELAYO ¹ , E. OKOTH ³ , J. MACHARIA ⁴ , R. BISHOP ³	59
EVOLUTION OF THE FIRST TUBERCULOSIS OUTBREAK DUE TO MYCOBACTERIUM BOVIS IN WILDLIFE IN FRANCE	61
G. ZANELLA ¹ , B. DURAND ¹ , J. HARS ³ , F. MOUTOU ¹ , B. GARIN-BASTUJI ² , A. DUVAUCHELLE ³ , M. FERMÉ ³ , C. KAROUI ² , M. BOSCHIROLI ²	61

ABSTRACTS FREE COMMUNICATIONS: THEME 7 RISK ASSESSMENT **63**

DEVELOPING A QUALITATIVE RISK ASSESSMENT FRAMEWORK FOR THE IMPACT OF CLIMATE CHANGE ON THE PREVALENCE OF LIVESTOCK DISEASES IN THE UK – WORK IN PROGRESS	65
GALE, P., ADKIN, A. AND SNARY, E.	65
METHODOLOGY AND PROCEDURES EMPLOYED AND OUTPUT GENERATED IN RISK ANALYSIS OF AVIAN INFLUENZA ENTRANCE IN SPAIN BY WILD BIRDS	66
I. IGLESIAS ¹ , M. MARTÍNEZ ¹ , A. DE LA TORRE ¹ , J. MANUEL SÁNCHEZ-VIZCAÍNO ² , J. FERNÁNDEZ ¹ , M. JESÚS MUÑOZ ¹	66
CSF IN WILDBOAR - A SURVEILLANCE DATA BASE FOR BELGIUM, FRANCE, GERMANY, LUXEMBOURG AND THE NETHERLANDS	67
C. STAUBACH, D. KLÖB, M. KRAMER, F. CONRATHS	67

ABSTRACTS POSTER PRESENTATIONS: THEME 4 DIAGNOSTICS **69**

DEVELOPMENT OF DIAGNOSTIC TECHNIQUES AND VACCINES AGAINST ASIA-1 FOOT AND MOUTH DISEASE IN CHINA	71
Q. ZHANG, X. LIU, Z. LIU, J. MA, D. LI, Y. SHANG, Z. LU, Y. CAO, G. WU, X. YAN	71
ASSESSMENT OF A FULLY AUTOMATED REAL-TIME RT-PCR DEVICE FOR THE DETECTION OF BOVINE VIRAL DIARRHOEA VIRUS	72
P. WAKELEY ¹ , D. MEALING ¹ , J. ERRINGTON ¹ , D. SQUIRRELL ² , G. MARTIN ² , M. LEE ²	72
RAPID AND SENSITIVE IDENTIFICATION OF H7 SUBTYPE INFLUENZA A VIRUSES USING MGB REAL TIME RT-PCR	74
S. VAN BORM ¹ , M. BOSCHMANS ¹ , O. OZHELVAZI ¹ , T. VAN DEN BERG ¹	74
THE GENOME SEQUENCE OF A NOVEL FOOT-AND-MOUTH DISEASE VIRUS BELONGING TO SEROTYPE O	75
J. STIRLING, N. KNOWLES	75
EVALUATION OF A PORTABLE THERMOCYCLER FOR THE DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS	76
S. REID ¹ , R. MISTRY ² , J. DUKES ¹ , K. EBERT ¹ , C. MILLS ² , L. BOSWELL ² , P. FERRIS ¹ , D. KING ¹	76
EVALUATION OF EMERGENCY FMD VACCINE IN SHEEP FOLLOWING AEROSOL CHALLENGE BY PIG	77
S. PARIDA, L. FLEMING, Y. OH, S. DAS, S. UPPADHYA, M. MOHAPATRA, J. GLOSTER, D. PATON	77

MONOCLONAL ANTIBODY BASED ELISAs TO DETECT ANTIBODIES AGAINST NEURAMINIDASE SUBTYPES 1, 2 AND 3 IN AVIAN SERA	78
P. CORDIOLI, A. MORENO MARTIN, D. LELLI, E. SOZZI, A. LUPPI, V. TRANQUILLO, E. BROCCHI	78
PHYLOGENY OF THE MYCOPLASMA MYCOIDES CLUSTER: CONSEQUENCES IN TAXONOMY AND APPLICATION FOR SPECIES IDENTIFICATION	80
L. MANSO-SILVÁN ¹ , X. PERRIER ² , F. THIAUCOURT ¹	80
DIVA ASSAYS: MULTIPCR VS MULTIPLEX PCR	81
L. LIU ¹ , F. WIDÉN ¹ , C. BAULE ¹ , S. BELÁK ¹	81
MOLECULAR EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE VIRUS TYPE C	82
N. KNOWLES, K. SWABEY, R. MIDGLEY, P. DAVIES, J. WADSWORTH	82
USE OF A NOVEL RECOMBINANT COWPEA MOSIAC VIRUS PARTICLE AS AN INTERNAL CONTROL FOR DIAGNOSTIC RT-PCR	83
D. KING ¹ , N. MONTAGUE ² , K. EBERT ¹ , S. REID ¹ , J. DUKES ¹ , L. SCHÄDLICH ² , G. BELSHAM ^{1,3} , G. LOMONOSOFF ²	83
DEVELOPMENT OF A N2-BASED DIVA TEST FOR DUCKS: CONSTRUCTION OF A RECOMBINANT BACULOVIRUS EXPRESSING N2 TYPE NEURAMINIDASE PROTEIN AVIAN INFLUENZA VIRUS	84
O. GUIONIE, V. JESTIN	84
UTILIZATION OF A MICROARRAY-BASED PADLOCK PROBE SYSTEM IN THE VIRAL DIAGNOSTICS	85
J. BANÉR ¹ , P. GYARMATI ¹ , A. YACOUB ¹ , M. HAKHVERDYAN ¹ , J. STENBERG ² , O. ERICSSON ² , M. NILSSON ² , U. LANDEGREN ² , S. BELÁK ^{1*}	85
PRELIMINARY VALIDATION OF A COMMERCIAL AVIAN INFLUENZA N1 ANTIBODY COMPETITIVE ELISA KIT THAT CAN BE USED AS PART OF A DIVA STRATEGY	86
WG DUNDON ¹ , C. TERREGINO ¹ , V. TUTTOILMONDO ¹ , M. PIZZUTO ¹ , L. BUSANI ² , M. MANCINI ² , G. CATTOLI ¹ , I. CAPUA ¹	86
COMPARISON OF ELISAs FOR ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS NON-STRUCTURAL PROTEINS IN CATTLE SERA BASED ON THE CONTINUOUS RESULTS	87
A. DEKKER ⁴ , D. SAMIN ⁸ , M. GREINER ⁵ , I. BERGMANN ⁹ , D. PATON ⁶ , S. GRAZIOLI ⁷ , K. DE CLERCQ ⁵ , E. BROCCHI ⁷	87
DEVELOPMENT OF A RT-LAMP ASSAY FOR THE DETECTION OF SWINE VESICULAR DISEASE VIRUS	88
A. BLOMSTRÖM ¹ , M. HAKHVERDYAN ² , S. BELAK ^{1,2} , M. BERG ¹	88
A NOVEL REAL-TIME RT-PCR FOR THE DETECTION OF BLUETONGUE VIRUS	89
A. SHAW ¹ , P. MONAGHAN ¹ , H. ALPAR ² , S. ANTHONY ¹ , K. DARPEL ¹ , C. BATTEN ¹ , S. CARPENTER ¹ , H. JONES ¹ , K. BANKOWSKA ¹ , C. OURA ¹ , D. KING ¹ , H. ELLIOT ¹ , P. MERTENS ¹	89
PRIMER AND PROBE DESIGN FOR FOOT-AND-MOUTH DISEASE TYPING	90
B. SAREYYÜPOĞLU	90
CLINICAL SIGNS IN BLUETONGUE VIRUS SEROTYPE 8 INFECTED SHEEP, GOATS AND CATTLE	92
A. BACKX, C. HEUTINK, E. VAN ROOIJ, P. VAN RIJN	92
A COMMON NEUTRALIZING EPITOPE ON ENVELOPE GLYCOPROTEIN E2 OF DIFFERENT PESTIVIRUSES: IMPLICATIONS FOR IMPROVEMENT OF VACCINES AND DIAGNOSTICS FOR CLASSICAL SWINE FEVER (CSF)?	93
P. VAN RIJN	93
EVALUATION OF PCR FAST FORMAT FOR THE RAPID DIAGNOSIS OF AFRICAN SWINE FEVER (ASF)	94
J. FERNÁNDEZ, C. GALLARDO, R. NIETO, A. ROBLES, C. PÉREZ, M. ARIAS	94
VALIDATION OF A COMMERCIAL ELISA KIT FOR DETECTION OF ANTIBODIES TO BLUETONGUE VIRUS IN INDIVIDUAL MILK SAMPLES OF DUTCH DAIRY COWS	95
H. KRAMPS ¹ , C. VAN MAANEN ² , J. MARS ² , J. POPMA ¹ , AND P. VAN RIJN ¹	95
DEVELOPING AND VALIDATION OF A BTV-8 SPECIFIC REAL-TIME RT-PCR	96
B. HOFFMANN, M. BEER	96
TRANSPLACENTAL TRANSMISSION AND PERSISTENCE IN FOETAL LAMBS FOLLOWING EXPERIMENTAL INFECTION OF PREGNANT EWES AT 90 DAYS GESTATION WITH FMDV	97
R. WATERS ^{1,2} , H. BROOKS ² , L. WEST ¹ , J. BROWNLIE ² , Z. ZHANG ¹	97
MOLECULAR EPIDEMIOLOGY OF AFRICAN SWINE FEVER AND PESTE DES PETITS RUMINANTS	98
O. KWIAITEK ¹ , V. MICHAUD ¹ , C. MINET ¹ , J. RAVAOMANANA ¹ , A. DIALLO ² , G. LIBEAU ¹ , E. ALBINA ¹	98
LONG-TERM STORAGE OF ANIMAL BLOOD ON FILTER PAPERS FOR DIRECT DETECTION AND GENOTYPING OF VIRUSES	99
V. MICHAUD ¹ , P. GIL ¹ , O. KWIAITEK ¹ , S. PROMÉ ¹ , L. DIXON ² , L. ROMERO ³ , M-F. LE POTIER ⁴ , M. ARIAS ³ , E. COUACY-HYMANNS ⁵ , F. ROGER ¹ , G. LIBEAU ¹ , E. ALBINA ¹	99

ABSTRACTS POSTER PRESENTATIONS: THEME 5 INTERVENTION STRATEGIES

101

CHIMERIC CALICIVIRUS-LIKE PARTICLES DISPLAYING B-CELL AND T-CELL EPITOPES FROM FOOT AND MOUTH DISEASE VIRUS	103
I. ANGULO ¹ , C. CUBILLOS ¹ , H. ALMANZA ¹ , M. MORALES ¹ , J. R. CASTÓN ² , F. SOBRINO ³ , E. BLANCO ¹ , J. BÁRCENA ¹	103
DNA VACCINES BASED ON FMDV-MINIGENES FUSED TO DIFFERENT SIGNAL PEPTIDES: ANTIGEN EXPRESSION AND <i>IN VIVO</i> IMMUNE RESPONSES DO NOT CORRELATE	104
B. BORREGO ¹ , J. ARGILAGUET ² , E. PÉREZ-MARTÍN ² , M. ESGUEVILLAS ¹ , M. GARCÍA-BRIONES ³ , B. ÁLVAREZ ³ , A. EZQUERRA ³ , F. SOBRINO ^{1,4} & F. RODRÍGUEZ ²	104
INVESTIGATION OF THE ROLE OF THE AFRICAN SWINE FEVER VIRUS A238L PROTEIN IN MODULATING HOST GENE TRANSCRIPTION USING PORCINE MICROARRAYS	105
R. SILK, F. ZHANG, C. ABRAMS, L. DIXON	105

TISSUE DISTRIBUTION OF A PSEUDORABIES VIRUS PLASMID DNA VACCINE AFTER INTRAMUSCULAR INJECTION IN SWINE	106
D. DORY, R. GRAVIER, A. JESTIN	106
ANTIGENIC AND GENETIC ANALYSIS OF SWINE INFLUENZA VIRUSES CIRCULATING IN SPAIN. CONSTRUCTION OF AN ATTENUATED H1N2 STRAIN	107
G. DEL REAL, M. MARTIN DEL BURGO	107
DIFERENTIAL EXPRESSION OF HOST GENES DURING EARLY AFRICAN SWINE FEVER VIRUS INFECTION	108
R. HERBERT, F. ZHANG, C. ABRAMS AND L. DIXON	108
TRANSCRIPTIONAL RESPONSE IN THE SKIN OF PIGS AFTER INTRA-DERMAL VACCINATION WITH DOE FORMULATED FMDV VACCINE.	109
M. HULST ¹ , S. DURAND ² , A. WIT ¹ , A. DEKKER ² , R. ZWART ¹ , N. STOCKHOFF ¹ AND P. EBLE ² .	109
CONSTRUCTION OF A QUADRUPLE RECOMBINANT BACULOVIRUS COEXPRESSING H5, N3, M, AND M2 PROTEINS OF A H5N3 LPAI VIRUS	110
A. PREL, G. LE GALL-RECULE, V. JESTIN	110
GENERATION OF VACCINES BASED ON RECOMBINANT VACCINIA VIRUS-EXPRESSED BLUETONGUE VIRUS ANTIGENS	112
E. CALVO, J. ORTEGO	112
<i>IN VIVO</i> COMPARATIVE STUDY OF IMMUNOPATHOGENY RELATED ON THE VIRULENCE OF THE CSFV STRAINS: PRELIMINARY SETTINGS IN PREPARATION FOR A TRANSCRIPTOMIC ANALYSIS	113
P. RENSON ¹ , R. CARIOLET ² , M. LE DIMNA ¹ , E. HUTET ¹ , M. LE POTIER ¹	113
INTERFERON ACTIVITY IN THE PLASMA, CULTURE SUPERNATANTS OF PBMC AND LEVEL OF IFN γ -mRNA IN PBMC FROM SHEEP INFECTED WITH BOVINE IMMUNODEFICIENCY VIRUS (BIV)	114
M. ROLA ¹ , M. MATERNIAK ¹ , M. KANDEFER-SZERSZEN ² , A. SZUSTER-CIESIELSKA ² , J. KUZMAK ¹	114
COINJECTION OF RAINBOW TROUT INTERLEUKIN 8 WITH THE GLYCOPROTEIN GENE FROM VIRAL HEMORRHAGIC SEPTICEMIA VIRUS (VHSV) MODULATES THE CC CHEMOKINE RESPONSE	115
E. SANCHEZ ¹ , J. COLL ² AND C. TAFALLA ¹	115
DIFFERENT EXPRESSION PATTERNS REVEALED BY MICROARRAY ANALYSES OF PORCINE ALVEOLAR MACROPHAGES INFECTED WITH AFRICAN SWINE FEVER VIRUSES OF DIFFERENT VIRULENCE	116
F. ZHANG ¹ , P. HOPWOOD ² , C. ABRAMS ¹ , A. DOWNING ³ , F. MURRAY ³ , R. TALBOT ³ , A. ARCHIBALD ³ , S. LOWDEN ² , AND L. K. DIXON ¹	116
INTELLIGENT DESIGN OF A VACCINE FOR AFRICAN SWINE FEVER VIRUS.	117
D. CHAPMAN ¹ , L. GOATLEY ¹ , H. TAKAMATSU ² , L. DIXON ¹	117
DEVELOPMENT OF DIVA VACCINE AGAINST PESTE DES PETITS RUMINANTS BY REVERSE GENETICS	118
C. MINET, P. GIL, G. LIBEAU, E. ALBINA	118
CONTROL OF MORBILLIVIRUS REPLICATION BY RNAi	119
R.S. ALMEIDA, D. KEITA, G. LIBEAU, E. ALBINA	119
RECOMBINANT CAPRIPOXVIRUSES EXPRESSING PROTEINS OF BLUETONGUE VIRUS: EVALUATION OF IMMUNE RESPONSE AND PROTECTION IN SMALL RUMINANTS	120
A. PERRIN ¹ , E. ALBINA ¹ , P. RUSSO ³ , S. ZIENTARA ² , C. CÊTRE-SOSSAH ¹	120
CONTROL OF AFRICAN SWINE FEVER VIRUS BY siRNA	121
D. KEITA, E. ALBINA	121
LYSSAVIRUS TRAFFICKING USING CO-CULTURE TECHNIQUES	122
C. J. FINNEGAN, S. M. BROOKES, A. R. FOOKS	122
DISTRIBUTION OF RABIES AND RABIES BAT VARIANTS WITHIN THE MOUSE BRAIN	123
D.M. HEALY ^{1,2} , S.M. BROOKES ¹ , A. NUNEZ ¹ , D. HICKS ¹ , S.L. COSBY ² AND A.R. FOOKS ¹ .	123

ABSTRACTS POSTER PRESENTATIONS: THEME 6 SURVEILLANCE AND EPIDEMIOLOGY

125

DIFFERENT EXPRESSION PATTERNS REVEALED BY MICROARRAY ANALYSES OF PORCINE ALVEOLAR MACROPHAGES INFECTED WITH AFRICAN SWINE FEVER VIRUSES OF DIFFERENT VIRULENCE	127
F. ZHANG ¹ , P. HOPWOOD ² , C. ABRAMS ¹ , A. DOWNING ³ , F. MURRAY ³ , R. TALBOT ³ , A. ARCHIBALD ³ , S. LOWDEN ² , AND L. K. DIXON ¹	127
INTRODUCING THE NETWORK "RODENT-BORNE PATHOGENS" IN GERMANY	128
S. ESSBAUER ¹ , J. SCHMIDT ² , F.J. CONRATHS ³ , B. EHLERS ⁴ , R.G. ULRICH ⁵	128
EMERGING OF A HIGHLY PATHOGENIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN CHINA	129
G-Z. TONG, Y-J. ZHOU, X-F. HAO, H-J. QIU, Z-J. TIAN, T-Q. AN	129
CLASSICAL SWINE FEVER IN CHINA	130
S-Q. SUN, X-T. LIU, S-H. YIN, Y-J. SHANG, H. TIAN, Y. JIN, H-X. ZHENG, Z-X. LIU, J-W. MA, X-Q.G HAN, H. YIN, X-P. CAI, Q-G. XIE	130
PRRSV: IMPACT OF TRADE BARRIERS ON VIRUS EPIDEMIOLOGY	131
T. STADEJEK ¹ , M. OLEKSIEWICZ ² , A. STANKEVICIUS ³ , D. POTAPCHUK ⁴	131
PROBE OF VARIATIONS OF 12 FIELD STRAINS OF CLASSICAL SWINE FEVER VIRUS E2 GENES IN CHINA	132
CQ. QIU, HC. GUO, H. HU	132
USING ARC HYDRO DATA MODEL TO SUPPORT THE AQUATIC ANIMAL DISEASE SURVEILLANCE	133
N. FERRE ¹ , M. BASSAN, F. BORGHEGAN, L. BORTOLOTTI, M. DALLA POZZA	133

SIGNIFICANT DIFFERENCE IN WITHIN- AND BETWEEN-PEN FOOT-AND-MOUTH DISEASE VIRUS TRANSMISSION AMONG PIGS	134
P. EBLÉ ¹ , A. DE KOEIJER ² , A. BOUMA ³ , A. DEKKER ¹ , A. STEGEMAN ³	134
SWINE VESICULAR DISEASE: ANALYSIS OF THE RISK FACTORS HIGHLIGHTED IN THE OUTBREAKS DETECTED IN LOMBARDIA REGION IN ORDER TO CARRY OUT BIOSECURITY GUIDELINE	135
S. BELLINI, G. ZANARDI, D. AVISANI, M. GRADASSI, C. NASSUATO, L. ALBORALI	135
FULL LENGTH SEQUENCE ANALYSES AND PHYLOGENETIC COMPARISON OF GENOME SEGMENT 2 OF BLUETONGUE VIRUS SEROTYPE 2 AND 9	136
S. MAAN, N. MAAN, C. BATTEN, A. SAMUEL, P. MERTENS	136
HIGH THROUGHPUT DIAGNOSIS OF AVIAN INFLUENZA: APPLICATION TO SURVEILLANCE OF WILD BIRDS	137
S. HAMMOUMI ¹ , P. GIL ¹ , N. GAIDET ¹ , T. DODMAN ² , D. MARTINEZ ¹ , E. ALBINA ¹	137
RISK FACTORS SIGNIFICANTLY INFLUENCING FUTURE AUJESZKY'S DISEASE ERADICATION PROGRAMME IN POLAND	138
A. LIPOWSKI, Z. PEJSK	138

ABSTRACTS THEME 5 MEETING 139

USE OF BACTERIOPHAGE LAMBDA PARTICLES AS POTENTIAL NOVEL DELIVERY SYSTEM OF MUCOSAL VACCINES	141
T. VAHLENKAMP, W. FUCHS, G. KEIL, T. METTENLEITER	141
NEW DNA VACCINE STRATEGIES AGAINST AFRICAN SWINE FEVER VIRUS	142
J. ARGILAGUET ^{1*} , E. PÉREZ-MARTÍN ^{1*} , C. GALLARDO ² , J. SALGUERO ² , M. PÉREZ-FILGUEIRA ³ , J. PUJOLS ¹ , M. NOFRARÍAS ¹ , N. FERNÁNDEZ-BORGES ¹ , E. BLANCO ² , J. ESCRIBANO ³ & F. RODRÍGUEZ ²	142
DEVELOPMENT OF A REVERSE GENETICS SYSTEM FOR PESTE DES PETITS RUMINANTS VIRUS (PPRV)	143
D. BAILEY, L. CHARD, A. BANYARD, P. DASH AND T. BARRETT	143
IMMUNIZATION OF RABBITS WITH RECOMBINANT CSFV E2 GLYCOPROTEIN LOADED PLGA MICROSPHERES	144
N. BRANDHONNEUR ¹ , C. LOIZEL ² , F. CHEVANNE ¹ , E. HUTET ³ , P. WAKELEY ⁴ , A. JESTIN ² , P. LE CORRE ¹ , M.F. LE POTIER ³	144
DEVELOPEMENT OF A SUBUNIT VACCINE AGAINST TUBERCULOSIS: PLANT-PRODUCED ESAT-6/AG85 FUSION ANTIGEN PROTEIN AND CATIONIC LIPOSOMES DELIVERY SYSTEM.	145
M. MOCKEY ¹ , U. CONRAD ² , P. HOLZMULLER ¹ , L. DEDIEU ¹ .	145
PHARMACOKINETIC AND TOXICITY STUDY IN PIGS OF AN IMIDAZOPYRIDINE, AN IN VITRO INHIBITOR OF THE REPLICATION OF CLASSICAL SWINE FEVER VIRUS	146
R. VRANCKEN ¹ , J. PAESHUYSE ² , G. PUERSTINGER ³ , J. ROZENSKI ² , M. WRIGHT ⁴ , J. NEYTS ² , F. KOENEN ¹	146
FOOT-AND-MOUTH DISEASE IMMUNOPROPHYLAXIS USING SINGLE-DOMAIN ANTIBODY FRAGMENTS	148
M. HARMSSEN ¹ , H. FIJTEN ¹ , A. DEKKER ² , P. EBLÉ ²	148

PARTICIPANTS ANNUAL MEETING EPIZONE 2007 149

Preface

Dear participants,

We welcome you all to the 1st Annual Meeting of our EU funded Network of Excellence, EPIZONE.

"EPIZONE INSIDE OUT"

The title "EPIZONE inside out" of this 1st Annual Meeting relates to the idea to organize this meeting in particular for the "inside" scientists of the EPIZONE partner institutes.

The title also expresses the mission of EPIZONE: Scientists of the EPIZONE partners "inside" go "out" their own institute during this meeting. So, you will have a meeting point here in Poland to look for collaboration with scientists of the other EPIZONE partners in order to make more progress in your research. You will find scientists involved in animal disease control to share ideas, thoughts and to discuss these extensively, etc. This will give you the chance to build new relationships and to strengthen the existing ones.

The title also highlights the intention of EPIZONE to share the "inside" strength of EPIZONE, built in the first year, to "outside" EPIZONE in the following years.

We hope with this program to fulfill your liking, with plenary lectures of invited speakers, presentations related to themes and their work packages, presentations and posters about a variety of subjects related to diagnosis and control of epizootic disease.

In addition to this program, time and meeting rooms are scheduled for work package meetings, free discussions and meetings between EPIZONE partners.

We are grateful to Dr Tadeusz Wijaszka and all involved members of NVRI for the generous hospitality, and the great and enthusiastic support in organizing this 1st Annual Meeting of EPIZONE.

We wish you all a successful and happy meeting

The scientific committee and the organizing committee

Scientific committee:

Piet van Rijn (CIDC)
Wim van der Poel (ID-L)
Martin Beer (FLI)
Ian Brown (VLA)
Linda Dixon (IAH)
Marie Frédérique Le Potier (AFSSA)
Søren Alexandersen (DFVF)
Laura Powell (VLA)
Louise Kelly (VLA)
Franz Conraths (FLI)

Organizing committee:

Katarzyna Szymanek (NVRI)
Krzysztof Niemczuk (NVRI)
Emmanuel Albina (AFSSA)
Jitty Oosterga-Land (CIDC)
Margriet Vedder-Rootjes (WUR)

Programme

"EPIZONE INSIDE OUT"

First Annual Meeting of the Network of Excellence EPIZONE

30th May – 1st June 2007

Lublin/Pulawy, Poland

"EPIZONE INSIDE OUT"

First Annual Meeting of the Network of Excellence EPIZONE
30th May – 1st June 2007
Lublin/Pulawy, Poland

Tuesday 29th May, Hotel Mercure, Lublin

	<i>Room D</i>	<i>Room C</i>
18.00 – 20.00	EC meeting (for TLs)	Work Package 7.4 meeting (for WP 7.4 participants)

20.00 – 22.00 Welcome reception
Registration
Presentations drop off
Poster set up

Wednesday 30 May 2007, Hotel Mercure, Lublin

08:00 – 09:00		Registration, Presentation drop off and Poster set up	
	Auditorium	Plenary session	
09:00 – 09:30		Chairman: Johan Bongers	
09:30 – 10:00		<ul style="list-style-type: none"> • Dr Piet van Rijn, Coordinator EPIZONE: "EPIZONE INSIDE OUT" • Dr Elisabeth Erlacher-Vindel, representative of OIE: "World organisation for animal health (OIE): presentation on its activities with special attention to EPIZONE related topics" 	
10:00 – 10:30		<ul style="list-style-type: none"> • Professor Julie Fitzpatrick, Scientific Director and Chief Executive, Moredun Research Institute, Scotland: "Disease Control: Challenges, Collaboration and Conflicts" 	
10:30 – 11:00		Coffee break	
	Auditorium	Plenary session: Theme 4	
11:00 – 11:30		Chairman: Dr Martin Beer	
11:30 – 12:30		<ul style="list-style-type: none"> • Key-note speaker: Professor Christian Griot, Director IVI, Switzerland: "Avian influenza in wild water fowl at the lake of Constance; a multi national study" 	
		Work Package Leaders presentations <ul style="list-style-type: none"> • WP 4.1 PCR-diagnostics • WP 4.2 DNA Chip-based diagnostics • WP 4.3 DIVA diagnostics • WP 4.4 Pen-side tests 	Dr Bernd Hoffmann Dr Yannick Blanchard Dr Áse Uttenthal Dr Philip Wakeley
12:30 – 14:00		Lunch	

14:00 – 15:30	Auditorium	Free communications: Theme 4 Chairman: Dr Martin Beer			
		<ul style="list-style-type: none"> • Dr Piet van Rijn "High throughput RRT-PCR diagnostics for Bluetongue based on genome segment 10" • Dr Jovita Fernández "A taqman-MGB real-time RT-PCR assay for the universal detection of African Horse Sickness Virus (AHSV)" • Dr Juliet Dukes "Development of a microarray for characterisation of veterinary viruses" • Astrid Gall "Towards microarray technology for influenzavirus diagnostics" • Dr Kris de Clercq "Workshop on the design and interpretation of post Foot-and-Mouth Disease (FMD)-vaccination serosurveillance by NSP tests; part I (dense cattle-pig countries)" • Dr Marek Slomka "Evaluation of lateral flow devices for rapid penside diagnosis of Avian influenza: a new strategy from FLUAID" 			
Parallel sessions		Room A	Room B	Room C	Room D
14:00 – 15:30		Meeting budget Theme 6 (for TL, Work Package Leaders of theme 6 and Jeroen Elzenaar)	Optional EAP meeting (for EAP members)	Theme 7 meeting (for TLs and WPLs of theme 7)	?
15:30 – 16:00	Coffee break				
16:00 – 16:30	Auditorium	Plenary session: Theme 7 Chair: Dr Louise Kelly and Dr Franz Conraths			
16:30 – 17:30		<ul style="list-style-type: none"> • Key-note speaker: Professor Katharina Staerk, Royal Veterinary College, United Kingdom: "Risk analysis in a dynamic environment – mission impossible?" 			
		Work Package Leaders presentations			
		<ul style="list-style-type: none"> • WP 7.1 Standardisation of import risk assessment • WP 7.2 European online database on epizootic diseases as an early warning system • WP 7.3 Decision support system for CSF • WP 7.4 Impact of environmental effects on the risk of the occurrence introduction of epizootic diseases in Europe: Identification and prioritisation 			<ul style="list-style-type: none"> Dr Larry Paisley Dr Franz Conraths Dr Klazien de Vos Dr Emma Snary
17:30 – 19:30		Poster session, Room A			
18:00 – 20:30		CF-meeting at Grand Hotel Lublinianka (for (dep)CF members)			
20:30	Dinner at Grand Hotel Lublinianka, Lublin				

Thursday 31 May 2007, Hotel Mercure, Lublin

09:00 – 10:30	Auditorium	<p>Free communications: Theme 7 Chair: Dr Louise Kelly and Dr Franz Conraths</p> <ul style="list-style-type: none"> • Dr Paul Gale “Developing a qualitative risk assessment framework for the impact of climate change on the prevalence of livestock diseases in the UK – work in progress” • Dr Jovita Fernández “Methodology and procedures employed and output generated in risk analysis of avian influenza entrance in Spain by wild birds” • Dr Franz Conraths “CSF in wildboar - a surveillance data base for Belgium, France, Germany, Luxembourg and the Netherlands” • Theme 7 discussion 			
Parallel sessions		<i>Room A</i>	<i>Room B</i>	<i>Room C</i>	<i>Room D</i>
09:00 – 10:30		Theme 5 meeting (for theme 5 participants)	Work Package 6.1 meeting (for WP6.1 participants)	Meeting budget Theme 4 (for TL, Work Package Leaders of theme 4 and Jeroen Elzenaar)	Work Package 6.3 meeting (for WP6.3 participants)
Coffee break					
11:00 – 11:30 11.30 – 12:30	Auditorium	<p>Plenary session: Theme 5 Chair: Dr Linda Dixon and Dr Marie-Frédérique Le Potier</p> <ul style="list-style-type: none"> • Key-note speaker: Professor Markus Czub, Faculty of (Veterinary) Medicine, University of Calgary, Canada: “Pathogenesis, prevention and diagnosis of Nipah virus” <p>Work Package Leaders presentations</p> <ul style="list-style-type: none"> • WP 5.1 Vaccine technologies Dr Alejandro Brun • WP 5.2 Host responses to infection Dr Thomas Vahlenkamp • WP 5.3 Adjuvants Dr Peter Mikael Heegaard • WP 5.4 Antivirals Dr Frank Koenen 			
Lunch					

14:00 – 15:30	Auditorium	<p>Free communications: Theme 5 Chair: Dr Thomas Vahlenkamp and Dr Frank Koenen</p> <ul style="list-style-type: none"> • Dr Alejandro Brun "Analysis of immune responses in sheep following vaccination with DNA plasmids encoding structural rift valley fever virus proteins" • Dr Günther Keil "Recombination vectors for gene transfer into vertebrate cells by baculoviruses" • Dr Gaëlle Kuntz-Simon "Classical swine fever virus induces activation of plasmacytoid and conventional dendritic cells in blood and secondary lymphoid organs of infected pigs" • Dr Noemi Sevilla "Interaction of Foot-and-mouth disease virus (FMDV) with dendritic cells" • Dr Zhi zhong Jing "Adjuvant Effects of recombinant plasmids of cP_g and Porcine Cytokines to FMD Vaccine in vivo" • Nesya Goris "Potent and selective inhibitors of the replication of foot-and-mouth disease and swine vesicular disease virus" 			
Parallel sessions		Room A	Room B	Room C	Room D
14:00 – 15:30		Meeting budget Theme 7 (for TL, Work Package Leaders of theme 7 and Jeroen Elzenaar)	Optional EAP meeting (for EAP members)	Work Package 6.2 meeting (demonstration of the GISAIID database)	Theme 4 meeting (for Theme 4 participants)
15:30- 16:00 Coffee break					
16:00 – 16:30 16:30 – 17:30	Auditorium	<p>Plenary session: Theme 6 Chairman: Professor Søren Alexandersen</p> <ul style="list-style-type: none"> • Key-note speaker: Manzoor Hussain, FAO Regional Epidemiologist, Islamabad, Pakistan: "Transboundary Animal Diseases and Their Control Strategies in Central Asian Countries" <p>Work Package Leaders presentations</p> <ul style="list-style-type: none"> • WP 6.1 Surveillance and epidemiology of viral diseases in aquaculture Dr Niels Jørgen Olesen • WP 6.2 Field epidemiology & surveillance of AI and APMV Dr Ilaria Capua • WP 6.3 Experimental Epidemiology Dr Phaedra Eblé • WP 6.4 Molecular Epidemiology Dr François Thiaucourt 			
18:00		Bus transport to Picnic			

Friday 1 June 2007, Pulawy

07:30 – 08:30		Bus transport from Lublin to Pulawy			
08:30 – 09:00		Coffee			
09:00 – 09:30 09:30 – 10:00	Auditorium	Plenary session Chairman Johan Bongers <ul style="list-style-type: none"> • Dr Tadeusz Wijaszka, Director NVRI, Pulawy • Dr Philip Mellor, IAH, Pirbright, UK : “Bluetongue: vectors, epidemiology and climate-change” 			
09:00 – 10:30		Parallel session Formal EAP meeting in room A			
10:00 – 11:30	Auditorium	Free communications Theme 6 Chairman: Søren Alexandersen <ul style="list-style-type: none"> • Dr You-Jun Shang “Study on biological characteristic of food-and-mouth disease type ASIA 1 viruses isolated from outbreaks in the mainland of china during recent two years” • Dr Shi-Qi Sun “Genetic typing of classical swine fever virus isolates from china” • Dr Phaedra Eblé “Quantification of transmission parameters of FMDV strain O TAIWAN among non-vaccinated and vaccinated pigs” • Dr Nick Knowles “Molecular epidemiology of swine vesicular disease viruses isolated in Italy over 15 years (1992-2006)” • Dr Carmina Gallardo “Molecular epidemiology of african swine fever virus (ASFV) isolates recovered from samples collected in kenya during 2006-2007 suspected outbreaks” • Gina Zanella “Evolution of the first tuberculosis outbreak due to Mycobacterium bovis in wildlife in France” 			
Parallel sessions		Room B	Room C	Room D	Room E
10:00 – 11:30		Meeting budget Theme 5 (for TL, Work Package Leaders of theme 5 and Jeroen Elzenaar)	Formal GB meeting (for GB members)	Work Package 7.1 meeting (for WP 7.1 participants)	Work Package 7.4 meeting (for WP 7.4 participants)

11:30 – 12:30		Excursion Institute Pulawy
11:30 – 12:30		Parallel session EC + EAP + GB meeting in room A
12:30 - 13:30		Farewell Lunch
		Bus transport to Warsaw airport

Abstracts invited/key note speakers

- Dr Elisabeth Erlacher-Vindel
- Professor Christian Griot
- Professor Katharina Stärk
- Professor Markus Czub
- Manzoor Hussain
- Dr Philip Mellor

WORLD ORGANISATION FOR ANIMAL HEALTH (OIE): PRESENTATION ON ITS ACTIVITIES WITH SPECIAL ATTENTION TO EPIZON-RELATED TOPICS

E. Erlacher Vindel, G. Brückner

World Organisation for Animal Health, OIE

Summary

The World Organisation for Animal Health (OIE) is an intergovernmental organisation created by an International Agreement of 25 January 1924. It is recognised by the World Trade Organization (WTO) as the reference organisation for international standards for trade in animals and animal products. In April 2007, the OIE totalled 168 Member Countries. The OIE maintains permanent relations with 20 other international and regional organisations and has a Regional Office on every continent.

To ensure transparency in the global animal disease and zoonoses situation, each Member Country has a commitment to report occurrences of OIE-listed animal diseases, including zoonoses, to the OIE at least every 6 months. Significant epidemiological events require urgent notification (within 24 hours). The OIE then disseminates the information to all the other countries to enable them to take the necessary measures to protect their animal disease status. The obligations of countries to notify epidemiological events and the OIE's active search and verification of information and rumours on epidemiological events occurring in Member Countries makes the OIE's Early Warning System very efficient. Annual reports provide further background information on animal health, on laboratory and vaccine production facilities, etc. The WAHID interface provides access to all data held within OIE's new World Animal Health Information System (WAHIS).

Through its renowned world-wide network of experts, mainly in the 21 Collaborating Centres and 161 Reference Laboratories, the OIE collects analyses and publishes the latest scientific information on control methods for animal diseases, including zoonoses. This information helps Member Countries and especially developing countries requiring assistance with the preparation and implementation of animal disease control and eradication programmes. The OIE also actively promotes the improvement of the capacity of the veterinary services of developing and in-transit countries through the Performance, Vision and Strategy (PVS) initiative – a tool for either the self-evaluation or evaluation by OIE-accredited experts of the shortcomings and needs in the delivery of veterinary services in these countries.

To ensure the safety of international trade in animals and animal products, the OIE develops standards that its Member Countries can use to protect themselves from disease incursions while avoiding unjustified sanitary barriers. OIE standards are recognised and referenced within the Sanitary and Phytosanitary Agreement of the WTO. Standards for trade are published annually in the *Terrestrial Animal Health Code* and the *Aquatic Animal Health Code*. Detailed information on diagnostic tests and vaccine production is published in the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* and the *Manual of Diagnostic Tests for Aquatic Animals*. The *Manuals* provide a harmonised approach to disease diagnosis by describing internationally agreed laboratory diagnostic techniques. All standards are science based and are prepared by internationally recognised scientists in the relevant fields.

The *Codes* include a section describing the components of import risk analysis and provides guidelines for conducting risk analyses. The methodology is further elaborated in an OIE Handbook on Import Risk Analysis, which discusses qualitative and quantitative approaches.

In the field of the safety of food of animal origin, the OIE focus is mainly on eliminating hazards existing prior to the slaughter of animals or the primary processing of their products (meat, milk, eggs, etc.) that could be a risk for consumers.

Due to the close relationship between animal health and animal welfare, the OIE promotes animal welfare through a science-based approach and has become, at the request of its Member Countries, the leading international organisation for animal welfare.

All information on the role, activities and publications of the World Organisation for Animal Health (OIE) is available on the Web site at: www.oie.int.

AVIAN INFLUENZA IN WILD WATER FOWL AT THE LAKE OF CONSTANCE; A MULTI NATIONAL STUDY

A. Baumer¹, B. Thür¹, M. Hofmann¹, I. Brunhart², M. Falk², K Stärk³, C. Griot¹

¹*Institute of Virology and Immunoprophylaxis, Mittelhäusern, Switzerland;*

²*Swiss Federal Veterinary Office, Bern, Switzerland;*

³*Royal Veterinary College, London, England*

Key words: Avian Influenza, Wild Birds, Monitoring, Modelling, and infection studies

1. Introduction and Objectives

Wild waterfowl is considered to be the major natural reservoir for avian influenza viruses (AIV). In particular, highly pathogenic avian influenza A (HPAI) subtype H5N1 poses a serious threat to animal health, wildlife conservation, human health and economic welfare in many regions of the world. Migratory birds are suspected of playing a role in the spread of H5N1; however, a review of the literature reveals that much remains unknown about the ecology and epidemiology of H5N1. Lake Constance, bordering Austria, Germany and Switzerland, is an important wetland area with 250 000 wild water birds over wintering annually. HPAI H5N1 was detected in several water birds found dead in the proximity of Lake Constance during the winter season 2006. As a consequence, the Swiss Federal Veterinary Office initiated the research project "Constanze", in collaboration with the German and Austrian authorities. "Constanze" consists of five scientific themes: i) wild bird monitoring for AIV infection, ii) wild water bird migration patterns, iii) pathogenesis of H5N1 in different species, iv) improved AIV diagnostics, and v) risk analysis of AIV transmission from wild water birds to domestic poultry. All themes have a specific focus on Lake Constance. Non-scientific themes are i) structure and management as well as ii) spreading of excellence.

2. Material and methods

Preliminary data related to the theme "wild bird monitoring for AIV infection" will be presented, including the adaptation process of the commercially available ELISAs for serum samples derived from caught wild water fowl such as swans and ducks as well as from sentinel ducks. In addition, the in "Constanze" involved national diagnostic reference laboratories (IVI, Mittelhäusern; AGES, Mödling) participated in an AIV antibody ring test (kindly provided by M Beer, FLI Riems) for harmonization and optimization purposes.

3. Results

Results of the validation process, including the approach taken will be presented. Furthermore, preliminary data of the other "Constanze" themes will also be discussed.

4. Discussion and Conclusions

Since this is a multi-national project with different diagnostic laboratories involved, it was important that commercially available ELISA systems were first standardised (according to ISO Norm 17025) for poultry sera samples in each of the laboratories. The testing strategy of the used ELISAs, which have been exclusively been validated with domestic poultry sera, needed to be modified in order to allow the testing of samples from the different wild waterfowl species. It was found that not all ELISAs were suitable for testing wild waterfowl and that the results were not always conclusive and possible misinterpretation needed to be handled with care.

5. Acknowledgements

The Swiss, Austrian and German federal and local governments fund the study. We would like to thank all involved governmental as well as non-governmental institutions for their generous support and valuable contributions to this research project.

6. References

Baumer A., Thür B., Hofmann M., Bachmann I., Griot C. Monitoring of sentinel and captured wild waterfowl at the Lake of Constance. 66th annual assembly of the SSM, Interlaken, 1-2 March, 2007.

RISK ANALYSIS IN A DYNAMIC ENVIRONMENT – MISSION IMPOSSIBLE?

Katharina D.C. Stärk, Dirk U. Pfeiffer

Royal Veterinary College, Department of Veterinary Clinical Sciences, North Mymms, UK

Key words: Avian influenza, risk assessment, risk management, risk communication

Summary

During the emergence of the highly pathogenic avian influenza virus of Asian lineage H5N1 (HPAI H5N1), numerous risk analyses were conducted in order to estimate the probability of the virus spreading into specific geographic regions or countries and the consequences of this spread. Most risk analyses were conducted according to the standards of the OIE with the risk assessment part including a release assessment and an exposure assessment, followed by a consequence assessment. An informal review of the risk assessments available on the internet showed that the outcome of most assessments conducted between June 2005 and June 2006 with a focus on HPAI H5N1 was expressed in qualitative terms. Most assessments were conducted at the level of national veterinary services. The risk question addressed by the assessments varied considerably; while some were general, others addressed only specific aspects of spread, e.g. the role of wild birds. Due to the dynamic nature of the situation from October 2006 onwards, frequent reviews and adjustments of the assessments were conducted, sometimes at monthly intervals.

In parallel to the national risk assessments, the European Food Safety Authority (EFSA) started a working group on the assessment of the risk related to the introduction of HPAI H5N1 into the European Union via migratory birds in November 2005, following a mandate of the Health and Consumer Protection Directorate General (DG SANCO). The EFSA assessment also followed the OIE standards and results were qualitative. A risk pathway was developed and data were collated, screened and interpreted by the working group members. At the same time, a report was mandated by the Directorate General Environment on bird migration. One objective was to identify so-called "high-risk" species with respect to the introduction of HPAI H5N1. The latter report was expected to provide important input to the EFSA risk assessment. The EFSA opinion was adopted by the Animal Health and Welfare Panel in May 2006 and updated in December 2006 (www.efsa.europa.eu).

During the time of the EFSA assessment, wild birds carrying HPAI H5N1 were found in several European countries. This event challenged the procedures of the working group in several aspects: 1) Working group members were often involved in aspects of avian influenza control or consultancy as part of their regular jobs. This situation may generally cause a shortage of expertise when risk assessments have to be conducted under emergency-type conditions. 2) Real-time surveillance data were not available from Member States and other regions. This type of data is essential for adequate updates of risk assessments that need to reflect a dynamic development. 3) Risk management had to be conducted without the final outcome of the assessment being available. In such a situation, the application of the precautionary principle can be justified. 4) Communication by risk managers had to be conducted without the final outcome of the assessment being available. This procedure may lead to revised messages over time or to contradictory messages due to insufficient communication between experts and risk managers. 5) Finally, the value of a risk assessment may be challenged in a situation where the undesired event of concern has already happened.

Despite these limitations, risk assessment maintains the advantage of a structured, flexible and transparent approach to decision making, even when conducted under severe time and resource constraints and in a dynamic environment.

PATHOGENESIS, PREVENTION AND DIAGNOSIS OF NIPAH VIRUS

H. Weingartl, Y. Berhane, B. Sawatsky, C. Ranadheera, M. Czub

¹*National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Canada*

²*Department of Medical Microbiology, University of Manitoba, Winnipeg, Canada*

³*National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, Canada*

⁴*Faculty of Veterinary Medicine, University of Calgary, Calgary, Alberta, Canada*

Key words: Emerging zoonotic infection, Henipavirus

Summary

Over the past decade, the previously unknown paramyxoviruses Hendra virus (HeV) and Nipah virus (NiV) have emerged in humans and livestock in Australia and Southeast Asia. Both viruses are contagious, highly virulent, and capable of infecting a number of mammalian species and causing potentially fatal disease. Due to the lack of a licensed vaccine or antiviral therapies, HeV and NiV are designated as biosafety level (BSL) 4 agents and are potential bioterrorist agents. The genomic structure of both viruses is that of a typical paramyxovirus. However, due to limited sequence homology and little immunological cross-reactivity with other paramyxoviruses, HeV and NiV have been classified into a new genus within the family Paramyxoviridae named Henipavirus.

TRANSBOUNDARY ANIMAL DISEASES AND THEIR CONTROL STRATEGIES IN CENTRAL ASIAN COUNTRIES

M. Hussain¹, G. Ferrari¹, S. Alexandersen², J. Klein², M. Ahmad¹

¹*Food and Agriculture Organization of the United Nations-Pakistan, NARC, Pakistan;*

²*National Veterinary Institute, Technical University of Denmark, Lindholm, Kalvehave, Denmark*

Key words: FMD, PPR, Rinderpest, epidemiology

Transboundary Animal Diseases (TADs) particularly foot & mouth disease (FMD), peste des petits ruminants (PPR) and Rinderpest are considered important diseases affecting both economies of the countries as well as livelihood of the livestock farmers. Keeping in view their significance, Food & Agriculture Organization (FAO) of the United Nations with the financial assistance of the Government of Italy, has launched a Regional Project, "Controlling Transboundary Animal Diseases in Central Asian Countries". The beneficiary countries are Afghanistan, Pakistan, Tajikistan, Uzbekistan and Turkmenistan. The main objectives are to complete the process of Rinderpest eradication and certification by OIE and to generate data about the occurrence of FMD and PPR leading to develop a Regional strategy for their control. Supported by FAO and EU, Pakistan has already achieved the status of Rinderpest disease and infection free country. According to the guideline provided by the Project, remaining four countries also announced provisionally free from Rinderpest during 2005 and are progressing well to achieve disease free status in 2008. Results of Rinderpest serosurveillance were quite encouraging. Analysis of blood samples collected from 6000 animals in randomly selected 300 villages in each beneficiary country (except Pakistan) further provided strong evidence about the absence of Rinderpest in the Region (<0.1 percent positive by eLISA).

In the absence of an efficient disease reporting system, the concept of participatory disease surveillance (PDS) was introduced in beneficiary countries except Turkmenistan. Results of PDS data collected from about 250 villages from each country indicated that Rinderpest has not been seen in the area for the last about 7 years. Other important diseases reported by the farmers are rabies, brucellosis, pasteurellosis and parasitic infestations. Foot & mouth disease and PPR were considered most devastating diseases of animals in Pakistan, Afghanistan and Tajikistan.

Foot & mouth disease is seriously affecting livestock sector in Pakistan, Afghanistan and Tajikistan. So far, serotypes A, O and Asia 1 have been detected. A collaborative study between the Regional Project, National Veterinary Institute Denmark and FMD-WRL UK has yielded useful information about the presence of new 'A' Iran-2005 and 'O' sub-types in Pakistan. During this experimentation, use of Real Time PCR was explored as an efficient method to determine the prevalence rate of FMD virus infection by analyzing oral swabs of apparently healthy animals. Epidemiological investigations are also carried out to better understand the occurrence of PPR in beneficiary countries.

It is expected that the information collected through various Project activities would help devise an effective strategy for the control of TADs in the Region.

BLUETONGUE: VECTORS, EPIDEMIOLOGY AND CLIMATE-CHANGE

Philip S. Mellor

Arbovirology Department, Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, GU24 0NF, UK

Key words: Bluetongue, *Culicoides*, Vectors, Epidemiology, Climate-change

Summary

The presentation will begin with a brief discussion of those climatic variables that are likely to influence the distribution and incidence of vector-borne diseases such as bluetongue. An explanation of how these variables may induce their own particular effects will be included.

The talk will then move on to describe recent changes in the world distribution of bluetongue virus and its vectors focussing on Europe from 1998 until 2006. It will be argued that the recent changes, both in terms of virus distribution and the species of vectors transmitting the virus can be linked to climate-change.

Suggestions of what this might mean for the future, in a time of on-going climate-change will be set out.

Abstracts work package presentations

- Dr Bernd Hoffmann, work package leader WP4.1
- Dr Áse Uttenthal, work package leader WP4.3
- Dr Emma Snary, work package leader WP7.4

Other presentations will be available on the Central Project Site:
[www.epizone-eu.net\cps](http://www.epizone-eu.net/cps)

PROGRESS OF THE WP 4.1-REAL TIME PCR IN THE LAST YEAR

B. Hoffmann, K. Utke, M. Beer

Friedrich-Loeffler-Institut, Institute of Diagnostic Virology, Greifswald-Insel Riems, Germany

Key words: real-time PCR, questionnaire, ring trial

1. Introduction and Objectives

The aim of the WP4.1 "Real-time PCR" in the EPIZONE project is the comparison of existing real-time PCR systems for the detection of veterinarian important viral pathogens and, if possible, the harmonisation and standardisation of useful real-time RT-PCR protocols. The objective of this work is to establish a platform for the participants to communicate protocols and information for an improving of the daily diagnostic work.

In the first 12 month the participants of the WP should be introduced and the core members should be defined. After selection of the important pathogens a review of the published protocols for the detection of these viruses should be produced by the core group members. Finally, in a first ring trial the standardisation process of real-time PCR assays should be started.

2. Material and methods

Two questionnaires were developed for the collection of data from the participants. In the first questionnaire we collected the general data of the participating institutes and there experience with the real-time PCR techniques. In the second questionnaire specific information about real-time PCR and protocols for the genome detection of the most important viruses were summarised. By the core group members the published real-time PCR assay for the selected viruses were reviewed and combined with a description of the real-time PCR technology in general.

3. Results

Based on the first questionnaire and EPIZONE meetings the participating institutes and the persons responsible were identified. As the currently most important viral pathogens in the veterinarian diagnostics CSFV, AIV, NDV, FMDV and BTV were selected and the comparison of real-time RT-PCR assays for the genome detection of these viruses were agreed. The second questionnaire pointed out, that the one step real-time RT-PCR based on TaqMan primer is the most popular detection system. Strongly differences exist by the enzymatic-chemical basis for the reverse transcription and the amplification. For the publication of a review article about the use of real-time RT-PCR in the veterinarian diagnostics of the most important viruses a contact with a peer review journal was established and the combining of the part of the manuscript is under the way. In a first ring trial in April/May 2007 the different real-time RT-PCR methods of the participants for the genome detection of CSFV should be compared.

4. Discussion and Conclusions

All participants of the WP 4.1 were highly interested on the forwardness of the project. The responses of the persons responsible were prompt in most cases. Helpful was the EPIZONE network in the autumn 2006 during the BTV-8 outbreak in central Europe.

Based on the second questionnaire and the review article a excellent starting point for the harmonisation and standardisation of real-time RT-PCR systems were developed. This information combined with the results of the first ring trial for CSFV assays should allow a first statement in regard to the sensitivity and specificity of the different CSFV genome detection assays.

WP 4.3 DIVA DIAGNOSTICS

Å. Uttenthal,

National Veterinary Institute, Department of Virology, Technical University of Denmark, Lindholm, DK-4771 Kalvehave, Denmark;

Key words: DIVA diagnostics, NSP, FMDV, CSFV, vaccination

1.Introduction and Objectives

This abstract presents the work performed in WP 4.3 DIVA diagnostics. The interests in DIVA diagnostics are increasing, as the "vaccinate to live" principle is more sound both ethically and economically. The culling of millions of healthy animals to prevent spreading of exotic diseases is unacceptable. To keep up the high health status of animals and animal products and to assure that the consumers will not resist marketing of meat from vaccinated animals the DIVA approach is a brilliant tool. The initial aim is to establish a network on DIVA diagnostics for several diseases; this workpackage has participation from the majority of the partners in EPIZONE.

3.Results 4.Discussion and Conclusions

In January 2007 a workshop entitled "Workshop on the design and interpretation of post Foot and mouth disease (FMD) vaccination serosurveillance by NSP tests" was conducted in Belgium with the participation of CVOs and laboratory personnel from countries with cattle and pig dense areas. Twelve countries were included: Spain, Ireland, United Kingdom, Germany, the Netherlands, Belgium, France, Italy, Slovenia, Denmark and Lithuania; representatives from Portugal were also invited but could not participate. A total of 41 participants and an additional 7 observers from OIE, EU, FAO EUFMD, CA-FMD-CSF and EFSA were invited, besides EFSA all other were represented. This joint workshop was supported by EPIZONE, CA-FMD-CSF and FAO EUFMD. During the workshop participants were trained and subsequently worked on 3 scenarios to improve the guidance and decision making in FMD serosurveillance in vaccinated animals. A report on this workshop is now available on the WP website and a scientific publication is considered. Further workshops for South east Europe and north east Europe with less cattle are planned. Extra funding from the non scientific WPs of EPIZONE for these two workshops have been granted to assure dissemination of results.

A first progress meeting or internal workshop was held in Copenhagen March the 26th 2007 with representatives from 11 partners. The minutes from the workshop are uploaded to the internal WP website. There were presentations on the state of the art for CSFV, FMDV, AIV and BTV; the presentations showed a large diversity in topics covered by the partners in the network. Several position papers are recently published and they will be part of the literature review of DIVA diagnostic. The availability of reference materials appears to be limited; for AIV no partners had excess materials whereas for CSFV a few partners had reference materials. Preliminary results on BTV DIVA diagnostics were presented and will be further elaborated. New ideas to be incorporated in the coming 18 months were presented.

Cooperation with SWP 5.1.5. "DIVA strategies for improved vaccines" is essential and has been initiated.

5.Acknowledgements

This workpackage have participation of 14 partners, the enthusiasm of the participants is invaluable for the progress! We are also grateful to the Coordinator for advice in the process of applying for support from the non-scientific work packages for dissemination of results.

6.References

IMPACT OF ENVIRONMENTAL EFFECTS ON THE RISK OF THE OCCURRENCE OF EPIZOOTIC DISEASES IN EUROPE: IDENTIFICATION AND PRIORITISATION

E. L. Snary¹, P. Gale¹ & EPIZONE WP7.4 participants

¹*Centre for Epidemiology & Risk Analysis, Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15
3NB, UK*

Key words: vectors, climate change, viruses, livestock, risk assessment

1.Introduction and Objectives

The introduction and spread of epizootic disease to the EU livestock population has the potential for large veterinary, economic and possibly human consequences. Epizone workpackage 7.4 focuses on the effect that climate change will have on the risk of introduction, establishment and consequence of new and emerging livestock viruses into Europe and, in particular, those that are vector-borne.

2.Material and methods

To assess the risk of introduction, establishment and consequence of new and emerging vector-borne disease due to climate change, a hazard identification is being undertaken. Each WP participant is inputting to this phase of the project using a prepared framework. The framework includes a review of climate change predictions for Europe and then for different vectors (midges, mosquitoes, ticks and biting flies) a number of sections that will lead to conclusions on the risk of introduction, establishment and consequence of a vector-borne virus in the EU, for the different predictions of climate change. The sections in the hazard identification, for each vector, are (i) Identification of epizootic viruses that could be spread by vector in EU; (ii) Impact of climate change in Europe on the vector population (range, establishment, behaviour and abundance); (iii) Possible routes of infected vector introduction into Europe which may act synergistically with climate change; (iv) Identification of factors (climatic or other) that affect susceptibility of a vector to replication of the virus, or to maintaining infection in the vector and (v) Host reservoir and immune status of host.

3.Results

Workpackage 7.4 will be ongoing at the time of the annual meeting and available results from the hazard identification will be presented. In particular, we will concentrate on information gained about climate change in Europe and its likely impact on the introduction, establishment and consequence of Bluetongue virus. This will demonstrate the approach to be taken for the other vector/virus combinations that are being considered in the Hazard Identification.

4.Discussion and Conclusions

Hazard identification can be used to identify both current and long-term risks. It is an identification stage of an early warning system. Using this methodology new and emerging viruses, which have the potential to enter and persist within the EU due to climate change, will be identified along with their possible routes of introduction. In addition, by using the framework discussed above we will also be able to comment on the relative likelihood of such viruses being introduced and to establish and also the consequences of such an event.

5.Acknowledgments

This project is funded by the EU Network of Excellence, EPIZONE.

Abstracts free communications: Theme 4 Diagnostics

- Dr Piet van Rijn
- Dr Jovita Fernández
- Dr Juliet Dukes
- Astrid Gall
- Dr Kris de Clercq
- Dr Marek Slomka

HIGH THROUGHPUT RRT-PCR DIAGNOSTICS FOR BLUETONGUE BASED ON GENOME SEGMENT 10

P. van Rijn, R. Heutink, R. van Gennip

Central Institute for Animal Disease Control, CIDC, Lelystad, The Netherlands

Keywords: Bluetongue, high throughput, PCR-diagnostics

A highly automated one-step real time reverse transcription polymerase chain reaction (RRT-PCR) protocol based on segment 10 was developed, optimized and extensively validated for the routine diagnosis for Bluetongue (BT). In this RRT-PCR assay many advantages are combined, such as robotized isolation, a closed system, and speed. The feasibility for application in BT-outbreaks is demonstrated as animals were detected as early as two days post inoculation, and earlier than serology or clinical signs. The RRT-PCR assay detects members of all 24 serotypes of BT-virus (BTV), viruses of different time of isolation and from different geographic origin. Genetically related viruses such as members of all 9 serotypes of African horse sickness viruses and several Epizootic hemorrhagic disease viruses are not detected. So, the RRT-PCR assay is highly sensitive and very specific. Performance of this RRT-PCR assay in ring trials has shown a diagnostic sensitivity at least equivalent to other RRT-PCR assays. This RRT-PCR assay has played a key role in the rapid diagnosis of the new and so far unknown variant of BTV-8. During the BT-outbreak in the Netherlands in 2006, the RRT-PCR assay has proven very reliable and robust for samples from cattle, sheep, and goats. The diagnostic sensitivity and diagnostic specificity in these three species are above 90% as compared to serology with a commercial ELISA.

A TAQMAN-MGB REAL-TIME RT-PCR ASSAY FOR THE UNIVERSAL DETECTION OF AFRICAN HORSE SICKNESS VIRUS (AHSV)

J. Fernández¹, E. Sotelo¹, P. Fernández-Pacheco¹, B. Rodríguez², A. Robles¹, M. Arias¹, J.M. Sánchez-Vizcaíno²

¹OIE Reference Laboratory for African Horse Sickness, CISA-INIA, Valdeolmos, Madrid, Spain;

²Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Spain

Key words: Real-time RT-PCR, AHSV detection

1. Introduction and Objectives

African Horse Sickness (AHS) is a non-contagious arthropod-borne infectious disease that affects equidae, caused by a double-stranded RNA orbivirus. It is a notifiable disease, and it is endemic in many African countries. Different conventional PCR methods have been already described for the AHS Virus (AHSV) detection^{1, 2, 3, 4, 5}. In this work, the development of a *TaqMan-MGB* real-time RT-PCR assay for the universal detection of AHSV is described.

2. Material and methods

The nine serotypes of AHSV were used in the study. For specificity assays, a collection of spleen homogenates from horses proceeding from Spanish AHS outbreaks (1987-1990), as well as Bluetongue Virus (BTV), Vesicular Stomatitis Virus (VSV), West Nile Virus (WNV), and Vero cell line culture were also employed. Total RNA was extracted from samples using commercial *High Pure Viral Nucleic Acid Kit*, following manufacturer's instructions (*Roche*).

A specific primer set and a *TaqMan-MGB* probe were designed from conserved regions of the VP7 viral gene using the *Primer Express™ 2.0 program* (*Applied Biosystems*) to amplify the different viral serotypes, delimiting an amplicon of 102 bp.

QuantiTect Probe RT-PCR kit (*Qiagen*) was used for AHSV nucleic acid amplification in a single-step protocol, that was adapted for its use both in capillary (*LightCycler, Roche*) and 96-well plate (*MX3005, Stratagene*) formats.

3. Results

The sensitivity of the developed real-time RT-PCR was determined using three replicates of 10-fold dilutions of a viral suspension of AHS serotype 4 Spain 1989 isolate (1.26×10^6 TCID₅₀/ml), grown in Vero cell line culture. Results showed that 0.01 TCID₅₀ can be detected using both capillary and 96-well plate thermal cyclers. A standard curve was constructed based on the observed Ct values for each viral dilution, showing a linear relationship of six orders of magnitude. Serotypes 1,2,3,5,6,7,8, and 9 were then analysed, obtaining a similar detection limit in all of them.

Specificity of the novel real-time RT-PCR was further evaluated using a collection of spleen homogenates from field AHSV infected and non-infected horses, VSV, BTV, WNV, and non infected Vero cell line. Only fluorescence signal was observed in AHSV positive field samples.

4. Discussion and Conclusions

Although several RT-PCR methods have been already reported for the AHSV detection, all of them are gel-based assays^{1, 2, 3, 4, 5}. This work describes for the first time a real-time RT-PCR method for the molecular diagnosis of AHS, that employes an specific primer set and a *TaqMan-MGB* probe. The completed studies prove that the real-time RT-PCR is a highly sensitive and specific method to detect and quantify any serotype of AHSV, and the definitive results can be ready in less than four hours. Furthermore, the positive results obtained with infected clinical material showed the presented real-time RT-PCR assay as a useful tool for the detection of AHSV in clinical samples to be applied in diagnostic laboratories.

5. Acknowledgements

This work has been funded by INIA project OT01-002 and EU project SSPE-CT2004-513645.

6. References

1. Díaz-Laviada, M; Sánchez-Vizcaíno, JM; Roy, P; Sobrino, F. (1997) *Invest. Agr. SA.*, 12: 97-102;
2. Koekemoer, JJ; Dijk, AA. (2004) *J Virol Methods.*, 122 (1): 49-56;
3. Sailleau, C; Hamblin, C; Paweska, JT; Zientara S. (2000) *J Gen Virol.*, 81 (Pt 3): 831-837;
4. Stone-Marschat, M; Carville, A; Skowronek, A; Laegreid, WW. (1994) *J Clin Microbiol.*, 32: 697-700;
5. Zientara, S; Sailleau, C; Moulay, S; Cruciere, C. (1994) *J Virol Methods.*, 46 (2): 179-188.

DEVELOPMENT OF A MICROARRAY FOR CHARACTERISATION OF VETERINARY VIRUSES

J. Dukes¹, A. Abu-Median², M. Watson², R. Card³, M. Banks³, P. Britton², D. King¹

¹*Institute for Animal Health, Pirbright Laboratory, Woking, Surrey, United Kingdom;*

²*Institute for Animal Health, Compton Laboratory, Newbury, Berkshire, United Kingdom;*

³*Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, United Kingdom*

Key words: Virus; characterisation; detection; micro array

1. Introduction and Objectives

Unambiguous viral identification with degenerate PCR is often complicated by the existence of highly homologous relatives. The capacity of micro arrays to perform numerous assays on the same sample increases experimental range by many orders of magnitude. Microarrays enable both broad-spectrum detection, and finer resolution fingerprinting. A second-generation micro array consisting of probes covering 290 viral 'species' has been developed. The array is being validated to serotype and subtype veterinary viruses using Foot and Mouth Disease Virus (FMDV) and Infectious Bronchitis Coronavirus (IBV) as models to optimise protocols for probe design, sample labelling, hybridisation and analysis.

2. Material and methods

Sequence data available both in the public domain and generated in-house was used as the basis to design 70 base oligonucleotide probes using publicly available freeware. Oligos were synthesised commercially (Operon) and spotted using a previously published protocol¹. Total RNA was extracted from cell-cultured FMDV and IBV and from epithelium of sheep 2 days post infection with FMDV, reverse transcribed, amplified and labelled with fluorophore and hybridised¹. Slides were scanned using GenePix[®] scanners (Axon/Molecular Devices) and fluorescence was quantified using GenePix[®] Pro 6.0 software. Data were normalised to total fluorescence and median signal: noise ratios were calculated for each hybridisation. Statistical analysis of data was carried out using T-tests within defined groups of oligos, using 'DetectiV' software developed in-house.

3. Results

Visual inspection of scanned images showed IBV-specific, pan-coronavirus and FMDV-serotype specific spots fluorescing when cell-culture viruses of the respective types were hybridised. Analysis revealed different hybridisation 'profiles' belonging to serotype A and serotype O of FMDV, which was supported by statistical analysis. Two FMDV viruses belonging to different topotypes also showed different hybridisation 'profiles'. Hybridisations of RNA isolated from clinical samples (FMDV) also showed statistical differences. Similarly, cell culture infection with IBV was also detected by the array.

4. Discussion and Conclusions

This first-generation microarray is capable of discriminating different viruses (IBV & FMDV). Furthermore, different serotypes of FMDV and different topotypes within a serotype also give different 'signatures'. Viral signatures are also detectable in 'real' samples from infected animals, and normalisation to an uninfected control reduces the majority of noise. A third-generation array has now been printed to expand the coverage to 308 viral species from 35 virus families. Current work at Pirbright is focused on validating this array and determining the resolution that can be achieved using further topotypes of FMDV, and related picornaviruses.

5. Acknowledgements

The authors would like to thank Geoff Hutchings, Nick Knowles, Jacquelyn Horsington, Nigel Silman, Karen Kempself, Jane Burton, James Oshota, David Stone and Neil Boonham. Funded in part by Defra projects SE1120, SD0443, FT5901 and SE4102.

6. References

1. Burton, J.E., Oshota, O.J., North, E., Hudson, M.J., Polyanskaya, N., Brehm, J., Lloyd G. and Silman, N.J. *Development of a multipathogen oligonucleotide microarray for detection of Bacillus anthracis Molecular and Cellular Probes* 19 (2005) 349–357.

TOWARDS MICROARRAY TECHNOLOGY FOR INFLUENZAVIRUS DIAGNOSTICS

A. Gall, B. Hoffmann, M. Beer

Friedrich-Loeffler-Institut, Institute of Diagnostic Virology, Greifswald-Insel Riems, Germany

Key words: Influenzavirus, microarray, diagnostics

Continuing outbreaks of Influenzavirus infections, especially the current epidemic of highly pathogenic avian influenza (HPAI) H5N1, indicate the urgent need for new diagnostic methods to accomplish a comprehensive diagnosis within a less time-consuming process. The most important objectives at present are a) identification of Influenza A virus, b) subtyping of Avian Influenza Viruses (AIV) on the basis of the haemagglutinin (H 1-16) and neuraminidase (N 1-9) and c) detection of highly pathogenic and low pathogenic H5 and H7 isolates. Low density microarray technology could accelerate molecular analyses by simultaneous detection of multiple genes and thus enable differentiated ad hoc-diagnostics.

The aim of this work is to develop a diagnostic microarray for AIV which addresses the main problems of Influenzavirus diagnostics. Initially, the real-time RT-PCR assay described by Spackman et al. (2002) for the detection of the M-gene was transferred to the NanoChip® 400 System (Nanogen) and validated for identification of Influenza A virus utilizing a panel of 82 isolates. A panHA RT-PCR amplifying an 176 bp (in case of HPAIV) fragment which encompasses the haemagglutinin cleavage site was established. About 90 % of Influenza A virus isolates from the tested panel scored positive with the current protocol. The development of a panNA RT-PCR yielding in a product of ~ 130 bp is in progress. The panHA and panNA RT-PCR products are to be differentiated by subtype specific capture probes on the microarray. Until today, probes for the subtypes H1 and H3 to H12 were established and are in validation. Furthermore, the sensitive detection of the HPAIV H5N1 circulating in Germany in 2006 can be ensured using a specific probe. Although the recent low density microarrays have limitations, this novel technology will become a future trend for faster and less expensive Influenzavirus diagnostics as well as molecular characterization.

WORKSHOP ON THE DESIGN AND INTERPRETATION OF POST FOOT-AND-MOUTH DISEASE (FMD)-VACCINATION SEROSURVEILLANCE BY NSP TESTS; PART I (DENSE CATTLE-PIG COUNTRIES)

K. De Clercq¹, K. Luyten¹, J. Westergaard²

¹ *Veterinary and Agrochemical Research Centre, Ukkel, Belgium;*

² *adc-consult, Horsholm, Denmark*

Key words: FMD-NSP, vaccinate-to-live

1.Introduction and Objectives

Given the current FMD-free status without vaccination in Europe and the possibility of a future outbreak with vaccinate-to-live used as an emergency measure, a workshop was organised which concentrated on: (1)the design and implementation of a survey to substantiate freedom from infection with a certain degree of confidence after vaccination, (2)the guidance to interpretation on the follow-up of seropositive animals/herds/flocks, (3)the guidance to use of laboratory test results in decision-making and (4)the identification of resources (laboratory, veterinarians) required.

2.Material and methods

Twelve invited countries and observers of OIE, EUFMD and EC were divided into 6 groups, each group having to (1)make the best possible survey design for a given scenario, (2)follow-up the seropositive herds/animals/flocks and (3)identify the necessary resources.

3.Results

Results were presented by each group in a plenary session. Despite the use of different working groups/countries, the approaches taken showed a clear degree of similarity.

4.Discussion and Conclusions

(1)The vaccinate-to-live policy with substantiating freedom from infection by a survey system including NSP testing is a realistic and achievable option in FMD control; (2)Because NSP assays are not sensitive enough (carriers), conclusions on infection status of vaccinated herds can only be based on a combination of clinical and serological surveys and epidemiological investigations; (3)Proving freedom from infection for vaccinated animals is impossible, in contrast to substantiating freedom from virus circulation or freedom from infection in non-vaccinated animals; (4)The current EU Directive (2003/85/EC) for FMD control mentions a survey for detecting the presence of FMD virus in the vaccination zone (Article 56) and a survey to regain freedom from infection after emergency vaccination (Article 61), where both articles should be revised; (5)The follow-up of herds with seroreactors by serological investigation has to be based on NSP assays with well-defined performance characteristics; (6)If specificity of the serological test system were known, only seroreactor rates above the Herd Cut Point could be considered, which is not in line with the EU directive; (7)A clinical surveillance combined with paired serology can detect holdings where virus circulation is ongoing, but all ruminants should be tested; (8)Testing all animals in a vaccinated population is not achievable in areas with a dense pig population or within big pig herds; (9)Vaccination of small herds remains controversial - 2 options were discussed.

5.Acknowledgements

Epizone, FMD-CSF co-ordination action, FAO EUFMD, FMD Improcon, A. Füssel, I. Minguéz, G. Bruckner, P. de Leeuw, K. Sumtison, T. Murray, D. Paton, A. Dekker, Y. Leforban, J. Westergaard, P. Houdart, K. Mintiens, P. Willeberg, C. Brasch-Andersen, W. Pelgrim, N. Goris, M. Greiner, P. Van Hoeyweghen, P. Kerkhofs.

6.References

1. Paton et al.. *Vaccine* 24 (2006) 6503–6512.

EVALUATION OF LATERAL FLOW DEVICES FOR RAPID PENSIDE DIAGNOSIS OF AVIAN INFLUENZA: A NEW STRATEGY FROM FLUAID

M. Slomka, J. Banks, L. Kerr, T. Pavlidis, V. Coward, I. Brown

Veterinary Laboratories Agency, VLA-Weybridge, Addlestone, United Kingdom

Key words: Avian influenza (AI), lateral flow device (LFD)

1. Introduction & Objectives

Recent & ongoing EU projects (*eg* AVIFLU, FLUAID & FLUTEST) have been developing & evaluating a variety of approaches to the accurate & harmonised diagnosis of avian influenza virus (AIV). AVIFLU delivered AI monoclonal antibodies (Mabs) which have been utilised within FLUAID for development of a rapid AI lateral flow device (LFD).

The goal is to demonstrate that the AI LFD is “fit for purpose” for rapid penside detection of AI in infected poultry. This study has (i) generated LFD evaluation data & (ii) critically examined the approaches which were employed. These outcomes will be discussed & agreed by the FLUAID consortium to plan a targeted & broader LFD assessment focusing on clinical specimens. Both EU & non-EU laboratories will be welcome to participate. This may serve as a template for future evaluations of other LFDs & / or molecular penside tests for a variety of veterinary pathogens.

2. Materials & methods

AI LFD: Produced by Forsite, a commercial partner within FLUAID. Design specification of the AI LFD was guided by interaction with VLA who provided reagents *ie* AI antigens & Mabs (from AVIFLU) plus AI “know how”, resulting in an assay specification chosen for manufacturing.

Other commercial rapid devices / LFDs: These include six existing commercial products from five companies, namely Becton Dickinson, Quidel, Synbiotics, Binax, & Anigen.

Reconstituted cloacal swabs: Prepared by spiking dilutions of inactivated AIV into chicken faeces & mixing, followed by dipping swabs into the mix. Swabs were extracted into the LFD extraction buffer, followed by LFD testing according to the manufacturer’s instructions. *Clinical specimens from infected poultry:* Swabs (buccal/tracheal & cloacal) were collected from poultry (i) infected during a recent H5N1 highly pathogenic (HP) AI outbreak in the UK (February 2007) & (ii) experimentally-infected with A/ostrich/Italy/99 H7N1 HPAI.

AI RealTime PCR: Used to test LFD extraction buffers to measure AIV titre.

3. Results

Sensitivity: Detection limits of different LFDs will be presented. Clinical sensitivity of *ca* 10^4 EID₅₀/ml suggests that AI LFDs may be restricted to diagnosing HPAI infections in poultry.

Specificity: Certain LFDs generate false positives with faecal-containing matter.

Limitations: Possible limitations for AI LFDs were considered. Includes infections (i) with low pathogenicity (LP) AI in poultry & (ii) in vaccinated poultry.

4. Discussion & Conclusions

- Importance of immediate testing of dry swabs containing clinical material.
- Identification of “flock sensitivity” as key criterion for use of AI LFDs, as opposed to clinical sensitivity in individual birds. Recognition of problems in assessing LFDs, limitations of simple evaluations which may not follow manufacturers’ protocols.
- Imperative to plan larger-scale evaluation of AI LFD which both (i) follows the manufacturer’s protocol and (ii) includes parallel AI testing by OIE/EU approved methods.
- New FLUAID LFD evaluation protocol will conduct a greater range of studies in poultry infected (i) experimentally & (ii) in outbreaks.
- Invitation for EU & non-EU labs to participate, so providing a larger number of clinical specimens for AI LFD assessment both (i) experimentally and (ii) in the field.

5. Acknowledgements

EU FLUAID laboratories for initiating discussion of the new evaluation protocol. Chris Danks & Jonathan Flint are thanked for providing the Forsite AI LFD, and the authors wish to acknowledge the EU for funding.

Abstracts free communications: Theme 5 Intervention strategies

- Dr Alejandro Brun
- Dr Günther Keil
- Dr Gaëlle Kuntz-Simon
- Dr Noemi Sevilla
- Dr Zhi zhong Jing
- Nesya Goris

ANALYSIS OF IMMUNE RESPONSES IN SHEEP FOLLOWING VACCINATION WITH DNA PLASMIDS ENCODING STRUCTURAL RIFT VALLEY FEVER VIRUS PROTEINS

G. Lorenzo¹, R. Martín¹, B. Borrego¹, F. Rodríguez², A. Brun¹

¹Centre for Animal Health Research, CISA, Valdeolmos, Spain;

²CReSA, Barcelona, Spain

Key words: DNA vaccine, RVFV

1. Introduction and Objectives

Rift Valley Fever virus is a mosquito-borne bunyavirus causing a severe disease in livestock species and man, in the form of fatal hemorrhagic fever⁴. The disease is endemic in sub-Saharan countries but northern spread has been proved in the recent years¹. Besides active surveillance and diagnostic control measures, vaccination has proven useful for ameliorating the spread of the disease after an outbreak⁵. Current available vaccines should be improved to avoid frequent deleterious side effects in cattle (abortions, teratogenicity in pregnant animals, etc)². The aim of this work was to evaluate the immune response elicited after immunization of sheep with different DNA vaccines encoding both viral segment M and/or the viral nucleoprotein N.

2. Material and methods

DNA fragments encoding the viral M segment (NsmG2/G1), and N ORFs were cloned into pCMV (Invitrogen) vectors. Expression was checked by western-blot, immunodot and immunocytochemistry using BHK-21 transfected cell extracts. Eight sheep were inoculated with the plasmid constructs mixed using by two different routes intradermal (ear) and intramuscular (hind limbs). Sheep received a total of three vaccine doses each one separated from the other by three weeks. Seroconversion was monitored by immunoprecipitation and/or ELISA assays. 5 months after the last DNA immunization the sheep received a single dose (10⁵ pfu) of the MP12 mutagenized strain³. RVFV Specific antibodies from sheep sera were analyzed by a virus capture ELISA and subjected to PRN tests. After stimulation IFN- γ and cellular responses, were measured by ELISA and [³H]-thymidine incorporation, respectively

3. Results

DNA vaccines encoding the nucleoprotein N were able to induce a detectable antibody response as after two rounds of immunization. In contrast no antibody response could be detected after immunization of DNA with the M construct. Although certain levels of IFN- γ were detected in some of the M vaccinated animals no cellular responses were observed in lymphoproliferation assays. However after boosting with the attenuated strain MP12, effector responses were induced earlier in the M vaccinated group when compared with controls indicative of vaccine priming. Interestingly, sheep vaccinated with the combination of DNA vaccines M+N showed higher levels of both antibody and cellular responses as measured by ELISA and lymphoproliferation assays, respectively.

4. Discussion and Conclusions

Altogether our data show that i) antibody responses can be efficiently induced against the viral nucleoprotein N by means of DNA vaccination and ii) a plasmid cocktail encoding the RVFV nucleoprotein and glycoproteins prime animals for an accelerated response upon attenuated RVFV boosting. Therefore, these results open new research venues to explore and improve mechanisms able to induce protective immunity in sheep against RVFV

6. References

- 1.-Ahmad, K. (2000). *Lancet* 356, 1422
- 2.-Botros et al (2006) *J Med Virol.* 75, 787-91
- 3.-Flick and Bouloy 2005. *Curr Mol Med.* 5, 827-34
- 4.-Caplen et al. (1985). *J Gen. Virol.* 66, 2271-7
- 5.-Morrill, J. C. and Peters, C. J. (2003) *Vaccine* 21, 2994-3002

RECOMBINATION VECTORS FOR GENE TRANSFER INTO VERTEBRATE CELLS BY BACULOVIRUSES.

G. Keil, C. Höhle, K. Vogt and K. Giesow

Friedrich-Loeffler-Institut, Institute of Molecular Biology, Insel Riems, Germany

Key words: Baculovirus, gene delivery

1. Introduction and objectives

Transient expression of proteins is frequently used to characterize biological functions of viral proteins involved e. g. in virus replication, morphogenesis or pathogenesis or to analyze their influence on cellular signalling cascades involved in inhibition or induction of apoptosis, in modulation of innate immune responses etc. Gene transfer into target cells is frequently performed by chemical transfection or electroporation of plasmid DNA. In the last years also several replication-defective virus systems have been developed for gene delivery which, however, often need transfection of plasmids into producer cell lines or helper virus infection. Gene transfer into vertebrate cells by recombinant baculoviruses, described already in 1985, is still rarely used although this technique yields high expression levels in close to 100% of exposed cells. In addition, cell lines which are largely resistant to chemical transfection or electroporation may be efficiently transduced. One reason for the hesitant application of the so called MamBac technology might be the laborious procedure for isolation of the recombinants especially when working with single plaque isolates is envisaged. The objective of this work was to construct transfer vectors to facilitate the procedure for isolation of recombinant baculoviruses and which enable efficacious transgene expression.

2. Materials and Methods

All cloning procedures followed established methods. The Bac-to-Bac System was used for the generation of recombinant baculoviruses. Transduction efficiency in vertebrate cells was monitored using green fluorescent protein (GFP)-expression.

3. Results

A series of pFastBacDual-based transfer plasmids was constructed which contain a polyhedrin promoter directed GFP expression cassette to monitor baculovirus replication in insect cells and 1.) the human CMV major immediate early promoter, the murine CMV ie1/ie2 dual promoter element for double expression and the murine CMV e1 promoter for inducible expression. Recombinant baculoviruses containing expression cassettes for the US3 protein kinases of pseudorabies virus and bovine herpesvirus 1, the P1-2A polyprotein and the 3C protease of foot and mouth disease virus, the hemagglutinin of avian influenza virus H5N1 and the G protein of viral haemorrhagic septicaemia virus. Expression of the respective proteins after transduction of a number of mammalian, avian and piscine cells was verified by indirect immunofluorescence, immunoblotting and FACS-analyses.

4. Discussion and conclusions

From the analyses done so far, our new transfer vectors facilitate isolation and plaque purification of recombinant baculoviruses which will be applicable for development of novel vaccines, elucidation of biological functions of viral proteins, analyses of immune responses, production of immunostimulatory proteins and testing of the mode of action of antivirals.

CLASSICAL SWINE FEVER VIRUS INDUCES ACTIVATION OF PLASMACYTOID AND CONVENTIONAL DENDRITIC CELLS IN BLOOD AND SECONDARY LYMPHOID ORGANS OF INFECTED PIGS

A. Jamin¹, S. Gorin¹, R. Cariolet², M. Le Potier¹, G. Kuntz-Simon¹

¹AFSSA-LERAPP, Swine Virology Immunology Unit, Ploufragan, France

²AFSSA-LERAPP, Section of Healthy Pig Production and Experimentation, Ploufragan, France

Key words: Classical swine fever virus, dendritic cells, alpha interferon, tonsil, spleen, blood

1. Introduction and objectives

Classical swine fever virus (CSFV) causes indirect lymphopenia and disrupts *in vitro* T-cell stimulation capacity. It can efficiently replicate in monocyte-derived dendritic cells (DC) and blood-isolated natural interferon producing cells (NIPC) without interfering with their immune reactivity (Carrasco et al., 2004; Balmelli et al., 2005) but no evidence has been found of a role of myeloid cells in the immunosuppression. Intending to better understand interactions between CSFV and DC in infected pigs, we investigated the activation of conventional DC (cDC) and plasmacytoid DC (pDC) in secondary lymphoid organs and blood in the early time course post-inoculation (pi), together with viral components dissemination and cytokine production in serum.

2. Material and methods

Antibodies: Anti-porcine CD1a, CD4, CD21 and CD172a were from SBA, anti-CD11R1 from Serotec, anti-human CD80/86 from Ancell, anti-porcine IFN- α , TNF- α and IL-12 from R&D, anti-IL-10 from Biosource. Secondary antibodies were from Dako, anti-E2 from Euroclone. Anti-NS3 was provided by Dr. Greiser-Wilke.

Animal infections: Twenty-four 7-week-old SPF pigs were infected by oronasal route with 10^6 TCID₅₀ of Eystrup strain (provided by Dr. Summerfield, Mittelhausern) and slaughtered in batches of 3 or 6 at 16, 24, 48, 72, 96 and 168 h pi (hpi). 10 control and 8 mock-inoculated pigs were included in assay. Blood, tonsils and spleens were collected at euthanasia.

Laboratory investigations: Fluorescence immunohistochemistry, flow cytometry analyses and measurement of cytokines in serum were performed as previously reported (Jamin et al., 2006).

3. Results

CSFV first replicated in tonsil T-cell areas and rapidly disseminated in blood system, reaching the spleen between 24 and 48 hpi. CSFV E2 antigen located in T cell areas within CD172a⁺ myeloid cells and IFN- α ⁺ cells, that could be DCs. Intriguing was the translocation of E2, but not viral particles, into germinal centres at 48 hpi. E2 did not locate within B lymphocytes there, but CD21⁺E2⁺ cells were detected in T areas at 72 hpi revealing B cell activation. CD172a⁺CD11R1⁺ cDC and CD172a⁺CD4⁺ pDC frequencies slightly decreased in tonsil between 16 and 24 hpi, while both DC numbers markedly diminished in spleen, whereas transiently for pDC. In blood, only cDC significantly decreased. Despite disappearance, cDC and pDC maturation and/or activation were demonstrated in blood and organs by down-regulation of CD1a, up-regulation of CD80/86 and increase in various cytokine expression. cDC essentially expressed TNF- α and IL-10, whereas pDC produced IFN- α and IL-12. High levels of IFN- α and TNF- α were measured in serum pi. IL-12 remained constant but IL-10, IL-18 and IFN- γ were transiently detected.

4. Discussion and conclusions

CSFV infection induced DC activation in blood and secondary lymphoid organ T-cell areas during the first hours pi, leading to initiation of innate immune responses through IFN- α and TNF- α production. IL-10 and IL-12 expression in spleen participated in induction of humoral and cellular responses, respectively. However, the uncommonly high levels of IFN- α and TNF- α would play a role in disruption of immune system cells, either inducing apoptosis, and/or impairing DC maturation and T-cell priming.

5.Acknowledgements

We thank all colleagues from AFSSA-LERAPP (Ploufragan) who participated in pig routine bleeding, slaughtering and organ sampling.

6.References

Balmelli et al. (2005) Eur J Immunol 35, 2406-15.

Carrasco et al. (2004) J Gen Virol 85, 1633-41.

Jamin et al. (2006) Vet Immunol Immunopathol 114, 224-37.

INTERACTION OF FOOT-AND-MOUTH DISEASE VIRUS (FMDV) WITH DENDRITIC CELLS

T. Rodríguez-Calvo¹, M. Sanz-Ramos^{1,2}, F. Díaz-San Segundo³, and N. Sevilla¹

¹Centre for Animal Health Research, CISA-INIA, Madrid, Spain;

²Centro de Biología Molecular Severo Ochoa, Cantoblanco, Madrid, Spain; ³Plum Island Animal Disease Centre, ARS, USDA, Greenport, USA

Key words: FMDV pathogenesis, dendritic cells, antigen presentation

1. Introduction and Objectives

Foot-and-mouth Disease Virus (FMDV) is the causative agent of a highly contagious vesicular disease of cloven-hoofed animals. We have previously described that FMDV serotype C infects lymphocytes *in vivo*, causing a profound lymphoid depletion (1). The animals are immunosuppressed during at least 14 days after inoculation. Dendritic cells (DCs) play a pivotal role in bringing forth innate and adaptive immune responses. We have studied the interaction between DCs and T cells during FMDV infection in swine to better understand the different parameters that drive immunosuppression in pigs infected with FMDV.

2. Material and methods

Animal, virus and experimental design. 16 Large White x Landrace pig female of 9 weeks old were used for this study. Animals were inoculated by intradermal route in the coronary band with 10⁵ PFUs of FMDV C-S8c1. FMDV C-S8c1 is a plaque-purified derivative of natural isolate C1-Sta Pau-Spain 70, a representative of the European subtype C₁ FMDV (2).

Generation of porcine DCs. Monocyte-derived DCs were generated as previously described (3). The expression of SLA and co-stimulatory molecules was detected by flow cytometry.

Mix lymphocyte reaction assay. T cell purification was done by SWC3⁺ cells depletion using MACS. T cells and DCs were co-cultured at 2 x 10⁵ T cells/well with increasing amounts of DCs. After 5 days 1 μCi [³H]thymidine was added to quantify T cell proliferation.

3. Results

In the present study we have found that FMDV infects DCs *in vitro* and interferes with their maturation. More interestingly, we have found that DCs isolated *ex vivo* from FMDV infected pigs have lost their maturation and antigen presenting capacity as evidenced by a significant reduction in the surface expression of SLA class II and CD80/86 molecules. The formation of immunological synapses between DCs and T cells from infected pigs was also diminished.

4. Discussion and Conclusions

DCs play a central role in initiating and directing the nature of adaptive antigen-specific T-cell responses against a pathogen. Functional disruption of DCs is an important strategy for virus to evade host defences. Our data suggest that FMDV cause immunosuppression partially due to the interference with DC function. This may allow the virus a window-time in which the virus replicates and spread. Surprisingly, although the virus infects DCs *in vitro*, we cannot detect viral infection *in vivo*. One may speculate that high viraemia *per se* may enhance the impairment of antigen-presenting cells. This may be due to a bystander effect mediated by viral antigen. We are currently investigate this hypothesis.

5. Acknowledgements

This work was supported by grants AGL2004-00499 and BMC2001-1823-C02-01.

6. References

- (1) *J. Virol.* (2006) **80**: 2369.
- (2) *Virology* (1983) **128**: 310.
- (3) *Immunology* (2001) **104**: 175.

ADJUVANT EFFECTS OF RECOMBINANT PLASMIDS OF CPG AND PORCINE CYTOKINES TO FMD VACCINE IN VIVO

Z. Jing, Y. Dou, X. Meng, G. Chen, X. Cai

State Key Laboratory of Veterinary Etiological Biology; Lanzhou Veterinary Research Institute, CAAS; Lanzhou, Gansu, China

Key words: CpG motif, Cytokine, Recombinant plasmid, FMD

1. Introduction and Objectives

Foot-and-mouth disease (FMD) is a highly contagious disease in cloven-hoofed animals and can cause a considerable socio-economic loss for affected countries. Up to date, the vaccination with inactivated FMDV is a major means to prevent and control this disease in most developing countries. However, the killed-vaccine have a serial side effect and stimulates limited immune responses. Though newly developed genetic engineered vaccines have become alternatives characterized by the advantages of being safer, its poorly immune responses to inoculated animal. Therefore, it is necessary to develop various immune adjuvants to enhance immune effects of vaccines. Recently, the concept of molecular adjuvant has been expanded to include the CpG motif and cytokine in newly vaccine design strategies. The objective of this work is investigated the immune modulation effects of recombinant plasmid of CpG or cytokines to FMD vaccine in vivo.

2. Material and methods

CpG motif and porcine cytokine of IL-4, IL-6 and IFN- γ gene was cloned and selected firstly, and subsequently constructed its recombinant plasmids in expressing or non-expressing vectors for mammalian cell. Of these recombinant plasmids, as molecular adjuvant, was carried out animal test with FMDV antigen or commercial killed vaccine in mice and pigs, respectively. The immune effects have been evaluated by detecting specific antibodies with blocking-liquid ELISA, secreting cytokine of the mouse IL-4 and IFN- γ by indirect ELISA, and immunity protective effect by FMDV challenging.

3. Results

Effect of CpG DNA: Using CpG DNA as an adjuvant of commercial 'O' type FMDV killed vaccine co-ministration to pigs, the results showed that CpG DNA group induce high antibody titer of 1:134 on 45 d after inoculation, is over 4 folds of commercial vaccine alone. Then using it with 'O' type FMDV purified antigen co-ministrated to pigs, the results showed that CpG DNA group could induce high antibodies titer and immunity protective effect than purified antigen vaccine alone. Immune protective rate of CpG DNA group is 100% and 75% in 1/3 and 1/9 dosage of vaccine, respectively, the PD50 value arrives above 13.00 is far high the control group of vaccine (PD50 value only 4.69).

Effect of Cytokines: Recombinant plasmids of IL-4, IL-6 and IFN- γ with 'O' type FMDV killed commercial vaccine co-ministrated 3 times to mice respectively, the results show that these cytokines all could enhance the level of immune response, among of IL-4 and IFN- γ groups both receive markedly high antibodies titer of 1:300 on day 90 of the first immunization, which is 6 fold of the control group of vaccine. Meanwhile, IL-4 group induce high level of secreting mouse IL-4 and IFN- γ on stimulated splenocyte than other groups. Then Using IL-4 and IFN- γ with commercial vaccine of 'Asia I' immunized mice respectively, proved both could enhance the level of immune response, and IFN- γ group that show the highest level of specific antibodies, but the changing tendency, cells population and relative value of CD4⁺ and CD8⁺ is similar in IL-4 and IFN- γ group each other.

4. Discussion and Conclusions

Now all known that short sequence of CpG motif and protein of cytokines is generally instable and easy to lose their activity, extracted from animal itself cells, and expressed protein of cytokines in prokaryotic or eukaryotic cell are difficultly to meet the needs of practice due to their defects. Many studies have proved that recombinant plasmids of CpG motif and gene of coded cytokine possess an adjuvant effect, and overcome to the defects above. Our work to FMD vaccine, demonstrated it too.

POTENT AND SELECTIVE INHIBITORS OF THE REPLICATION OF FOOT-AND-MOUTH DISEASE AND SWINE VESICULAR DISEASE VIRUS

N. Goris¹, A. De Palma², J. Neyts², K. De Clercq¹

¹Veterinary and Agrochemical Research Centre, Virology Department, Epizootic Diseases Section, Brussels, Belgium;

²Katholieke Universiteit Leuven, Rega Institute for Medical Research, Leuven, Belgium

Key words: FMD, SVD, 2'-C-methylcytidine, disease control

1. Introduction and Objectives

The foot-and-mouth disease (FMD) virus (FMDV) causes one of the most contagious and devastating animal diseases. With the adoption of Council Directive 2003/85/EC greater emphasis has been put on emergency vaccination to control future FMD outbreaks with the Community. However, FMD vaccines are serotype and subtype specific and confer complete clinical protection against homologous live virus challenge only 4-7 days post vaccination. Swine vesicular disease (SVD) is endemic in southern regions of Italy and, thus, continues to pose a threat to other European countries. Add to this that no commercial SVD vaccines are available at present. The objective of this work was to develop alternative/supplementary disease control tools such as antiviral agents that could overcome the "immunity-gap" during the initial stages of an FMD and/or SVD outbreak.

2. Material and methods

Anti-FMDV, anti-SVDV and cytotoxicity assays were performed to assess the *in vitro* antiviral activity of the nucleoside analogue, 2'-C-methylcytidine (2-CMC), and of two thiazolobenzimidazoles, CHI-67 and CHI-68. The effect on viral RNA yield was investigated using a real-time RT-qPCR. The activity of 2-CMC was compared to that of a known anti-FMDV agent, ribavirin, and additional virus yield and plaque reduction assays were set up, as well as time of drug-addition studies to obtain information on the mode of action of 2-CMC.

3. Results

The 50% and 90% effective concentrations (EC₅₀ and EC₉₀) for 2-CMC inhibition of FMDV-induced cytopathic effect were 6.4±3.8 µM and 10.8±5.4 µM, making it 100-140 times more potent than ribavirin. Moreover and in contrast to ribavirin, 2-CMC proved active against the SVD virus (EC₅₀ = 45.2±0.5 µM; EC₉₀ = 71.0±0.1 µM). CHI-67 and CHI-68 proved active against FMDV at EC₅₀ values of 18.3 and 2.4 µg/ml, respectively. Comparable EC₅₀ values for inhibition of viral RNA synthesis were observed. Treatment of FMDV-infected cells with 77 µM 2-CMC resulted in a 1600-3200 fold reduction of infectious virus yield. Time of drug addition results suggest that 2-CMC interacts with FMDV replication at a time point that coincides with the onset of viral replication.

4. Discussion and Conclusions

In contrast to emergency vaccination, potent and selective antiviral agents may provide almost immediate (prophylactic/therapeutic) protection against infection and, thus, constitute an important alternative/supplementary option to contain outbreaks such as those caused by FMDV and SVDV (Goris et al., 2007). Although resistant viral mutants remain to be established and further *in vivo* studies are urgently needed, the present study clearly underlines the *in vitro* proof-of-principle for FMD and SVD control using antiviral agents.

5. Acknowledgements

This study was supported by the Belgian Science Policy grant 60.11.45.23 from the Scientific and Technical Information Service and by the FP6 VIZIER project (LSHG-CT-2004-511960).

6. References

Goris N, De Palma A, Toussaint J-F, Musch I, Neyts J, De Clercq K. 2007. 2'-C-Methylcytidine as a potent and selective inhibitor of the replication of foot-and-mouth disease virus. *Antiviral Res.*, 73(3): 161-16

**Abstracts free communications:
Theme 6 Surveillance and Epidemiology**

- Dr You-Jun Shang
- Dr Shi-Qi Sun
- Dr Phaedra Eblé
- Dr Nick Knowles
- Dr Carmina Gallardo
- Gina Zanella

STUDY ON BIOLOGICAL CHARACTERISTIC OF FOOD-AND-MOUTH DISEASE TYPE ASIA 1 VISUSES ISOLATED FROM OUTBREAKS IN THE MAINLAND OF CHINA DURING RECENT TWO YEARS

Y. SHANG, X. LIU, Z. LIU, H. YIN, J. MA, H. CHANG, Y. WANG, Q. ZHANG, D. LI, Z. LU, H. BAO, J. GUO, Q. XIE

State Key Laboratory of Veterinary Etiological Biology and Key Laboratory of Animal Virology of Ministry of Agriculture, Lanzhou Veterinary Research Institute, Chinese Academy of Agriculture Sciences, Gansu, China

Key words: foot-and-mouth disease type Asia1, molecular epidemiology, infectivity, persistence

1. Introduction and Objectives

In recent years (1998-2005), 13 countries in Asia and two countries in Europe were reported having outbreaks of type Asia1. Outbreak of type Asia1 ever occurred in Yunnan province, China, in 1958. Since then, this disease had not been found in China, however, the outbreaks of type Asia1 suddenly occurred in China at the beginning of May 2005, and spreaded in 10 provinces. Although only cattle were infected so far, and no clinic syndrome were found in sheep and swine, it is very necessary to know whether the FMD type Asia1 can infect sheep, goats and swine in order to ensure the vaccination breeds. In this work, we attempted to learn biological characteristic of these Asia1 virus isolates such as genotype, virulence, host tropism, pathogenic, antigenic, persistent infectivity. The ultimate objective of this work is to provide scientific advices to veterinary administration for making a design of controlling and eradicating FMD type Asia1.

2. Material and methods

All vesicular samples were collected from field cattle, and probang samples were collected from experimental animals including cattle, goat and pig.

Complete VP1 genes of FMD type Asia1 virus isolates, which were collected in China during May 2005-January 2006, were amplified by RT-PCR, followed sequencing and analysis of nucleotide differences between these isolates and reference virus strains.

Virulence of virus was assessed by measuring dose of virus which is enough to kill fifty percent sucking mice or infect fifty percent cell monolayer culture when injected.

The virus neutralization test was performed to analyse the antigen relationship between field isolates and vaccine strain, then the r -Value was calculated and verified.

There are two animal experiments in this work, one is FMD type Asia1 virus injected infection experiment to sheep or pigs, and the other is cohabitation infection experiment of FMD type Asia1 virus injected cattle to health sheep and pigs. Probang samples from convalescent experimental animals was subjected to detection by virus isolation and RT-PCR.

3. Results

3.1 Analysis of complete VP1 sequence data showed that FMD type Asia1 virus isolates from outbreaks in the mainland of China during May 2005-January 2006 were very different from Hong Kong isolate with more than 16% nucleotide differences.

3.2 There is a close antigen relationship between vaccine strain and Wuxi isolates ($r=0.72$).

3.3 The rates of infection on injection condition is higher than those on coinhabitation condition, the rate of infection of pigs is higher than goats. Some cattle and goats could carry virus up to 60 days post-infection.

4. Discussion and Conclusions

Whether susceptible animals could be infected depend not only on exposure to enough high level of virus dose but also on routes of exposure. The vaccine strain could be used to produce a vaccine rightly against the recent outbreaks; Goats and pigs could be infected with FMDV type Asia1, although few was infected in recent outbreaks so far.

5. Acknowledgements

This work was supported by a grant from 973, Major State Basic Research Development of China (No. 2005CB523201). This work was also partly supported by the EU-funded Inco-Dev project "INCOME" (INCO-CT-2005-515915).

6. References (omitted)

GENETIC TYPING OF CLASSICAL SWINE FEVER VIRUS ISOLATES FROM CHINA

**S-Q. Sun, X-T. Liu, S-H. Yin, Y-J. Shang, H. Tian, Y. Jin, H-X. Zheng,
Z-X. Liu, X-Q. Han, H. Yin, X-P. Cai, Q-G. Xie**

Key Laboratory of Animal Virology of Ministry of Agriculture, State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China

Key words: classical swine fever virus, Genetic typing, Chinese isolates

1. Introduction and Objectives

Classical swine fever (CSF) is probably the economically most important viral infectious disease of domestic pigs in various parts of the world. The disease is caused by the CSF virus (CSFV), a member of the genus Pestivirus within the family Flaviviridae. Genotyping has been widely used to assist epidemiological studies. According to previous studies three major genetic groups of CSF virus are known. Groups 1 and 2 are composed of three subgroups (1.1, 1.2, 1.3 and 2.1, 2.2, 2.3) and group 3 of four subgroups (3.1, 3.2, 3.3 and 3.4) (Paton *et al.*, 2000). Records of CSF in China date back to 1920s. Owing to a wide use of C-strain, CSF is well controlled in China. However, sporadic outbreaks were still reported every year. The aim of this work was to analyze the phylogenetic relationship of CSFV isolates originating from China, based on the partial nucleotide sequences of the E2 gene.

2. Material and methods

All 73 field isolates were collected from suspected-CSF cases during 1984 and 2006 in different regions of the mainland China. Phylogenetic analysis was carried out on a 190 nucleotide fragment of the E2 gene using Neighbor-Joining method with PHYLIP package.

3. Results

All Chinese viruses were segregated into two major groups and subdivided into four subgroups. Most of isolates (61.6%) were belonged to group 2 and were further divided into three subgroups, subgroup 2.1, 2.2 and 2.3. Subgroup 2.1 was the largest subgroup which contained 46.6% of isolates, whilst subgroup 2.3 was the smallest subgroup which contained only one isolate (1.4%). The remaining 38.4% of isolates were classified into subgroup 1.1 within group 1. However, none group 3 and subgroup 1.2 and 1.3 viruses were found.

4. Discussion and Conclusions

Group 1 comprise of vaccine strains together with most of the historical strains and some recent isolates. Group 2 contain most of the world-wide current viruses, which segregates into subgroup 2.1, 2.2 and 2.3 (Paton *et al.*, 2000). Previous studies showed that subgroup 2.1 and 2.2 predominated in the recent epizootics in Asia (Blacksell *et al.*, 2004; Pan *et al.*, 2005; Kamakawa *et al.*, 2006). However, a quite distinct situation has been reported in Europe where subgroup 2.3 is common (Lowings *et al.*, 1996). Our study also supported the above idea. Group 3 contain disparate viruses restricted in some regions (Paton *et al.*, 2000). This group virus seems to become "silent" type that rarely causes epizootics in recent year.

5. Acknowledgements

This work was supported by grant from The National Basic Research Program (973) No.2005CB523201 and National key Technology R&D Program No. 2006BAD06A03.

6. References

1. Blacksell, S.D., Khounsy, S., Boyle, D.B., *et al.*, 2004. Phylogenetic analysis of the E2 gene of classical swine fever viruses from Lao PDR. *Virus Research* 104, 87–92;
2. Kamakawa, A., Thu, H.T.V., Yamada, S., 2006. Epidemiological survey of viral diseases of pigs in the Mekong delta of Vietnam between 1999 and 2003. *Vet. Microbiol.* 118, 47–56;
3. Lowings, P., Iбата, G., Needham, J., Paton, D., 1996. Classical swine fever virus diversity and evolution. *J. Gen. Virol.* 77, 1311–1321;
4. Pan, C.H., Jong, M.H., Huang, T.S., Liu, H.F., Lin, S.Y., Lai, S.S., 2005. Phylogenetic analysis of classical swine fever virus in Taiwan. *Arch. Virol.* 150, 1101–1119;
5. Paton, D.J., McGoldrick, A., Greiser-Wilke, I., *et al.*, 2000. Genetic typing of classical swine fever virus. *Vet. Microbiol.* 73, 137–157.

QUANTIFICATION OF TRANSMISSION PARAMETERS OF FMDV STRAIN O TAIWAN AMONG NON-VACCINATED AND VACCINATED PIGS

P. Eblé¹, A. de Koeijer², M. de Jong², B. Engel², A. Dekker¹

¹Central Institute for Animal Disease Control Lelystad, CIDC, Lelystad, The Netherlands

²Division of Infectious Diseases, Animal Sciences Group Wageningen UR, Lelystad, The Netherlands

Keywords: foot-and-mouth disease, reproduction ratio, transmission rate, modelling, pigs

1. Introduction and Objectives

Quantified transmission parameters of Foot-and-Mouth Disease Virus (FMDV) are needed as input for epidemic models used for the development of control and surveillance programmes.

The aim of this study was to provide additional estimates of main parameters for the transmission of foot-and-mouth disease virus (FMDV) strain O Taiwan (3/97).

2. Material and methods

We used the data of previous experiments in non-vaccinated and vaccinated pigs and combined the data of experiments with the same treatment(s). First, we quantified the reproduction ratio R for the various groups using a final-size method. Secondly, we used the daily results of virus excretion to quantify the transmission rate β , using Generalized Linear Modelling, and the infectious period T , using survival analysis. With the results of β and T estimates for R could be made more precisely as compared to the final size method, also for the groups for which a finite estimate could not be obtained using a final size method.

3. Results

The final size results predicted that vaccination with a 4-fold vaccine dose (but not with a single dose) at one week before inoculation (-7dpi) would reduce R compared to the non-vaccinated group. The modelling results predicted that β for non-vaccinated, for single-dose and 4-fold dose groups would be 6.1, 2.0 and 0.4 day⁻¹, T at 6.5, 5.3 and 2.3 days and R at 40, 11 and 1.0 respectively. For as well the single as the 4FD -7 dpi vaccinated groups β , T and R in the vaccinated groups were significantly reduced as compared to the non-vaccinated group.

4. Discussion and Conclusions

These results predicted that both vaccination with a 4-fold vaccine dose and with a single dose at -7dpi would reduce β , T and R , thus indicate that vaccination will reduce transmission of FMDV significantly already one week post vaccination. The modelling method that we described can be used to obtain more precise estimates of transmission parameters.

5. Acknowledgements

This work was supported by the Ministry of Agriculture, Nature and Food Quality, The Netherlands.

MOLECULAR EPIDEMIOLOGY OF SWINE VESICULAR DISEASE VIRUSES ISOLATED IN ITALY OVER 15 YEARS (1992-2006)

E. Brocchi¹, S. Grazioli¹, I. Barbieri¹, M. Bugnetti¹,
G. Wilsden², N.J. Knowles²

¹Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy;

²Institute for Animal Health, Pirbright Laboratory, Pirbright, Woking, Surrey, GU24 0NF, United Kingdom

Key words: SVDV, Italy, molecular epidemiology, sequencing

1. Introduction and Objectives

Swine vesicular disease (SVD) is a highly contagious viral disease of pigs, with lesions indistinguishable from those of foot-and-mouth disease. Epidemics of SVD have occurred in European countries and Eastern Asia during the 1970s and early 1980s; then the disease has continued to persist in Italy until the present day and has reappeared in the European Union (EU), outside Italy, on sporadic occasions since 1992. Although SVD virus occurs as a single enterovirus serotype, four congruent groupings were found in both the genetic and antigenic properties of the virus (1). The most recent group consists of viruses isolated from the EU since July 1992. The objective of this study was to phylogenetically analyse isolates collected over 15 years (1992-2006) to evaluate the epidemiology and variability of SVDV in the field.

2. Material and methods

RT-PCR amplification and nucleotide sequencing of the VP1-coding region of more than 100 SVD viruses, isolated mainly in Italy during 1992-2006, were performed. A phylogenetic tree was constructed using the program MEGA 3.1 (2).

3. Results

All SVD viruses occurring since 1992 were clustered in a unique genomic lineage, corresponding to the fourth and most recent group (1). However, two genomic sub-lineages were distinguishable. One includes the older cluster of SVD viruses, isolated in different EU countries between 1992 and 1995, and another cluster of closely related viruses detected in Portugal in 2003/2004 and later in southern Italy in 2004 and 2006. The second sub-lineage was comprised of isolates occurring only in Italy from 1998 to 2006. Interestingly, from 2004, members of the two different sub-lineages have been present simultaneously in Italy. In 2006, an epidemic of SVD affected northern regions, caused by viruses typical of those evolved in Italy (second sub-lineage), while SVD strains more closely related to the Portuguese isolates (older sub-lineage) spread in the Southern endemic regions.

4. Discussion and Conclusions

Phylogenetic analyses of SVD viruses isolated during 15 years proved evidence of a substantial genomic stability: all isolates belong to the same genetic lineage, first detected in the EU in 1992 and still circulating. Yet, even minor changes may help in understanding virus epidemiology and tracing infection. In fact, the present study suggests the following assumptions: a) the SVD virus occurred in Portugal in 1995 may have persisted for many years; it resurfaced in Portugal in 2003 and was probably later introduced into Italy; alternatively, this could represent a reintroduction from outside the European Community, where subclinical infection may be missed in absence of active surveillance; b) two genetic sub-lineages have been recently circulating in Italy: SVD viruses genetically related to isolates detected in previous years were responsible for the 2006/2007 epidemic in northern Italy; the "Portuguese" sub-lineage is present in Italy since November 2004, and from Abruzzo region it has spread to further southern regions.

5. References

1. Brocchi E., Zhang G., Knowles N., et al. (1997). Molecular epidemiology of recent outbreaks of swine vesicular disease: two genetically and antigenically distinct variants in Europe, 1987-94. *Epidemiol. Infect.* 118: 51-61.
2. Kumar S, Tamura K, Nei M. (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* 5: 150-163.

MOLECULAR EPIDEMIOLOGY OF AFRICAN SWINE FEVER VIRUS (ASFV) ISOLATES RECOVERED FROM SAMPLES COLLECTED IN KENYA DURING 2006-2007 SUSPECTED OUTBREAKS

C. Gallardo^{1,3}, D. Mwaengo², V. Pelayo¹, E. Okoth³, J. Macharia⁴, R. Bishop³

¹*Centro de Investigación en Sanidad Animal, CISA-INIA. Valdeolmos, Madrid, Spain;*

²*Dept. Of Medical Microbiology, College of Health Sciences, University of Nairobi, Kenya;*

³*International Livestock Research Institute, ILRI, Kabete, Naitobi, Kenya;*

⁴*Central Veterinary Laboratory, Kangemi, Nairobi, Kenya*

Key words: ASFV, epidemiology, genotype, p72, p54, TRS

1. Introduction and Objectives

African Swine Fever (ASF) is an important disease of domestic pigs caused by an icosahedral double stranded DNA virus classified within the *Asfarviridae* family, genus *Asfivirus*. ASF has been reported from most countries in southern and eastern Africa, where the virus is maintained in a sylvatic cycle. The Republic of Kenya is situated on Africa's east coast. It is bordered to the northeast by Somalia, to the north by Ethiopia, to the northwest by Sudan, to the west by Uganda, and to the south by Tanzania. According to data from the Office International des Epizooties (World Organisation for Animal Health), the last occurrence of ASF in Kenya was recorded in August 2001. However, the occurrence of frequent monthly outbreaks of ASF in neighbouring Uganda since 2003 poses a great threat to the pig industry in Kenya. On May 2006, ASF was suspected to have hit the Kenya-Uganda border district of Busia leaving nine pigs dead. Quarantine was imposed on the movement of pigs and their products within Busia municipality to avoid its spread. On February 2007 a new outbreak of AFS was reported in Uasin Gishu district bordering with Uganda. Field samples were collected in these districts and several ASFV isolates were obtained after the inoculation of leucocytes. The main aim of this study is to contribute to the clarification of the epidemiological situation of ASF in Kenya during 2006-2007 suspected outbreaks. We report here the epidemiological studies of Kenyan viral isolates by a first genotyping sequencing of the gene encoding the VP72 (Bastos et al., 2003) and VP54 protein to place isolates into major subgroups, following by sub-typing analysing three variable regions of the ASFV genome marked by the presence of tandem repeats sequences (TRS). This study attempts to provide a first assessment of intra- and inter-genotypic variability of ASFV field isolates from Kenya, and to uncover epidemiological links that may exist between outbreak viruses from Kenya and neighbouring countries.

2. Material and methods

Viruses used in this study: 13 Kenyan ASFV isolates named Ken06 (1-6) and Ken07 (1-7) were recovered from domestic pigs in the outbreaks occurred in Busia (2006) and Uasin Gishu (2007) districts. In this study were also include more than 58 viruses selected from the CISA virus collection, native to Europe, Africa and America, collected between 1961 and 2003.

Nucleic acid extraction and genomic amplification: DNA was extracted from tissue sample homogenates and sera using a nucleic acid extraction kit (Nucleospin/ Machery-Nagel -Cultek) following the manufactures procedures. A PCR assay was used to confirm the presence of ASFV DNA (Aguero et al 2003). The p72 genotyping was achieved by PCR as described Bastos et al 2003. The complete gene encoding VP54 protein was amplifying by PCR using the primer set PPA89-PPA722 (PPA89 5' - TGTAATTCATTGCGCCACAAC-3' ; PPA722, 5' - CGAAGTGATGTAATAAACGTC - 3'). For amplifying the TRS by PCR, different set of primers were selected (Table 1).

Nucleotide sequencing and analysis: The PCR products were analyzed by electrophoresis on 2% agarose gels. Bands of the correct size were excised and purified by Quiaex gel extraction (QUIAGEN) and cloning into the pGMT easy vector according the manufactures instructions and sequenced using an automated sequencer 3730 DNA analyzer" (Applied Biosystems). Sequence alignment was performed with the CLUSTAL W package and the phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1.

Table 1. PCR primers used for TRS analysis

REGION	PRIMER	SEQUENCE (5'→3')	GEN NAME	ASFV GENOMIC LOCALIZATION
R1 (ir)	ECO1A (s)	CTATTTATCCCCRCCTTTGG	I73R	155,210 nts.....155,431 nts
	ECO1B (a)	CTCGTGTCTGAGGATGACGA	I329L	155,667 nts.....156,656 nts
R2 (cg)	1196L1 (s)	AGCAGGGAAAAAGGGGATTA	1196L	158,175 nts.....158,765 nts
	1196L2 (a)	CRTCAATAAACCTAAAAAGCCA		
R3 (pg)	ORF9L-F (s)	AATGCGCTCAGGATCTGTTAAATCGG	9L	84,634 nts.....85,027 nts
	ORF9L-R (a)	TCTTCATGCTCAAAGTGGTATACCT	(B602L)	

cg (complete gene); ir (intergenic region); (pg) partial gene (s) sense (a) antisense.

3.Results

A PCR assay using the ASF diagnosis primers PPA1/PPA2 which delimits an amplicon of 257 bp for the p72 protein was used to confirm the presence of ASFV DNA in the Kenyan samples analyzed. The specificity of the amplicons obtained was confirmed using *Nde I* restriction endonuclease that specifically cut the ASFV collected from East African isolates.

The Kenyan viruses isolated were first genotyped by partial p72 gene characterization. All of them were classified into the genotype IX, which includes viruses from Uganda. The same result was obtained sequencing the complete gene that encodes the p54 protein where no differences were found at amino acid level between the Kenyan isolates and Ugandan isolates. Although the p72 and p54 genes are useful for identifying the major genotypes, higher resolution of virus relationships is required to uncover epidemiological links. To this end, sequences of three variables regions characterized by the presence of TRS were generated from Kenyan isolates showing a high degree of homology with the last viruses characterized in Uganda in 2003. In addition, comparison of amino acid tetrameric repeats located in the variable CVR within the B602L ORF (Nix et al 2006) revealed different groups between Kenyan isolates due to the absence of one internally located tetrameric repeats (CADT).

4.Discussion and Conclusions

Currently, there is not available information about recent outbreaks in Kenya, but the biggest problem at this time is the presence of the disease in neighboring countries as Uganda where the virus appears to be more virulent with a higher morbidity rate in the last outbreaks occurred. Epidemiological studies and sequence identity of the 13 Kenyan isolates, compared here, classify these isolates with viruses recovered in Uganda from domestic pigs. This fact suggests that the disease may have been introduced recently in Kenya-Uganda border district either through swill from boat or plane, through pork products, or through live pigs. Furthermore, the results obtained sub-typing these isolates, which included interesting differences, could be reflecting the co-circulation of different ASFV strains during the suspected outbreaks occurred in Kenya.

5.References

1. Bastos A.D.S. et al., (2003). *Arch Virol* 148 (4), 693-706;
2. Agüero M. et al, (2003). *J Clin Microbiol*, Sept p. 4431-4434;
3. Malmquist and Hay (1960). *Am. J. Vet. Res.* 21, 104-10;
4. Nix et al., (2006). *Arch Virology Dec*; 151(12):2475-94.

EVOLUTION OF THE FIRST TUBERCULOSIS OUTBREAK DUE TO MYCOBACTERIUM BOVIS IN WILDLIFE IN FRANCE

**G. Zanella¹, B. Durand¹, J. Hars³, F. Moutou¹, B. Garin-Bastuji²,
A. Duvauchelle³, M. Fermé³, C. Karoui², M. Boschirolli²**

¹Unité Epidémiologie and ²Unité Zoonoses Bactériennes, Agence française de sécurité sanitaire des aliments, Maisons-Alfort, France;

³Unité Sanitaire de la faune, Office National de la Chasse et de la faune sauvage, Gières, France

Key words: *Mycobacterium bovis*, red deer, wild boar, molecular typing, diagnostic tests

1. Introduction and Objectives

At the beginning of 2001, three hunted-killed red deer (*Cervus elaphus*) in the Brotonne and Mauny forests, Normandy (France) disclosed tuberculosis-like lesions, from which *Mycobacterium bovis* was isolated. In subsequent hunting seasons two epidemiological surveys were carried out in the area. Our objectives are to report the results of those surveys, to assess the efficiency of the control measures implemented after the outbreak discovery (reduction of red deer population, ban on supplemental feeding and destruction of viscera of hunted animals), to study the infectious link with neighbouring cattle outbreaks by molecular typing and to quantify the performance of necropsy used as a diagnostic criterion in wild red deer.

2. Material and methods

Red deer, roe deer (*Capreolus capreolus*), wild boars (*Sus scrofa*), red foxes (*Vulpes vulpes*) and badgers (*Meles meles*) were analysed. Sampling of visible gross tuberculosis-like lesions and of the respiratory tract and retropharyngeal lymph nodes was undertaken. Culture was used to detect *M. bovis* in the samples and molecular typing techniques were applied to the totality of *M. bovis* isolates. Sensibility, specificity and predictive values were calculated for gross tuberculosis-like lesions using culture as the gold standard from red deer data.

3. Results

In the first survey (2001/2002 hunting season), 9 (12.5%) out of 72 red deer sampled tested positive to *M. bovis*. In the 2005/2006 hunting season, the prevalence of *M. bovis* infection increased to 23.9% ($\chi^2 = 3.85$, $df = 1$, $P = 0.05$) in overall red deer (out of 138 sampled). While remaining stable in juveniles, the prevalence increased significantly in adults: from 13.3% in 2001-2002 to 31.7% in 2005-2006 ($\chi^2 = 5.13$, $df = 1$, $P = 0.02$). Wild boars were found to be heavily infected in both surveys. One roe deer and one red fox also tested positive in the second survey. *M. bovis* was not isolated from badgers. Spoligotyping and MIRU-VNTR analysis demonstrated that all wildlife *M. bovis* strains had the same genotype. Sensitivity and positive predictive value of the presence of macroscopic lesions increased from one survey to the other in adult red deer (50% to 96% and 38% to 92%, respectively), while the positive predictive value remained stable (75%) and the sensitivity decreased in juveniles (from 100% to 75%). These variations by age class were observed neither for the specificity (94%) nor for the negative predictive value (96%).

4. Discussion and Conclusions

In spite of the control measures taken in 2002, overall infection prevalence in red deer increased. However, if we consider the prevalence of juveniles as an incidence indicator of recent infection in the red deer population, we can assume that the implemented control measures had some impact as it did not increase. Molecular typing findings indicate not only that a unique strain has been the cause of the wildlife outbreak but also that it is the same one circulating in nearby cattle herds since at least 1995. Sensitivity, specificity and predictive values of macroscopic lesions' presence suggest that it could be used as a diagnostic criterion for routine follow up of the disease in wild red deer populations in regions where *M. bovis* infection is enzootic.

Abstracts free communications: Theme 7 Risk Assessment

- Dr Paul Gale
- Dr Jovita Fernández
- Dr Franz Conraths

DEVELOPING A QUALITATIVE RISK ASSESSMENT FRAMEWORK FOR THE IMPACT OF CLIMATE CHANGE ON THE PREVALENCE OF LIVESTOCK DISEASES IN THE UK – WORK IN PROGRESS

Gale, P., Adkin, A. and Snary, E.

Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, Weybridge, Surrey, UK

Key words: Climate change, qualitative risk assessment, vector

1. Introduction and Objectives

Climate change potentially offers unique opportunities for the emergence and increase in prevalence of livestock diseases. This objective of this paper is to describe the development of a qualitative risk-based framework to assess the potential, future impact in the UK of climate change on the prevalence of a given livestock disease.

2. Material and methods

The approach has been to review host, pathogen, vector and epidemiological information for a wide variety of livestock diseases across the world and collate those factors which not only affect their emergence and prevalence, but also are influenced by climatic variables. The factors include vectors, environmental parameters, zoological considerations (including movement and distribution of wildlife, pets and imported exotic animals), changes in farming practice and management, human activities and extreme weather events. Consideration is given to formation of micro-environments which favour establishment of particular vectors and/or reservoirs. Central to assessing the impact of climate change on livestock diseases is an understanding of how farming practices may adapt. Requirements for water, food and shade may affect not only the intensity of livestock farming, but also the movement and transport of livestock around the UK.

3. Results

A variety of livestock pathogens are used to develop and populate the framework. These include bluetongue virus (vector *Culicoides* midges), African swine fever virus (tick vector), *Fasciola* trematode flukes (vector lymnaeid snails), and Venezuelan equine encephalitis virus (mosquito vector). The main modules of the framework are: Routes of entry to the UK (if not endemic): Impact of climate change on host reservoirs: The role of vectors (if any): The effect of climate change on direct contact with other livestock and wildlife, and: The impact of climate change on environmental transmission (if appropriate). A case study will be presented.

4. Discussion and Conclusions

A feature of climate change is that different processes change at different rates, so that new ecological niches offering favourable conditions may be created. For example, while the summer temperatures in the UK in 2080 may resemble those currently in southern France, there may also be a decline in insectivorous birds enabling non-indigenous arthropod vectors to establish and increase in abundance. Any assessment of the impact of climate change on livestock diseases requires understanding of a broad combination of factors and events, many of which interact together.

5. Acknowledgements

This is part of a project (SD0441) funded by Defra, UK, to investigate the impact of climate change on agricultural pathogens and chemical contaminants.

METHODOLOGY AND PROCEDURES EMPLOYED AND OUTPUT GENERATED IN RISK ANALYSIS OF AVIAN INFLUENZA ENTRANCE IN SPAIN BY WILD BIRDS

I. Iglesias¹, M. Martínez¹, A. de la Torre¹, J. Manuel Sánchez-Vizcaíno², J. Fernández¹, M. Jesús Muñoz¹

¹Centre for Animal Health Research, CISA, Valdeolmos, Spain;

²Veterinary faculty, Complutense University of Madrid, Spain.

Key words: Avian influenza, wild birds, risk analysis

1.Introduction and Objectives

As part of a global study about the prediction of avian influenza (AI) entrance and spread in Spain and for the knowledge of those partners who can be interested in, we present the methodology and procedures that are being currently employed and the outputs generated in the epidemiological study and risk assessment of currently relevant animal diseases as AI.

2.Material and methods

At first, gathering of information relative to the disease, the virus, the susceptible host animals and the biological, environmental and physical variables involved, is carried out consulting scientific literature and different database such as national and international scientific, geographic, climatologic, biologic and sanitary databases. Secondly, the risk factors are identified and analysed by using geographic spatial analysis (ArcGIS 9.1 and ArcView 3.2) and statistical analysis of the data (SPSS 14.0). Finally, a probabilistic risk analysis is carried out based on the Covella-Merkhoffer model. For this approach a statistical analysis and mathematical fit of the data is also carried out and specific softwares as @Risk palisade corp 4.0 are used.

3.Results

The data output is generated by: a) specific maps representing:

- Geographical position and world-wide evolution of the disease including information about the number and type of outbreaks, species in which AI was diagnosed, etc
 - European migratory scenarios of bird flyways affecting Spain.
 - The most relevant wetlands in the migratory scenarios, selected by biological criteria.
 - Movements of wintering wild birds in Spain (for 27 avian species).
 - Distribution and abundance of these species in Spain.
 - Probable number of infected birds coming to the Spanish provinces.
 - Risk of entrance by wild birds classified in risk levels by Spanish provinces.
- and b) specific databases including information about:
- Outbreak data considering affected species, virus serotype, etc.
 - Number of bird species by Spanish province.
 - Range of European temperatures and virus survival.
 - Estimation of the biological variables like the number of stopovers by species during the migratory flyway and the time spent at each stop-over.
 - Outdoor poultry farm density by Spanish province.
 - Ringing data considering the percentage of bird movements coming from each country and reaching each Spanish province.

4.Discussion and Conclusions

During the last year we have been working in the risk assessment of the entrance of avian disease in Spain by wintering wild birds. This work has been developed in collaboration with Dr. Sánchez-Vizcaíno from the Veterinary Faculty (Complutense University of Madrid). The results obtained have been presented at different congresses and in scientific papers.

6.References

1. Sánchez-Vizcaíno et al (2006). Spanish Journal of Agricultural Research 4(2):140-145.
- 2.-Martínez et al (2006). Avian diseases, 50 (en prensa).
- 3.-Martínez et al (2006). 6th International Symposium on Avian Influenza. 24 April. Cambridge.
- 4.- Martínez et al (2006). 7th International Congress of Veterinary Virology. 24-27 September. Lisbon
- 5.-Martínez et al (2007). IMED Vienna, Austria. 24 February, 2007.

CSF IN WILDBOAR - A SURVEILLANCE DATA BASE for BELGIUM, FRANCE, GERMANY, LUXEMBOURG AND THE NETHERLANDS

C. Staubach, D. Klöß, M. Kramer, F. Conraths

Friedrich-Loeffler-Institut, Institute of Epidemiology, Wusterhausen, Germany

Key words: Classical Swine Fever, Surveillance, Data base, Risk Assessment

1.Introduction and Objectives

A web based data base containing surveillance data of Classical swine fever (CSF) in wild boar of Belgium, France, parts of Germany, Luxembourg and The Netherlands was developed and used as central data source with the aim to describe and analyse the course of infection in the wild boar population.

2.Material and methods

The data base has been set up as an internet website. The use of the data base is therefore exclusively possible with a web browser. Access of the website is protected by security mechanisms (user name and password). All users are allowed to read the data base contents, but only a defined user group of each member state can add, edit or delete records of their own country. The data base is implemented as client-/server-system. The clients (users) in each participating country using a web browser to record data for each shot or dead found wild boar via HTML form or import data via a defined text file. The latter made it possible to import data from specialized data bases in the laboratories of the member states.

Data visualisation for all users is based on HTML pages in the internet browser. The internet server produces a table view of the data base which can be restricted according to time period, NUTS levels (e.g. member states, federal states, districts) and laboratory results. Furthermore, it is possible to create a summary report for each month or any other time period stratified by age, carcass, type of restriction area, vaccination (yes/no) and virological and serological results. An internet map server displays the wild boar data on topographical maps of the participating member states.

An extension of the data base to other countries is possible at any time.

3.Results

A total of 284,278 records of wild boar tested for CSF in Belgium, France, Germany, Luxemburg and The Netherlands have been entered and analysed in the data base (as per 23 March 2007).

4.Discussion and Conclusions

This is the first supra-national data base providing information on the complete surveillance data (all positive and negative diagnostic results) on an OIE listed disease. Its technology has recently be extended to avian influenza and can also be used for many other diseases of interest.

**Abstracts poster presentations:
Theme 4 Diagnostics**

DEVELOPMENT OF DIAGNOSTIC TECHNIQUES AND VACCINES AGAINST ASIA-1 FOOT AND MOUTH DISEASE IN CHINA

Q. Zhang, X. Liu, Z. Liu, J. Ma, D. Li, Y. Shang, Z. Lu, Y. Cao, G. Wu, X. Yan

Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China

Key words: Foot and Mouth Disease, Asia1, Diagnosis, Vaccines

1. Introduction and objectives

Foot and mouth disease is still endemic in large areas of African, Asia and South America and has shown an extraordinary ability to cross international boundaries and cause epidemics in previously free areas, as evidenced by the 2001 epidemic in the UK and continental Europe as well as the outbreaks in year 2000 in Japan and South Korea. FMDV serotype Asia1 was identified in a sample from Pakistan in 1957, and still remains endemic in many parts of Asian countries. Since 2005, it caused a FMD outbreak in Shandong, Jiangsu and Xinjiang provinces in China. Interestingly, there were rarely reports that pigs were found to be infected. It is unclear whether the virus caused this FMD outbreak is a cattle-adapted virus or not. A series of methods to detect foot and mouth disease Asia1 antigen and antibody were developed in our laboratory. At the same time, we carried out experimental studies in pigs, cattle and sheep in order to understand its pathogenicity in different species. On these data, vaccines against foot and mouth disease type Asia1 was developed.

2. Material and methods

2.1 Development of immunological diagnostic methods. Isolated the Asia1 virus, the virus was passaged on BHK21 cells, then purified by sucrose density gradient centrifugation. The rabbit antiserum and guinea pigs antiserum prepared by inoculating pure FMDV 146S antigen was used in antigen trapping ELISA to detect FMDV antigen and in LPB-ELISA to detect FMDV antibody, and a pen side method-colloidal gold test paper was established at the same time.

2.2 Nucleic acid recognition methods. Specific primers have been designed to for a multi-PCR and for a real time PCR to distinguish O, A and Asia1.

2.3 Common practice vaccines researching. Virus is used to infect a monolayer cell culture and the resulting preparation is clarified, inactivated with ethyleneimine and emulsified with adjuvant.

2.4 Genetically engineering vaccine. Attempt to study genetically engineering vaccines, for example live carrier vaccines.

3. Results

3.1 A LPB-ELISA, trapping ELISA have been used to detect FMDV antibody and antigen respectively, and the colloidal gold test paper is used for quickly test.

3.2 Developing a multi-PCR and a type identifying PCR have been used, researcher was establishing the real time PCR.

3.3 Inactivated bovine FMD Asia 1 vaccine, bovine FMD(O,Asia1) bivalent vaccine and bovine FMD(O,A,Asia1) trivalent vaccine have been developed and used in some area. The animal trials showed that the Asia 1 isolate for vaccine is safe, it is could not cause FMD in cattle and sheep, and neither in pigs, so a swine FMD Asia1 vaccine has been studying.

3.4 FMDV P1-2A3C gene was inserted into adenovirus carrier and capripox virus carrier. The animal experiments were carrying out now.

4. Discussion and Conclusions

We have developed a series diagnostic methods and bovine inactivated vaccines.

5. Acknowledgements

This work was supported by The National Basic Research Program 973-2005 CB 523201.

6. References

1. Amaral-Doel et al., *Vaccine* 1993(11), 415-21
2. Kitching, R.P., *Vaccine* 1999(17), 1772-4
3. Hamblin, C. et al. *J Immunol Methods* 1993, 123-9

ASSESSMENT OF A FULLY AUTOMATED REAL-TIME RT-PCR DEVICE FOR THE DETECTION OF BOVINE VIRAL DIARRHOEA VIRUS

P. Wakeley¹, D. Mealing¹, J. Errington¹, D. Squirrell², G. Martin², M, Lee²

¹Veterinary Laboratories Agency, VLA, Addlestone, Surrey, United Kingdom

²Enigma diagnostics Ltd. Tetricus Science Park, Salisbury, Wiltshire, United Kingdom

Key words: Bovine Viral Diarrhoea Virus, Real-time RT-PCR, Field trial

1. Introduction and Objectives

Bovine viral diarrhoea virus (BVDV) belongs to the genus pestivirus which also includes Border disease virus of sheep and Classical Swine fever virus of pigs. Pestivirus genomes are positive sense, single stranded RNA molecules of approximately 12.5kb in length consisting of a single open reading frame and a 5' and 3' un-translated region (UTR). The single polypeptide is processed by host and viral encoded proteases during viral replication into viral structural and non-structural proteins. BVDV can be separated into two main genotypes, BVDV1 and BVDV2, based on sequence differences in the 5'UTR of the genome.

In collaboration with Enigma Diagnostics (a spin-out company of Dstl) we have developed a real-time, reverse transcription polymerase chain reaction employing a TaqMan[®] fluorogenic probe specific for the 5'UTR of BVDV (both genotypes 1 and 2). BVDV is endemic in ruminants in the UK allowing field testing for a virus with characteristics similar to that of a virus (CSFV) causing a notifiable disease.

2. Material and methods

The design of the Enigma-FL instrument was originally specified by the Ministry of Defense, UK for rapid, on-site biological agent detection and identification. The instrument allows automated template extraction (RNA), rapid PCR thermal cycling and automated result calling. It is portable, "ruggedised", requires minimal operator training and can be powered either by mains electricity or vehicle battery. The matrix chosen for extraction and analysis was bovine whole heparin treated blood. Nucleic acids can be extracted and analysed using the real-time RT-PCR TaqMan[®] in approximately 40 minutes.

Cattle were sampled on three separate farms by minimally trained veterinarians. Extractions, molecular amplifications and final calls (positive or negative) were performed by the Enigma-FL instrument. The results were confirmed on the same samples using our laboratory based real-time, RT-PCR TaqMan[®] test performed by highly trained personnel.

3. Results

Only results of farm 3 are shown as all other results were negative on both the Enigma-FL instrument, confirmed as the correct result using the laboratory based method.

Sample	Enigma-FL	Lab Result
Calf1 (farm)	+	+
Calf2 (farm)	-	-
Calf3 (farm)	-	-
Pool1 (farm)	+	+/-/-
Pool2 (farm)	-	-/-/-
Pool3 (lab)	-	-/-/-
Pool4 (lab)	-	-/-/-
Pool5 (lab)	-	-/-/-

BVDV RNA was detected in both single blood samples and pooled samples of three bloods (where only 1 was positive). No false positives were detected using the Enigma-FL instrument.

4. Discussion and Conclusion

Despite the low number of samples analysed no false negatives or false positives were identified. Where low numbers of positive cases are required for diagnosis the Enigma-FL may prove valuable for detection of virus causing notifiable disease "on site". Confirmation of presence of pathogen is required in a recognised competent laboratory.

5. Acknowledgements

VLA Seedcorn funding (SC 0146), Enigma Diagnostics and NoE EPIZONE (EUF6).

RAPID AND SENSITIVE IDENTIFICATION OF H7 SUBTYPE INFLUENZA A VIRUSES USING MGB REAL TIME RT-PCR

S. van Borm¹, M. Boschmans¹, O. Ozhelvaci¹, T. van den Berg¹

¹*Veterinary and Agrochemical Research Institute, VAR, Brussels, Belgium*

Key words: real time RT-PCR, Influenza A, H7, minor groove binder

1. Introduction and Objectives

Real time RT-PCR is becoming a key technique in avian influenza diagnosis. Well-validated generic influenza A assays targeting conserved regions of the influenza genome, and several H5 subtype specific RRT-PCRs have been reported. However, currently available H7 tests have a limited phylogenetic scope (i.e. do not detect the complete variety of H7 strains). The objective of this work is the development of an H7 RRT-PCR with a wide phylogenetic scope.

2. Material and methods

A total of 210 H7 hemagglutinin sequences, including 107 Eurasian isolates and 91 American isolates were downloaded from public databases (accession codes available on demand) and aligned using the ClustalW algorithm. Conserved regions were identified and a MGB Taqman probe and primers were defined with the help of Primer Express software. The specificity was confirmed using a BLASTn search as well as a panel of 21 influenza A viruses (including 4 H7s) and one influenza B virus. The detection limit of this new test (*H7mgb*) was determined using serial tenfold dilutions of A/Ck/BE/06600/2003 (H7N7) and A/Ck/It/1067/v99 (H7N1) and compared with the detection limit of the generic influenza A RRT-PCR (*M1*, Spackman *et al* 2002) and H7 specific RRT-PCR tests (*H7VLA*, Slomka and Brown 2007) recommended in the EU Diagnostic Manual for Avian Influenza.

3. Results and Discussion

This new H7 real time RT-PCR had a wide phylogenetic scope. The MGB probe proved 100 % conserved for all available H7 sequences, and the degenerate primers were conserved for all Eurasian sequences. When using an alternative set of primers, all available American H7 sequences could theoretically be detected using the same probe. The analytical specificity of *H7mgb* was confirmed as no signal was detected in any of the 18 non-H7 strains in the panel. The detection limit of *H7mgb*, as well as of *M1* and *H7VLA* depended on the virus strain used. When using A/Ck/BE/06600/2003 (H7N7) and A/Ck/It/1067/v99 (H7N1) dilutions, *H7mgb* was able to detect 0.14 and 2.3 EID₅₀/reaction respectively. The new test proved slightly less sensitive compared to *M1* (resp. 0.014 and 2.3 EID₅₀/reaction), and equivalent to the *H7VLA* test (resp. 1.41 and 0.23 EID₅₀/reaction).

We are currently expanding our strain collection in order to investigate 2 remaining questions: (1) the use of primers targeting American isolates, and (2) further proof for specificity against H15 and H11 viruses is needed as the conserved probe sequence seems to occur in some viruses of these subtypes. Specificity in these cases seems, however guaranteed by at least 3 mismatches in primer sequence. Validation of *H7mgb* on clinical samples is currently ongoing.

4. Acknowledgements

I. Capua (IZVS, Padova) and I. Thomas (IPH, Brussels) provided influenza viruses or their RNA. We thank M. Decaestecker and M. Sayouti for excellent technical assistance.

5. References

1. Slomka and Brown. 2007. Protocol for detection of H7 avian influenza using realtime RT-PCR (9 February 2007), personal communication;
2. Spackman, E., D. A. Senne, *et al.* 2002. *J. Clin. Microbiol.* 40: 3256-3260.

THE GENOME SEQUENCE OF A NOVEL FOOT-AND-MOUTH DISEASE VIRUS BELONGING TO SEROTYPE O

J. Stirling, N. Knowles

Institute for Animal Health, Pirbright Laboratory, Pirbright, Woking, Surrey, United Kingdom

Key words: Foot-and-mouth disease virus, Sequence analysis

1. Introduction and Objectives

Foot-and-mouth disease (FMD) was thought to have disappeared from Africa following the rinderpest pandemic of late 19th Century in which possibly 90% of cloven-hooved animals died (2). It is also thought that the European FMDV serotypes (O, A and C) were subsequently re-introduced into Africa from either Europe or Asia, while the three Southern African Territories (SAT) serotypes re-emerged from isolated African buffalo (*Syncaerus caffer*) populations where these viruses were able to persist covertly. During a study of the molecular epidemiology of FMD virus type O in Africa, a virus (which had been isolated from an outbreak in Sudan in 1963) was discovered to possess a highly divergent VP1 sequence. To determine the relationship of this virus to other FMD viruses its complete genome sequence was determined and phylogenetic analyses performed.

2. Material and methods

Virus RNA extracted from cell culture grown FMDV isolate O/SUD/62/63 was reverse transcribed and the resultant cDNA amplified by PCRs which covered the whole genome except for the poly(C) tract. Each amplicon was sequenced on both strands and the sequence assembled using Vector NTi. Phylogenetic analyses, using previously published FMDV sequences, were performed using MEGA 3.1 (1).

3. Results

The VP1 nucleotide sequence O/SUD/62/63 differed from other type O viruses by 27.7 to 34.4%, types A, C and Asia 1 by 34.5 to 38% and types SAT1, SAT2 and SAT3 by 46 to 48%. In the non-structural protein coding regions, both upstream and downstream of the capsid, O/SUD/62/63 clustered with East African SAT viruses rather than with O, A, C or Asia 1.

4. Discussion and Conclusions

Although the maximum difference observed in the VP1 gene between individual serotype O isolates can be as much as 26%, the minimum distance of O/SUD/62/63 to all type O's was nearly 28%. Phylogenetic analysis of the sequence placed it much closer to the branching of the O, A, C and Asia 1 serotypes than any other type O virus. To confirm this divergent relationship the sequence of the complete P1 capsid-coding region was determined and found to substantiate the VP1 data. Sequences outside the capsid-coding region do not contain serotype-specific information and in these regions O/SUD/62/63 was more closely related to SAT viruses. It is not clear if O/SUD/62/63 represents a novel virus generated by recombination between an early lineage of type O and a SAT virus, or if it is a relic of the early evolution of serotype O, which may have evolved from one of the SAT serotypes.

5. Acknowledgements

This work was partially supported by grants from Defra, UK (nos. SE2921 and SE2935).

6. References

1. Kumar S, Tamura K, Nei M. (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* 5: 150–163;
2. Mack, R. (1970). The great African cattle plague epidemic of the 1890's. *Trop. Anim. Hlth. Prod.* 2: 210-219.

EVALUATION OF A PORTABLE THERMOCYCLER FOR THE DETECTION OF FOOT-AND-MOUTH DISEASE VIRUS

S. Reid¹, R. Mistry², J. Dukes¹, K. Ebert¹, C. Mills², L. Boswell², P. Ferris¹, D. King¹

¹Institute for Animal Health, Pirbright, Surrey, United Kingdom;

²Smiths Detection, Bushey, Watford, United Kingdom

Key words: PCR, FMDV, Field detection, point of care diagnostics

1. Introduction and Objectives

Control of foot-and-mouth disease (FMD) outbreaks relies upon rapid and accurate diagnosis. The time taken to transport suspect clinical material to a central laboratory delays the feedback of results to decision-makers in the field. Improvements to diagnosis might therefore be achieved using tests that can be deployed *in situ* (or close to a suspect premises), without transferring the samples to a central laboratory. The aim of this study was to evaluate a portable thermocycler (Bioseeq™; Smiths Detection, Watford) that could be used for field detection of FMD virus (FMDV).

2. Material and methods

The Bioseeq™ is a portable, battery operated real-time PCR machine weighing approximately 3 Kg with 6 independently programmable modules. The instrument can be operated in the field employing preset protocols and the in-built LED screen to present assay results. A real-time RT-PCR [1] was adapted as a one-step assay for use on the Bioseeq™ using primers and probes that have undergone validation for diagnostic sensitivity [2]. Reactions contained 6 mM MgSO₄, 0.2 mM of each dNTP, 20 pmols of each primer, 7.5 pmol FAM-TAMRA dual labeled fluorogenic probe and 1 µl of the enzyme SuperScript™ III RT/Platinum® Taq mix. RNA template was amplified using a single thermocycling programme: RT step (60°C for 600 s) followed by 50 cycles of PCR amplification (95°C for 20 s and 60°C for 30 s).

3. Results

RT-PCR products of the expected size (97 bp) could be visualized in a 2% agarose-gel indicating that the correct region of the FMDV genome had been amplified successfully. For subsequent assays, the Bioseeq™ Support Software (ver 1.24, Smiths Detection) was used to assign cycle threshold (C_T) values for the RT-PCR. The limit of detection of the real-time RT-PCR on the Bioseeq™ was comparable to the laboratory-based diagnostic assay, although the C_T values produced on the Bioseeq™ were higher for the corresponding RNA samples. The variability of the Bioseeq™ modules was assessed by testing aliquots of an RNA sample prepared from the reference FMDV isolate O₁ Manisa. These results showed that mean *inter*-module variability (CV) was 6.5% (all 6 modules) in comparison with mean *intra*-module variability of 4.7% (*n* = 5 separate runs).

4. Discussion and Conclusions

Although further development and validation is required, these preliminary results are encouraging and indicate that it is possible to develop a sensitive RT-PCR assay for FMDV on the Bioseeq™ platform which can generate a result within 60 minutes. Clearly, effective preparation of template RNA is an important consideration for the deployment of this technology in the field, since the presence of tissue-derived factors may inhibit the RT-PCR. This aspect was previously recognised as a limitation of molecular methods for field detection of FMDV [3]. Future work will address the development of a combined simple-to-use extraction protocol in conjunction with a novel FMDV assay.

5. Acknowledgements

This work was funded by DEFRA project SE1121 and EU project LAB-ON-SITE (SSPE-CT-2004-513 645).

6. References

1. Reid, et al., (2003) *J. Virol. Meths* 107, 129-139;
2. King, et al., (2006) *JVDI* 18, 92-96;
3. Hearps et al. (2002) *Vet. Rec.* 150, 625-628.

EVALUATION OF EMERGENCY FMD VACCINE IN SHEEP FOLLOWING AEROSOL CHALLENGE BY PIG

S. Parida, L. Fleming, Y. Oh, S. Das, S. Uppadhya, M. Mohapatra, J. Gloster, D. Paton

Institute for Animal Health, Pirbright laboratory, Surrey, United Kingdom

Key words: Emergency FMD vaccination, sheep, sub-clinical detection of FMD

1. Introduction and Objectives

The 2001 outbreak in the UK showed that strict movement controls combined with the stamping out of infected and contact animals are not always sufficient to eradicate FMD quickly, have high economic costs and cause great public alarm. In future, a policy of vaccinate-to-live may be included in the repertoire of control measures and in support of this approach, we have investigated: a) The efficacy of emergency FMD vaccine in sheep, b) The reduction in virus excretion from vaccinated and then infected sheep, c) The ability to detect sub-clinical infection in vaccinated sheep, d) The possibility of transmission of the FMDV from vaccinated sub clinically infected sheep to naive vaccinated sheep and e) The prevalence of persistence of FMDV in the oropharynx of sheep.

2. Material and methods

Two groups (n=10 per group) of O1 Manisa vaccinated sheep were exposed to indirect airborne challenge from O1 UKG 2001 FMDV infected pigs for nine hours at 4 (group 1) and 10 (group 2) days after vaccination. Another 8 sheep served as unvaccinated controls, 4 animals being allocated to each vaccine challenge group. A further 8 sheep were used as vaccinated sentinels. Four were vaccinated at the same time as group 1 and mixed with this group two days after their challenge. Another 4 were vaccinated at the same time as group 2 and were mixed with this group two days after challenge. Thereafter, the sheep were monitored for a minimum 39 days. Excretion of virus in nasal and oro-pharyngeal secretions and in exhaled air will be measured by virus isolation and real time RT-PCR. Two commercial ELISAs for antibodies to FMDV non-structural proteins were evaluated for detection of infection. Cell mediated immune responses will be measured and correlated to protection.

3. Results

Nine hours aerosol challenge infected all 8 unvaccinated control sheep. All the vaccinated sheep were clinically protected, although body temperatures were elevated in 4 day vaccinated animals (n=4) for one day. Although vaccination could not prevent virus replication, it reduced virus excretion in comparison to unvaccinated sheep. Sub-clinical infection in vaccinated sheep was detected by non-structural protein antibody tests. Analysis of real time RT-PCR and virus isolation from nasal and probang samples are on going and from the preliminary results it is evident that viral RNA/live virus was more readily detectable in challenged sheep vaccinated 4 days before compared to 10 days before. Although virus/ viral genome was detected from the sentinels introduced to the 4day vaccinated group these sheep did not seroconvert to non-structural proteins.

4. Discussion and Conclusions

A) Although emergency vaccination could provide complete clinical protection in sheep, it did not protect from sub-clinical infection. However, it reduced virus excretion in sub-clinically infected sheep (Barnet et al., 2004 and Cox et al., 1999). B) Transmission from vaccinated sub-clinically infected sheep to other vaccinates was not convincingly demonstrated. C) Sub-clinical infection in vaccinated sheep was detected by non-structural protein antibody tests as well as by real time PCR. D) Prevalence of carrier in vaccinated sheep will be assessed after evaluation of all samples.

5. Acknowledgements

This work is supported financially by Defra, UK (Project SE1122).

6. References

1. Barnett, P.V., P. Keel, S. Reid, et al. 2004. Evidence that high potency foot-and-mouth disease vaccine inhibits local virus replication and prevents the carrier states in sheep. *Vaccine* 22, 1221-1232;
2. Cox S.J, P.v Barnet, P. Dani et al 1999. Emergency vaccination of sheep against FMD: protection against disease and reduction in contact transmission. *Vaccine*. 17, 1858-1868.

MONOCLONAL ANTIBODY BASED ELISAs TO DETECT ANTIBODIES AGAINST NEURAMINIDASE SUBTYPES 1, 2 AND 3 IN AVIAN SERA

P. Cordioli, A. Moreno Martin, D. Lelli, E. Sozzi, A. Luppi, V. Tranquillo, E. Brocchi

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, IZSLER, Brescia, Italy

Key words: Avian influenza, N1-N2-N3 antibodies, ELISA, monoclonal antibodies

1. Introduction and Objectives

Since 1997 epidemic waves of Avian Influenza (AI) have occurred in Italy; control measures adopted in high density populated areas included vaccination with AI strains of the same haemagglutinin (H) and different neuraminidase (N) subtype combined with the detection of specific anti-N antibodies to discriminate vaccinated from infected animals (1). Objective of this study was the development and the evaluation of diagnostic performances of competitive ELISA assays based on monoclonal antibodies for the detection of anti-N1, -N2, -N3 antibodies in avian sera.

2. Material and methods

Serum samples

A total of 1450 sera from different avian species (610 from chicken, 727 from turkeys, 89 from ducks, 14 from quails and 10 from ostriches) were used. Samples included: 854 negative field sera and 596 sera from vaccinated or infected animals (185 sera positive to N1-AIVs, 136 sera positive to N2-AIVs, 219 sera positive to N3-AIVs, 56 sera positive to other N). In addition, some sera (n. 109) were tested in parallel by the three ELISA tests and NI assays.

Liquid phase blocking ELISA (LPBE)

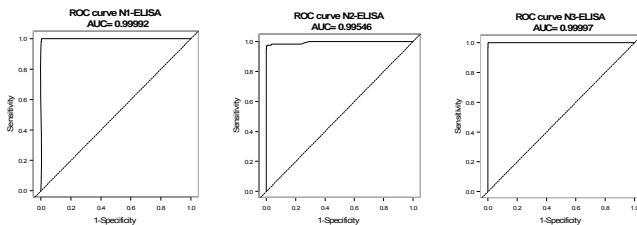
The same anti-N1, -N2, -N3 specific MABs, selected for the development of Neuraminidase-typing ELISAs (2) were also used to design competitive-based ELISAs for antibodies detection. For LPBE, detergent-treated N1- or N2- or N3- AIV reference strains are pre-incubated with test sera and transferred into ELISA plates coated with an homologous MAB; after incubation and washing, the second peroxidase conjugated MAB is distributed.

Statistical analysis

The discriminating power of ELISAs was evaluated by ROC (Receiver Operating Characteristic) curves. The agreement between ELISA and Neuraminidase Inhibition (NI) tests was assessed by Kappa value.

3. Results

All 1450 sera were tested in parallel by the three LPBEs. The relevant ROC curves and "Area Under Curve" values showed that the tests are very close to 100% sensitivity (Se) and specificity (Sp). At the unique cut-off (75% inhibition) selected for the three tests, the high levels of Se and Sp reported in the annexed table were found. The preliminary comparison of the ELISA and NI assays showed an almost perfect agreement (K=1) between N2-LPBE and NI for anti-N2 antibodies detection and a substantial agreement between N1-LPBE and NI (K=0,76) and between N3-LPBE and NI (K=0,8). The lower correlation was mainly caused by infected duck sera that resulted positive in ELISA and negative in NI tests.



		% inhib	Se	Sp
ELISA	N1	75%	1	0.99
	N2	75%	0.97	0.99
	N3	75%	0.99	1

4. Discussion and Conclusions

The validation process of the three developed ELISAs proved excellent diagnostic performances, with high specificity and sensitivity. The discriminating potential between antibodies elicited against homologous and heterologous N make the tests validated for use as "DIVA" assays, to distinguish between vaccinated and infected birds in vaccination programs based on vaccines containing a heterologous N.

5. References

1. Capua I, Terregino C, Cattoli G, Mutinelli F and Rodriguez JF. (2003) *Av. Pathol.*, 32: 47-55;
2. Lelli D, Cordioli P, Moreno A, Gamba D, Sozzi E and Brocchi E. (2006) *Proc 7th International Congress ESVV, Lisboa – Portugal*, pp.194.

PHYLOGENY OF THE MYCOPLASMA MYCOIDES CLUSTER: CONSEQUENCES IN TAXONOMY AND APPLICATION FOR SPECIES IDENTIFICATION

L. Manso-Silván¹, X. Perrier², F. Thiaucourt¹

¹CIRAD BIOS, UPR 15, Control of exotic and emerging animal diseases, Montpellier, Cedex, France

²CIRAD BIOS, UPR 75, Biomathematics: Genetic improvement of vegetatively propagated crops, Montpellier, Cedex, France

Key words: M mycoides cluster, phylogeny, taxonomy

1. Introduction and Objectives

The *Mycoplasma mycoides* cluster consists of six closely related mycoplasmas that are pathogenic for ruminants and which are currently referred to as: *Mycoplasma mycoides* subsp. *mycoides* biotypes Small Colony (MmmSC) and Large Colony (MmmLC), *M. mycoides* subsp. *capri* (Mmc), *M. capricolum* subsp. *capricolum* (Mcc), *M. capricolum* subsp. *capripneumoniae* (Mccp) and a group of strains that remain unassigned called *Mycoplasma* sp. bovine group 7 of Leach (MBG7). Since the members of this cluster share many genotypic and phenotypic traits their classification and evolutionary relationships have been difficult to establish. In addition, the position of species such as *M. putrefaciens*, *M. cottewii* and *M. yeatsii* has not been ascertained as yet, although they are considered related to the *M. mycoides* cluster. Phylogenetic analysis based on 16S rRNA sequences relied on too few nucleotide variations to construct a robust phylogenetic tree. It was then proposed that phylogenetic studies be based on sequence analysis of alternative genes.

2. Material and methods

Five housekeeping genes were identified (*fusA*, *glpQ*, *gyrB*, *lepA* and *rpoB*) that met the requirements for phylogenetic studies. A sample of strains from various geographical origins was carefully chosen, adapting their number to the known variability within each group. The choice of primers for PCR amplification was based on alignments from published sequences, as well as sequences from ongoing genome sequencing projects. The resulting PCR products were sequenced and alignments were obtained by ClustalW. Phylogenetic analyses, carried out with Darwin 5.0 included calculation of distance matrices and creation of phylogenetic trees by the neighbour-joining method.

3. Results

A robust phylogeny of the *M. mycoides* cluster was inferred from a set of concatenated sequences from these five genes. This phylogeny was reinforced by detailed analysis of the congruence of the phylogenies derived from each gene sequence independently. Two subclusters were distinguished. The *M. mycoides* subcluster comprised MmmSC, MmmLC and Mmc, where MmmLC and Mmc could not be clearly separated. The *M. capricolum* subcluster included Mcc, Mccp and MBG7, with MBG7 constituting a distinct branch. *M. cottewii* and *M. yeatsii* clustered in a group that was distinct from *M. putrefaciens* and they were all clearly separated from the *M. mycoides* cluster.

4. Discussion and Conclusions

This approach has permitted to clarify the phylogenetic positions of all the members of the *M. mycoides* cluster and related species and has put in evidence the need to adjust the existing taxonomy, as proposed: (i) MmmLC and Mmc may be united into a single subspecies and named collectively *M. mycoides* subsp. *capri*, as previously proposed. (ii) MBG7 may be established as a new subspecies of *M. capricolum*. Furthermore, this method may be used as a reference technique to unequivocally assign a position to any particular strain related to this cluster and may eventually lead to the development of new techniques for rapid species identification.

5. Acknowledgements

We are grateful to Valerie Barbe from Genoscope (Evry, France) for MmmLC strain 95010-C1 whole genome sequencing and to François Poumarat and Florence Tardy, from AFSSA (Lyon, France) for characterisation of the strains by dot immunobinding.

DIVA ASSAYS: MULTIPCR VS MULTIPLEX PCR

L. Liu¹, F. Widén¹, C. Baule¹, S. Belák¹

¹*Joint R&D Division in Virology, The National Veterinary Institute & The Swedish University of Agricultural Sciences, Uppsala, Sweden*

Key words: CSFV, DIVA

1. Introduction and Objectives

Classical swine fever (CSF), a notifiable disease to OIE, is a highly contagious viral disease affecting both domestic pigs and wild boars. Wild boars are playing an important role in the primary outbreaks in domestic pigs in several European countries, as they are potential reservoir of Classical swine fever virus (CSFV). To control CSF in wild boars, one of the marker vaccine candidates has been developed and evaluated (1). The purpose of this study is to compare performances of both gel-based and real-time RT-PCR DIVA assays in both multiPCR and multiplex PCR formats.

2. Material and methods

Viruses used in this study included 16 BVDV-1 (strain NADL and other 15 isolates), 3 BVDV-2, 6 BDV, 30 CSFV strains, and an atypical pestivirus D32/00-“HoBi”. In vitro transcribed RNA from the vaccine construct was used for this study. The RT-PCR assays for wild-type CSFV have been described previously (2). For detection of vaccine candidate, one-step gel-based and real-time RT-PCR assays were developed, using the same commercial kit and cycling profiles as for wild-type assays. MultiPCR was performed in a two-tube format (one for wild-type and another for vaccine candidate) with same composition, while multiplex PCR was carried out in a one-tube format (two reactions in one tube).

3. Results

The performances of two assays in two formats were same in terms of specificity. All assays detected only corresponding targets out of all tested viruses and RNAs. The sensitivity of multiPCR assays were expressed as copies per reaction based on in vitro transcribed RNA standards. The detection limits for real-time PCR were 50 copies for both wild-type and vaccine. And for gel-based assays the detection limit was 50 for wild-type and 500 copies for vaccine candidate. However, detection limits were severely affected by multiplexing PCR, a decrease of 10-100 fold comparing to multiPCR.

4. Discussion and Conclusions

Multiplexing PCR has been described for genetic detection and differentiation of multiple targets in one tube. It has advantages of cost-effective, reduced handling of samples and the opportunity for contamination. Therefore it is desirable whenever there is a possibility for choosing target genes given a suitable real-time PCR instrumentation or different amplicon size. However one major drawback is loss of sensitivity. In this study, despite extensive optimisation, the sensitivity of the multiplex PCR was still reduced around 10-100 fold. Since the vaccine candidate is a BVDV backbone with replacement of CSFV E2 gene, the freedom of choosing targets was limited, which restricted positioning primers and probe for differentiation. Rapid, sensitive, and specific detection and differentiation of CSFV from vaccine weight much more than cost-consideration, therefore multiPCR is still a format of choice for DIVA assays.

5. Acknowledgements

This work has been funded by the EU project (SSP1-501599).

6. References

1. Koenig P, Lange E, Reimann I, Beer M. 2007. doi: 10.1016/j.vaccine.2006.12.052;
2. Liu L, Widen F, Baule C, Belak S. 2007. doi: 10.1016/j.jviromet.2006.10.007.

MOLECULAR EPIDEMIOLOGY OF FOOT-AND-MOUTH DISEASE VIRUS TYPE C

N. Knowles, K. Swabey, R. Midgley, P. Davies, J. Wadsworth

Institute for Animal Health, Pirbright Laboratory, Pirbright, Woking, Surrey, United Kingdom

Key words: Foot-and-mouth disease virus, Molecular epidemiology

1. Introduction and Objectives

Foot-and-mouth disease (FMD) is considered one of the most threatening diseases for the livestock industry. FMD virus (FMDV) exists as seven immunologically distinct serotypes named O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3. The epidemiology of FMDV serotype C is not well understood. In the past outbreaks due to this serotype in South America, Africa and Asia were far less prevalent than the other serotypes which occur in these regions. Since 1995, only about seven suspected outbreaks of FMDV C have been reported throughout the world. Laboratory confirmation has been obtained for cases in Nepal in 1996, Kenya in 1996, 1998, 2000 and 2004, and Brazil in 2004. However, a suspected outbreak in Pakistan in 2004 was not confirmed. To extend our knowledge of the epidemiology of FMDV C the complete VP1 sequences of over 130 isolates from Europe, South America, Asia and Africa were determined and compared with those already published.

2. Material and methods

Virus RNA was extracted from cell culture grown virus isolates and the VP1-coding region was amplified using a one-step RT-PCR. Resulting amplicons were sequenced using the Beckman CEQ8000 system. Phylogenetic analyses were performed using MEGA 3.1 (1).

3. Results

Phylogenetic analyses revealed four major lineages. The first contained the classical C₁ and C₂ subtypes which were found in mainly in Europe. The second was one of two C₃ lineages which occurred almost exclusively in South America, apart from one isolate from Angola in Africa. The third lineage contained the other South American C₃ lineage (as represented by C₃/Resende/Brazil/55) with additional isolates from Europe and the Middle East from the period 1969-70. This lineage also contained isolates from the Philippines (1976 to 2004). The fourth major lineage contained viruses from Asia (Bangladesh, Bhutan, India, Kuwait, Nepal, Saudi Arabia, Sri Lanka and Tajikistan) and Africa (Ethiopia, Kenya and Uganda).

4. Discussion and Conclusions

The eradication of FMDV C from Europe and South America can be explained by vigorous control measures including vaccination; however, the reason for its disappearance from Africa and Asia is less clear. FMDV C was present in some East African countries from the late 1950's through to the early 1970's; however, viruses from later outbreaks (which occurred only sporadically) in Kenya and Ethiopia appear to be either vaccine-related re-introductions or cases of laboratory contamination. The sudden disappearance of FMDV C from the Indian sub-continent in 1996 contrasts with the occurrence of O, A and Asia 1 which have remained despite vaccination. FMDV C had been introduced into the Philippines in 1976 (from South America) and was well established; however, it completely disappeared following the introduction of type O from Hong Kong in 1994. Monitoring for new outbreaks of FMDV C and establishing their origin will assist in the control and global eradication of this serotype.

5. Acknowledgements

This work was partially supported by grants from Defra, UK (nos. SE2921 and SE2935).

6. References

1. Kumar S, Tamura K, Nei M. (2004). MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* 5: 150–163.

USE OF A NOVEL RECOMBINANT COWPEA MOSIAC VIRUS PARTICLE AS AN INTERNAL CONTROL FOR DIAGNOSTIC RT-PCR

D. King¹, N. Montague², K. Ebert¹, S. Reid¹, J. Dukes¹, L. Schädlich², G. Belsham^{1,3}, G. Lomonosoff²

¹*Institute for Animal Health, Pirbright, Surrey, United Kingdom*

²*Department of Biological Chemistry, John Innes Centre, Norwich, United Kingdom*

³*Current address: National Veterinary Institute, Technical University of Denmark, Kalvehave, Denmark*

Key words: RNA, internal control, real-time RT-PCR, capsid

1. Introduction and Objectives

The OIE guidelines for the validation and quality control of PCR tests recommend that internal controls are included into the assay to verify negative results [1]. These controls confirm that substances that are inhibitory to the enzymatic RT-PCR steps (which would yield false-negative results) are not present in the samples. Artificial RNA constructs can be generated by *in-vitro* transcription for use in diagnostic real-time RT-PCR assays. Unfortunately these "naked" RNA transcripts are relatively unstable since they are prone to degradation by RNases. Furthermore, since a lysis step is not required to release the mimic RNA, these transcripts only provide limited validation of the RNA extraction process where viral genome is typically encapsidated or resides in intra-cellular compartments. The aim of this project was to generate an encapsidated RNA suitable for use as an internal control in diagnostic RT-PCRs for foot-and-mouth disease virus (FMDV) and swine vesicular disease virus (SVDV).

2. Material and methods

Cowpea Mosaic Virus (CPMV) is a non-enveloped, RNA plant virus with similarities to picornaviruses such as FMDV and SVDV. CPMV has a bipartite single-stranded positive sense genome of 9.4 kb: each of the segments (5.9 kb and 3.5 kb respectively) is packaged separately in the mature capsid. A cassette containing sequences of 2 diagnostic primer sets for FMDV and a single set for SVDV was engineered into a full-length cDNA clone of the RNA-2 segment of CPMV. After co-inoculation with a plasmid that expressed CPMV RNA-1, recombinant virus particles containing the expected RNA were rescued from Cowpea plants (*Vigna unguiculata*). For passaging, virus in the sap of agroinoculated leaves was first concentrated by precipitation with polyethylene glycol before applying the extract to further, healthy cowpea plants. Virus particles were purified and, where required, the components were separated by centrifugation on Nycodenz (Nycomed) gradients.

3. Results

RNA contained in these particles was successfully amplified using 2-colour real-time RT-PCR with a VIC™ labelled probe specific for the engineered construct. When added directly to clinical samples, the recombinant CPMV did not reduce the analytical sensitivity of the real-time RT-PCR assays to detect FMDV or SVDV. Stability experiments showed that there was no significant degradation of the encapsidated RNA at room-temperature over a 33 day period. Furthermore, the RNA within the CPMV particle was resistant to digestion by RNase A (20 µg/ml), in comparison to equivalent *in-vitro* RNA transcripts.

4. Discussion and Conclusions

This system provides an attractive solution to the production of internal controls for real-time RT-PCR assays since the particles are thermostable at room temperature, RNase resistant and CPMV grows to high yields in plants. Simple purification of RNA-2 containing capsids (based on differential density) can yield a non-infectious particle preparation with similar structural properties to picornaviruses such as FMDV and SVDV.

5. Acknowledgements

This work was funded by DEFRA project SE1121, EU project LAB-ON-SITE (SSPE-CT-2004-513 645) and the BBSRC.

6. References

1. Belak S., Thoren P., 2001. *Expert Rev. Mol. Diagn.* 1: 434.

DEVELOPMENT OF A N2-BASED DIVA TEST FOR DUCKS: CONSTRUCTION OF A RECOMBINANT BACULOVIRUS EXPRESSING N2 TYPE NEURAMINIDASE PROTEIN AVIAN INFLUENZA VIRUS

O. Guionie, V. Jestin

AFSSA, Swine and Poultry Research Laboratory, Avian And Rabbit Virology Immunology and Parasitology Unit, Ploufragan, France

Key words: Avian Influenza, N2 subtype, recombinant baculovirus, DIVA strategy

1. Introduction and Objectives

Besides the risk of introduction of HP H5N1 AIV that fluctuates according to the season and the world epidemiological situation, France has to control the potential infection of duck and goose flocks with LP H5 AIV, as reported in the results of AI national surveys. Among them H5N2 AIV are the most frequent. So, if a preventive vaccination campaign was implemented to prevent H5 LP infection using an heterologous NA as the vaccinal strain, it might be useful to have got a reliable test able to detect N2 antibodies in these species (ducks and geese) for the DIVA strategy. For this purpose we intend to set on an immunofluorescence antibody test (iIFAT) and an ELISA test using a recombinant N2 protein expressed in the baculovirus system.

2. Material and methods

The first step consisted to select the N2 type neuraminidase. Neuraminidase inhibition assays allowed to test the specificity of several N2 strains against heterologous AI antibodies. This technique was also used to select the strain giving the strongest responses using N2 sera. The second step was to put the N2 gene in an expression system. The "Bac-to-Bac Baculovirus Expression System" (Invitrogen) was used to produce a recombinant baculovirus. The N2 gene was cloned into a bacmid where its expression is controlled by the polyhedrin promoter. Construct was checked by nucleotide sequencing. The third step was to transfect the recombinant bacmid DNA into Sf9 insect cells to generate a recombinant baculovirus. The baculoviral stock was amplified and used to infect cells for large-scale expression of the N2 neuraminidase protein. The protein expression will be checked by Western Blot and/or neuraminidase inhibition assay. This protein will be used as antigen to prepare an iIFAT and an ELISA test.

3. Results

12 HxN2 strains from our collection lab were tested. The use of 7 N2 antibodies allowed to select a H5N2 strain called A/Duck/France/05057b/2005. The N2 gene was cloned and absence of mutation was verified in the final construct. At this date, we are producing the "master seed". In parallel, we are constructing a bacmid coding for the same N2 protein without the cytoplasmic and transmembrane domains, in order to facilitate the protein excretion in Sf9 cells.

4. Discussion and Conclusions

After cell infection using the "master seed", presence of N2 protein will be checked by Western Blot. However, previous results obtained in the lab and other teams showed that the NA protein could be present without having been detected by this technique. That's why a neuraminidase inhibition assay will be performed in order to detect the full length protein in cells and the truncated protein in cell supernatant. The next steps will be to seed infected Sf9 cells expressing the recombinant N2 protein in plates and to test and optimise (cell density, MOI, serum and conjugate's concentrations, etc ...) the iFAT using serum samples. An ELISA test is also programmed, using the truncated N2 protein.

5. Acknowledgements

We thank Béatrice Grasland and Anne-Cécile Nignol (GVB unit, AFSSA Ploufragan) for insect cell culture.

UTILIZATION OF A MICROARRAY-BASED PADLOCK PROBE SYSTEM IN THE VIRAL DIAGNOSTICS

J. Banér¹, P. Gyarmati¹, A. Yacoub¹, M. Hakhverdyan¹, J. Stenberg², O. Ericsson²,
M. Nilsson², U. Landegren², S. Belák^{1*}

¹ The National Veterinary Institute and the Swedish University of Agricultural Sciences, Uppsala, Sweden;
² Uppsala University, Uppsala, Sweden

Keywords: foot-and mouth disease; swine vesicular disease; vesicular stomatitis; padlock probes; microarray

1. Introduction and Objectives

Padlock probes are circularizable oligonucleotides that offer significant advantages in multiplex analyses. The circular structure can be formed through intramolecular ligation of the probe ends upon hybridization to a target sequence. This allows several different types of padlock probes (i.e. recognizes different targets) to be used simultaneously without cross-reactions. All probes have a common sequence that allows amplification of circularized padlock probes with the same primer/s, either by rolling circle amplification (RCA) or by PCR. Each type of probe also contains a unique sequence by which the amplification products can be analysed on a microarray, where oligonucleotides complementary to the unique sequences have been positioned in a predetermined manner.

Here we present a simultaneous, complex and rapid method using 36 padlock probes and one microarray for the detection of three vesicular viruses. The assay gives information about the potential presence of Foot-and-mouth disease virus (FMDV), Swine vesicular disease virus (SVDV), and Vesicular stomatitis virus (VSV), as well as serotype information in case of VSV, in a single reaction tube. In summary, this method offers more effective diagnosis for viruses causing similar vesicular symptoms.

2. Material and methods

Padlock probes were designed towards target sequences in conserved or semi-conserved regions within the three viruses. Nineteen FMDV, 15 SVDV, and 7 VSV cDNA samples were used to optimize assay performance. The probes were added to the samples, ligated then amplified twice by RCA and PCR. Then each reactions were transferred to microarray slides into subarrays and hybridized. The slides were scanned and the images analyzed.

3. Results

Virus identities of all FMDV and SVDV samples were correctly determined, as well as of the 5 VSV samples classified as informative.

4. Discussion and Conclusions

A powerful novel method is described for the simultaneous and rapid detection of multiple viruses in the vesicular disease complex of swine, using padlock probes with a microarray-based read-out. The assay principle is straightforward comprising a few internally controlled reaction steps in a single vessel. Run-times are comparable to real-time PCR, but with the benefit that the presence of several viruses and their various serotypes can be analyzed within the same reaction.

5. Acknowledgements

Dr. Donald King, Institute for Animal Health (Pirbright Laboratory, UK), Dr. Montserrat Agüero and Dr. Jovita Fernandez, Centro de Investigación en Sanidad Animal (CISA-INIA, Valdeolmos, Madrid, Spain), kindly provided samples used in the study.

6. References

1. Baner et al, 2003: Parallel gene analysis with allele-specific padlock probes and tag microarrays. *Nucleic Acids Res* 31, e103;
2. Baxi et al., 2005: Microarray-based detection and typing of foot-and-mouth disease virus. *Vet J.* 172(3):473-81.

PRELIMINARY VALIDATION OF A COMMERCIAL AVIAN INFLUENZA N1 ANTIBODY COMPETITIVE ELISA KIT THAT CAN BE USED AS PART OF A DIVA STRATEGY

**WG Dundon¹, C. Terregino¹, V. Tuttoilmondo¹, M. Pizzuto¹, L. Busani²,
M. Mancin², G. Cattoli¹, I. Capua¹**

¹OIE\FAO and National Reference Laboratory for Avian Influenza and Newcastle Disease, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, PD, Italy ;

² Centro Regionale di Epidemiologia Veterinaria, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, PD, Italy

Key words: DIVA, Avian influenza, kit validation

1.Introduction and Objectives

The use of vaccination as part of a set of coordinated measures to combat AI is deemed to be successful in achieving the goal of eradication if it allows for the "DIVA" (Differentiation of Infected from Vaccinated Animals) principle (1). These systems enable the detection of field exposure in vaccinated flocks and through this, infected flocks may be properly managed. One of the systems that enables the detection of field virus in a vaccinated population is based on the use of a vaccine containing a seed virus of the same H subtype but of a different N subtype to the field virus (eg H5N9 vaccine against H5N1 field virus). Cross-protection is ensured by the same H group and antibodies to the N of the field virus are a result of field infection. In the framework of Workpackage 4.3 of EPIZONE (DIVA diagnostics) a commercially available competitive ELISA kit (ID Screen®, ID-VET, France) which is designed to specifically detect antibodies directed against the N1 antigen was validated using poultry sera from the OIE\FAO and National reference laboratory for Avian influenza and Newcastle disease, Padova Italy.

2.Material and methods

The ID Screen® ELISA kits were used according to the manufacturer's instructions. The sera tested were from (A) chickens vaccinated with an H7N5 subtype virus and challenged with an H7N1 subtype virus (n=33); (B) turkeys (n=13) and chickens (n=8) negative for type A influenza virus; (C) unvaccinated turkeys infected with H7N1 subtype virus (n=41); (D) unvaccinated turkeys infected with H7N3 subtype virus (n=40). Each sera was tested in duplicate. The results obtained from the ID Screen® ELISA were compared to the indirect immunofluorescence antibody assay (iIFA) which was taken as the gold standard (2). Cohen's Kappa statistic (K) value was calculated to assess the agreement between the tests.

3.Results

The overall sensitivity of the ID Screen® ELISA test as compared to the gold standard iIFA was 93.0% (CI 95% 85.0-98.0), 91% for chickens (CI 95% 76.0-98.0) and 95% for turkeys (CI 95% 83.0-99.0). The overall specificity of the ID Screen® ELISA test as compared to the gold standard iIFA was 100% (CI 95% 94.0-100.0), 100% for chickens (CI 95% 63.0-100.0) and 100% for turkeys (CI 95% 93.0-100.0). The K value was calculated as 0.9264 indicating "excellent agreement" between the two tests according to Landis & Koch (3).

4.Discussion and Conclusions

The preliminary validation of the ID Screen® ELISA test has revealed an almost perfect agreement with the iIFAT, with excellent specificity and a sensitivity of 93%. This is most probably due to the competitive nature of the test. Optimisation and further validation studies will be carried out using a larger number of sera from a larger variety of birds (e.g. ducks, quail, ostriches etc).

5.References

1. Capua I, Cattoli G, Marangon S. (2004) DIVA--a vaccination strategy enabling the detection of field exposure to avian influenza. *Dev Biol* 119:229-33;
2. Cattoli, G., Terregino, C., Brasola, V., Rodriguez, JF, Capua, I (2003) Development and preliminary validation of an ad hoc N1-N3 discriminatory test for the control of avian influenza in Italy. *Avian Dis.* 47: 1060-2;
3. Landis J, Koch G. (1977) An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. *Biometrics.* 33:363-74.

COMPARISON OF ELISAs FOR ANTIBODIES AGAINST FOOT-AND-MOUTH DISEASE VIRUS NON-STRUCTURAL PROTEINS IN CATTLE SERA BASED ON THE CONTINUOUS RESULTS

**A. Dekker^a, D. Samin^b, M. Greiner^c, I. Bergmann^d, D. Paton^e, S. Grazioli^f,
K. de Clercq^g, E. Brocchi^f**

^aCentral Institute for Animal Disease Control Lelystad, CIDC, Lelystad, The Netherlands;

^bCentral Veterinary Research Laboratory, Co. Kildare, Ireland;

^cFederal Institute for Risk Assessment (BfR, Berlin, Germany);

^dCentro Panamericano de Fiebre Aftosa OPS/OMS, PANAFTOSA, Rio de Janeiro, Brazil;

^eInstitute for Animal Health, Pirbright Laboratory, Surrey, United Kingdom;

International EpiLab, Morkhoj, Denmark;

^fIstituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, IZSLER, Brescia, Italy;

^gCODA-CERVA-VAR, Ukkel, Belgium

Key words:

1. Introduction and Objectives

Six tests for antibodies against FMDV non-structural proteins were compared at a workshop in Brescia, Italy in 2004 using dichotomous results. Continuous results were also available from the validation study, therefore we extended the comparison by using ROC analysis and estimation of the likelihood ratio using these data.

2. Material and methods

From the dataset 1337 sera were selected, 1100 from non-infected cattle and 237 from exposed cattle. In the analysis the sera from exposed cattle were considered true positive and from non-infected cattle as true negative. Using the results we produced ROC curves and computed the likelihood ratio for each possible result.

3. Results

ROC analysis showed that the IZS-Brescia and the Ceditest ELISA had a significantly higher sensitivity than the other commercial ELISAs at 99% specificity. At 97.5 and 95% specificity, NCPanaftosa, IZS-Brescia, Ceditest and Svanovir ELISAs were comparable and had the highest sensitivity. The likelihood ratio analysis provides information for each test on the likelihood ratio for a positive result. However the various tests produced different likelihood ratio's when used on the same serum.

4. Discussion and Conclusions

The ROC analysis confirms the previous finding that the NCPanaftosa, IZS-Brescia and the Ceditest ELISAs have both a high sensitivity combined with a high specificity. When a confirmatory test is available, as in the case of the Panaftosa western blot technique, a higher sensitivity can be achieved without the consequent loss in specificity. The analysis of the likelihood ratios provides information which can be used in decision making, but the results should be interpreted with caution. Titration of sera will probably provide more consistent results.

DEVELOPMENT OF A RT-LAMP ASSAY FOR THE DETECTION OF SWINE VESICULAR DISEASE VIRUS

A. Blomström¹, M. Hakhverdyan², S. Belak^{1,2}, M. Berg¹

¹*Department of Biomedical Sciences and Veterinary Public Health, Swedish University of Agricultural Sciences, Uppsala, Sweden;*

²*Joint Research and Development Division in Virology of the National Veterinary Institute, SVA, and the Swedish University of Agricultural Sciences, SLU*

Key words: SVDV Detection, loop-mediated isothermal amplification (LAMP)

1. Introduction and Objectives

Swine vesicular disease virus (SVDV) causes disease in pigs characterised by an increased body temperature and the development of vesicles. Mortality is rare and the animal usually recover within 2-3 weeks but the symptoms make it clinical indistinguishable from Foot-and-mouth disease (FMD) (Lin and Kitching 2000). Therefore it is important with rapid methods to detect SVDV and distinguish it from FMDV and other vesicular diseases. Loop-mediated isothermal amplification (LAMP) is a fast and simple method for detection of RNA and DNA were the result can be seen directly by the naked eye through the addition of an intercalating dye (Notomi, Okayama et al. 2000). The objective with this study was to set up a one-step reverse transcriptase-LAMP (RT-LAMP) assay for the detection of different SVDV strains.

2. Material and methods

Primers were design using PrimerExplorer v3 from the highly conserved 3D polymerase gene. The LAMP system was set up and optimised using in vitro transcribed RNA. The reaction is run for 1h at 63°C after which the result can be seen either directly through the addition of SybrGreen to the product or through a regular gel run. The RNA copy number was calculated and dilutions series were made in order to test the sensitivity. Also a time test study was made to see how long amplification time was needed for a positive result, this was done by taking out one sample every 5 minutes. The specificity was checked using RNA from 15 different strains of SVDV, also RNA from FMDV and VSV was used.

3. Results

The positive samples gave as expected a ladder-like pattern on the gel and the product turned green after the addition of SybrGreen while negative samples was brown. The time test showed that already after 25 minutes a strong positive signal was shown in both the gel run and in the SybrGreen test. All the SVDV strains used in the specificity test were positive and all FMDV and VSV samples were negative. The sensitivity was shown to be approximately 50 copies of RNA.

4. Discussion and Conclusions

Rapid detection methods are important for the detection of SVDV; LAMP has shown to be a fast and reliable detection method for different pathogens. The advantage to this kind of method compared with for example classical or real-time PCR is that it is very simple with no need for any special equipment like a PCR machine or computers; that the result can be seen directly by the naked eye. This makes it a good method that could be used under more primitive conditions like in a field laboratory. Since this is a one-step RT-LAMP without the need for a cDNA synthesis step this further simplifies the method and also decreases the risk of contamination. This assay has shown to be fast, SVDV specific and sensitive.

5. References

1. Lin, F. and R. P. Kitching (2000). "Swine vesicular disease: an overview." *Vet J* **160**(3): 192-201;
2. Notomi, T., H. Okayama, et al. (2000). "Loop-mediated isothermal amplification of DNA." *Nucleic Acids Res* **28**(12): E63.

A NOVEL REAL-TIME RT-PCR FOR THE DETECTION OF BLUETONGUE VIRUS

A. Shaw¹, P. Monaghan¹, H. Alpar², S. Anthony¹, K. Darpel¹, C. Batten¹, S. Carpenter¹,
H. Jones¹, K. Bankowska¹, C. Oura¹, D. King¹, H. Elliot¹,
P. Mertens¹

¹Pirbright Laboratory, Institute for Animal Health, Pirbright, Surrey, United Kingdom;

²-Centre for Drug Delivery Research, University of London, London, United Kingdom

Key words: RT-PCR, BTV

1. Introduction

Bluetongue virus (BTV) is the type member of the Orbivirus genus. BTV causes a haemorrhagic disease in ruminants which can result in significant economic loss. Recently BTV has been moving northwards into Europe, with severe outbreaks in 2006 in France, Belgium, Germany and the Netherlands. Traditionally BTV has been diagnosed by antibody detection by ELISA, however recently real-time RT-PCR (rRT-PCR) assays for the detection of BTV RNA have become valuable tools for early detection of BT disease.

2. Materials and Methods

Viral RNA was extracted either manually (Qiagen), or robotically (MagNA Pure LC robot). Existing Seg-1 sequences for BTV (and related orbiviruses) were downloaded from the GenBank database and a suitably conserved region for the assay identified. Further sequence data was generated by directly sequencing PCR products encompassing the proposed amplicon.

Suitable primers (Sigma) and probes (Eurogentec) were designed according to standard TaqMan conditions. The Invitrogen SuperscriptIII Platinum Taq one-step rRT-PCR kit was used for all rRT-PCR reactions. The assay was tested on a diverse range of BTV isolates as well as a 10-fold dilution series of *in vitro* transcribed RNA.

3. Results

It was found that BTV Seg-1 sequences divide into eastern and western genotypes. An eastern and western primer set were designed with universal probes. Initially, two probes were required to detect all viruses, The resulting assay is capable of detecting every BTV tested without cross-reacting with related orbiviruses (Shaw et al., 2007). Once automated, this assay was used extensively as a front-line tool during an outbreak of BTV-8 in Northern Europe in 2006.

4. Summary and Conclusions

The assay has been found to be sensitive and specific for the BTV serogroup. Furthermore, the assay now represents a diagnostic tool being used at the community reference laboratory for BTV (Pirbright, UK), and in other laboratories across Europe.

5. References

1. Shaw, A.E., Monaghan, P., Alpar, H.O., Anthony, S., Darpel, K.E., Batten, C.A., Guercio, A., Alimena, G., Vitale M., Bankowska, K., Carpenter, S., Jones, H., Oura, C.A.L., King, D.P., Elliott, H., Mellor P.S., Mertens, P.P.C. (2007) Development and initial evaluation of a real-time RT-PCR assay to detect bluetongue virus genome segment 1. *J. Virol Methods* [submitted].

PRIMER AND PROBE DESIGN FOR FOOT-AND-MOUTH DISEASE TYPING

B. Sareyyüpoğlu

Foot and Mouth Disease Institute, Ankara, Turkey

Key words: FMDV, serotyping, primer- probe, design

1.Introduction and Objectives

Foot and Mouth Disease (FMD) is a contagious viral disease of domestic and wild ruminants, which causes negatively effects animal trade between countries because of the economic losses encountered. Foot and Mouth Disease Virus (FMDV), has mainly 7 serotypes and many subtypes especially of serotype A. There is no cross immunity among the serotypes but partial cross immunity can be observed among the subtypes. FMD is known to be included OIE list and found 2/3 of countries of OIE. Serotypes O and A are endemic in Turkey and vaccination is the main measure for disease control. Since FMDV has variable serotypes and subtypes, and animal transport is not controlled appropriately difficulties were encountered in the control and prevention of the disease control. Besides, there is no rapid laboratory test applied in Turkey for FMDV diagnosis. Rapid and precise determination of serotypes causing FMDV epidemics fasten diagnosis (2). Recently, as like many disease, different PCR techniques are being evaluated for the detection and typing of FMDV serotypes, despite the lack of literature on molecular typing of the serotypes of FMDV by real time RT-PCR.

Efficiency of PCR firstly depends on primer and probe quality. A poorly designed primer can result in little or no product, due to the nonspecific amplification and/or primer-dimer formation leading to reaction failure although other PCR reaction conditions are optimized perfectly. When designing primers, they should have a GC content should be %40-60 in the primer sequence, T_m (melting temperature) values should be close to each other, preferably the same; should not be lower than 50°C, they should not have intra- and inter complementary bases (1).

The objective of this work is designing primers and probes for the detection and typing of FMDV by Real time RT-PCR techniques, precisely for O and A serotypes which are endemic in Turkey.

2.Material and methods

All steps of primer and probe design were performed with MacVector Program for Macintosh computer.

Establishing a sequence database for FMDV

For this purpose, all VP1 sequences of FMDV Turkey isolates and all complete FMDV genome sequences (GenBank) were investigated and gathered for alignment. Since there are not sufficient (only one for each serotype of FMDV) whole sequences of FMDV strains isolated in Turkey, sequences of FMDV strains isolated in other parts of the world were used.

Creating the alignments of the sequences

Creating Consensus Files

VP1, a structural protein of FMDV, containing the highly variable regions was used as the target sequence for the molecular differentiation of FMDV serotypes. Since the VP1 region has sincere heterogeneity amongst the FMDV serotypes, it was not easy to get enough consensus sequences. Therefore, suitable 5 sets of primer and probes were defined from VP1 region. Thus, sequences of nonstructural proteins (containing fewer variable regions) such as 2A and 2B were also searched for primer and probe design. By the help of the genome of nonstructural protein sequence (4250 bp), I have identified the consensus sequences of serotypes A and O of FMDV. Following the alignment of all consensus files, I have searched for the suitable primer sets (forward and reverse) from 4250 bp nonstructural protein region and the VP1 region. I have detected suitable 5 primer and probe sets from nonstructural protein region of FMDV.

Primer and Probe Design

Suitable primer sets were established from the consensus sequences manually not with the help of automatic search engines of program. Most suitable primer pairs were determined following several trials.

BLAST confirmation

As a final step, each suitable primer sets were confirmed via PubMed GenBank by Blast (www.ncbi.nlm.nih.gov/PubMed/Nucleotide/Blast) search. In other words, each primer sets were compared to FMDV sequences those submitted to GenBank before. Each primer sets were searched for cross-reaction between the O and A FMDV serotypes so each primer set was checked for their appropriateness.

3.Results

As a result, nearly 120 primer and almost 20 probe sets were determined following *in-silico* analyses. All were checked with Blast programs for their feasibility and finally only 10 sets of them were accepted for FMDV typing.

4. Discussion and Conclusions

Since FMDV serotype A mutates continuously, this primer design for this serotype was more difficult than the others. Another handicap of primer and probe design of FMDV Turkey isolates was lack of enough sequences for primer and probe design. As a conclusion, primer and probe sets were developed for the former in-house and the recent real-time PCR for molecular discrimination of FMDV serotypes in Turkey. I believe that, they have the potential of discriminating the most prevalent serotypes by molecular typing with PCR and/or real-time PCR for the first time in Turkey, which should yet to be investigated.

5. Acknowledgements

I would like to thank Dr. Chris Helps for his technical assistance in primer and probe design for FMDV typing during my visit to University of Bristol, Langford School of Veterinary Sciences as a co-researcher.

6. References

1. Dieffenbach, C.W. (2003). *PCR Primer a laboratory Manual*, Ed: Dieffenbach, C.W and Dveksler, G.S, In: Newyork, Inc: Gold Spring Harbor Laboratory Press, 2003;
2. Callens, M., Clercg, K.D., (1997); *Differentiation of the seven serotypes of foot-and-mouth disease virus by reverse transcriptase polymerase chain reaction. Journal of Virol. Methods.* 67:35-44.

CLINICAL SIGNS IN BLUETONGUE VIRUS SEROTYPE 8 INFECTED SHEEP, GOATS AND CATTLE

A. Backx, C. Heutink, E. van Rooij, P. van Rijn

Central Institute for Animal Disease Control Lelystad, CIDC, Lelystad, The Netherlands

Key words: Bluetongue virus serotype 8, clinical signs, experimental infection

1. Introduction and Objectives

The confirmation of Bluetongue virus serotype 8 (BTV-8) in the Netherlands on August 17th 2006 marks the onset of the first outbreak of bluetongue (BT) in Northwest Europe. Clinical signs of the disease were seen in sheep and cattle, but not in goats. The objective of this study was to assess the course of disease in experimentally BTV-8 infected sheep, goats and cattle.

2. Material and Methods

The washed cell fraction of EDTA-blood from BTV-8 field infected donor sheep and cows was injected in three sheep, two goats and three heifers originating from different farms in the Netherlands. Blood from two experimentally infected heifers was withdrawn to inoculate seven pregnant multiparous cows late in gestation. Two sheep, two goats and eight pregnant cows were included to serve as negative control animals. Blood and serum samples were collected from all the animals at set days post inoculation (dpi) and tested on BTV with a real-time Polymerase Chain Reaction (PCR) and a blocking Enzyme Linked Immuno Sorbent Assay (ELISA). Body temperature was measured and clinical signs were observed and recorded daily.

3. Results

All the inoculated animals but one heifer became PCR positive for BTV nucleic acid from 3 to 9 dpi and BTV-antibody positive from 7 to 14 dpi. Transient fever was measured in all the experimentally infected animals. Clinical signs of bluetongue were seen in all the inoculated animals but the heifers and one goat. These signs included serous to bloody nasal discharge, erosions and ulcers of oral and nasal mucosa, oedema, coronitis and lameness. The negative control animals stayed negative in BTV PCR and ELISA and didn't show any clinical signs.

4. Conclusions

Sheep, goats and cattle were successfully infected with bluetongue virus serotype 8 from field infected sheep and cows. Clinical signs were produced in cattle, sheep and one goat.

5. Acknowledgements

The authors thank the animal care takers who participated in the described research for their assistance.

A COMMON NEUTRALIZING EPITOPE ON ENVELOPE GLYCOPROTEIN E2 OF DIFFERENT PESTIVIRUSES: IMPLICATIONS FOR IMPROVEMENT OF VACCINES AND DIAGNOSTICS FOR CLASSICAL SWINE FEVER (CSF)?

P. van Rijn

Central Institute for Animal Disease Control Lelystad, CIDC, Lelystad, The Netherlands

Keywords: pestivirus, serological differential epitope, E2, antigenic structure

The Pestivirus genus within the family of Flaviviridae consists of at least four groups; Classical swine fever virus (CSFV) found in swine and wild boar, Bovine viral diarrhoea virus type 1 and type 2 (BVDV-I and BVDV-II) mainly isolated from cattle, and Border disease virus (BDV) preferably replicating in ovine species. Many features demonstrate differences between CSFV and other pestiviruses, BVDV-I, BVDV-II, and BDV, here defined as nonCSFV, whereas other features show similarities between all different groups of pestiviruses. Focusing on the major envelope glycoprotein E2, the immunodominant protein of pestiviruses, CSFV seems to be a more distinct group within the pestivirus genus. Here we confirm on one hand the more separated grouping of CSFV by isolation of monoclonal antibodies (MAbs) raised against E2 of BVDV-I and BVDV-II. None of these MAbs recognize E2 of CSFV strains. On the other hand, only one MAb, MAb 912, was isolated against E2 of BDV. MAb 912 binds to E2 of CSFV strains and partly neutralizes CSFV. The epitope of MAb 912 is mapped in antigenic domain B of CSFV-E2. This common epitope of CSFV strains and non CSFV strains could have implications for development of DIVA vaccines and serological diagnostics for CSF.

References

1. Dekker, A., Wensvoort, G., Terpstra, C., 1995, Six antigenic groups within the genus pestivirus as identified by cross neutralization assays. *Vet Microbiol* 47, 317-329;
2. Deregt, D., Van Rijn, P.A., Wiens, T.Y., Van den Hurk, J., 1998b, Monoclonal antibodies to the E2 protein of a new genotype (type 2) of bovine viral diarrhoea virus define three antigenic domains involved in neutralization. *Virus Res* 57, 171-181;
3. Paton, D.J., Sands, J.J., Lowings, J.P., Smith, J.E., Ibata, G., Edwards, S., 1995, A proposed division of the pestivirus genus using monoclonal antibodies, supported by cross-neutralisation assays and genetic sequencing. *Vet Res* 26, 92-109;
4. Van Rijn, P.A., Miedema, G.K., Wensvoort, G., Van Gennip, H.G., Moormann, R.J., 1994, Antigenic structure of envelope glycoprotein E1 of hog cholera virus. *J Virol* 68, 3934-3942;
5. Van Rijn, P.A., van Gennip, H.G., Leendertse, C.H., Brusckke, C.J., Paton, D.J., Moormann, R.J., Van Oirschot, J.T., 1997, Subdivision of the pestivirus genus based on envelope glycoprotein E2. *Virology* 237, 337-348.

EVALUATION OF PCR FAST FORMAT FOR THE RAPID DIAGNOSIS OF AFRICAN SWINE FEVER (ASF)

J. Fernández, C. Gallardo, R. Nieto, A. Robles, C. Pérez, M. Arias

EU, OIE Reference Laboratory for African Swine Fever, CISA-INIA, Valdeolmos, Madrid, Spain

Key words: Fast PCR, ASF, Rapid diagnosis

1. Introduction and Objectives

African Swine Fever (ASF) is a highly contagious infectious disease that affects swine, caused by a complex DNA virus of the *Asfarviridae*. It is a notifiable disease, that produces great economic losses in the affected regions. Currently, it is endemic in more than twenty Sub-Saharan African countries, and in Sardinia. As no vaccine is available, control and eradication strategies are mainly based on rapid and accurate laboratory diagnosis and on the enforcement of strict sanitary measures. Different PCR methods have been already described for the ASF Virus (ASFV) detection^{1, 2, 3}. In this work, two previously described conventional¹ and real-time² PCR methods have been modified to a fast format, and they have been evaluated for their application in the rapid molecular diagnosis of ASF.

2. Material and methods

Serum and EDTA-blood samples collected from ASFV experimentally infected pigs were employed in the assays. A collection of 80 clinical samples of pigs proceeding from ASF recent African outbreaks, and 30 serum samples of pigs and wild boars from the Spanish ASF surveillance program were also used in this study. Total DNA was extracted from samples using *High Pure PCR Template Preparation Kit (Roche)*, following manufacturer's instructions.

Described conventional PCR¹ was performed using *TaqGold DNA polymerase (Applied Biosystems)*, and real-time PCR previously reported² was achieved using *FastStart Taq DNA polymerase (Roche)*. Fast PCR protocols were optimised using *Qiagen Fast Cycling PCR* and *Quantifast Probe PCR kit (Qiagen)* for conventional and real-time assays, respectively.

3. Results

The detection of ASFV using all the compared conventional and real-time PCR assays was observed since the second day post-inoculation in EDTA-blood and the third day in serum of experimentally infected pigs. When field clinical samples from pigs and wild boars, including EDTA-blood, serum, and tissue homogenates were tested, similar results were also obtained comparing the standard¹ and fast conventional PCR protocols, as well as the standard² and fast real-time assays.

4. Discussion and Conclusions

Highly sensitive and specific conventional and real-time PCR methods have been reported for the ASFV detection^{1, 2}, and they are currently applied in diagnostic laboratories. The first results of an evaluation study comparing the mentioned PCR assays with the conventional and real-time PCR modified protocols using reagents for a fast DNA amplification, are presented. The ability for an early detection of ASFV and the similar results obtained when field clinical samples are analysed, showed the modified PCR systems as powerful tools for a very rapid, sensitive and specific molecular diagnosis of ASF. The presented PCRs only take less than one hour, so the definitive results can be finished in less than two hours for real-time or three hours for conventional PCR since samples arrive in the laboratory. Moreover, the use of the reagents for a fast DNA amplification doesn't mean a significant increase in the cost and can be used in any conventional or real-time thermalcycler.

5. Acknowledgements

This work has been funded by INIA project OT01-002 and ASF EU Reference Laboratory.

6. References

1. Agüero M., Fernández J., Romero L., Sánchez C., Arias M., Sánchez-Vizcaíno J.M. (2003) *J Clin Microbiol*, 41 (9): 4431-4434;
2. King D.P., Reid S.M., Hutchings G.H., Grierson S.S., Wilkinson P.J., Dixon L.K., Bastos A.D.S., Drew T.W. (2003) *J Virol Methods*, 107: 53-61;
3. Zsak L., Borca M.V., Risatti G.R., Zsak A., French R.A., Lu Z., Kutish G.F., Neilan J.G., Callahan J.D., Nelson W.M., Rock D.L. (2005) *J Clin Microbiol*, 43 (1): 112-119.

VALIDATION OF A COMMERCIAL ELISA KIT FOR DETECTION OF ANTIBODIES TO BLUETONGUE VIRUS IN INDIVIDUAL MILK SAMPLES OF DUTCH DAIRY COWS

H. Kramps¹, C. van Maanen², J. Mars², J. Popma¹, and P. van Rijn¹

¹ *Central Institute for Animal Disease Control Lelystad, CIDC, Lelystad, The Netherlands;*

² *Animal Health Service Ltd., GD, Deventer, The Netherlands*

Keywords: Bluetongue, ELISA, milk

In 2006, an outbreak of bluetongue occurred in the Netherlands, Belgium, Germany, France and Luxemburg. Routine laboratory diagnosis has successfully been performed in the Netherlands by an in-house real-time RT-PCR (reverse transcription and polymerase chain reaction) on nucleic acid isolated from EDTA-blood samples (PCR) and by serology using a commercial blocking ELISA (ID.VET). Positive PCR results can be found in cattle up to 200 days post infection or more. Using the commercial ELISA, seroconversion has been detected starting at about 14 days post infection.

In accordance to EU legislation, control measures must be taken and disease monitoring and surveillance is of fundamental importance to assess the risk posed by animal movements. For dairy cattle, testing for antibodies in milk samples rather than in serum samples would be very cost-effective because of the easy availability of the former sample type.

In this study, the performance of an indirect ELISA developed by ID.VET for the detection of BTV-specific antibodies in bovine milk samples (mELISA) was compared to that of the routinely used blocking ELISA on serum samples (sELISA).

During the bluetongue outbreak in the Netherlands in 2006, caused by a variant of serotype 8, paired serum- and milk samples were obtained from 470 individual cows from 10 BTV-infected farms with an over-all seroprevalence of 57%. In addition, bulk milk samples of the same farms, and historically BT-negative samples were tested. Compared to the ELISA for sera, the relative specificity and sensitivity of the ELISA for milk samples is 97.5%, and 98.9%, respectively. Titres in positive individual milk samples ranged from 1 to 2048 with a mean titre of 117. Bulk milk samples contained antibodies with titres ranging from 64 to 512. The ELISA for milk samples with a cut-off value of 50%, a detection limit of 20%, and a high over-all accuracy of 98.3% is very reliable and robust. This diagnostic tool is very useful, and may replace testing of serum samples in order to get insight into the status of lactating individual animals with respect to BTV infection.

DEVELOPING AND VALIDATION OF A BTV-8 SPECIFIC REAL-TIME RT-PCR

B. Hoffmann, M. Beer

Friedrich-Loeffler-Institut, Institute of Diagnostic Virology, Greifswald-Insel Riems, Germany

Key words: real-time PCR, questionnaire, ring trial

1. Introduction and Objectives

In August 2006 an outbreak of Bluetongue Virus (BTV) of the serotype 8 was ascertained in central Europe. Beside the detection of antibodies of BTV based on commercial competitive ELISA the antigen detection by RT-PCR is the most useful diagnostic technique. At this moment the published conventional or real-time RT-PCR systems were not optimal for high-throughput applications or BTV-8 detection. Thus, it was necessary to develop a sensitive real-time RT-PCR system for the transfer to the regional laboratories in the federal states of Germany.

2. Material and methods

Selection of primer and probes were performed using the Beacon Designer software version 3.1 from Premier Biosoft and synthesized by MWG-Biotech. For the amplification and detection of the BTV-8 specific fragments the QuantiTect Probe RT-PCR kit (Qiagen) and the MX3005p real-time PCR cyclers (Stratagene) were used. For viral RNA extraction from whole blood by hand or robotics the Viral RNA Mini kit (Qiagen) and Virus 96 RNA kit (Machery-Nagel) were used.

3. Results

Using the outer primers from the OIE protocol a PCR fragment of the NS1 segment of current BTV-8 strain was amplified and the sequence was used for selection of specific primer and probes. 4 primer and 2 probes were selected and tested for earliest Ct values combined with a maximum of ratio-to-noise. The optimal primer-probe combination was combined with a universal internal control system (Hoffmann et al., 2006) for the identification of inhibition factors. The new developed BTV-8 duplex real-time RT-PCR was validated on samples from an animal experiment and more than 2000 field samples. The assay detects BTV-8 genome one or two days post infection in whole blood samples from cattle and sheep. The diagnostic sensitivity of the assay based on serological positive BTV samples in an early stage of infection was more than 99.7%. The fit for purpose of the assay was confirmed by the BTV8 specific ring trial of the CRL in autumn 2006. All samples were correctly detected; here the sensitivity and specificity were 100%.

4. Discussion and Conclusions

The aim of our work was the fast establishing of a high sensitive real-time RT-PCR system for the robust detection of the BTV-8 strain circulating in central Europe. The validation data based on samples from an animal experiment and from the field as well as the results of the European ring trial performed by the CRL confirmed the successful developing. The integration of an internal control system improves the diagnostic safety and robustness especially in context with high-throughput applications. The transfer of the optimized protocol of the BTV-8 duplex real-time RT-PCR to the regional laboratories was unproblematic. This statement was supported by the results of two national ring trials performed in November 2006 and January 2007. At the moment 22 regional laboratories perform the BTV-8 specific duplex assay successfully and thus PCR testing capacities of several thousand runs were established.

TRANSPLACENTAL TRANSMISSION AND PERSISTENCE IN FOETAL LAMBS FOLLOWING EXPERIMENTAL INFECTION OF PREGNANT EWES AT 90 DAYS GESTATION WITH FMDV

R. Waters^{1,2}, **H. Brooks**², **L. West**¹, **J. Brownlie**², **Z. Zhang**¹

¹*Institute for Animal Health, Pirbright Laboratory, Pirbright, Woking, Surrey, United Kingdom;*

²*Royal Veterinary College, Hatfield, Herts, United Kingdom*

Foot-and-mouth disease (FMD) is a highly contagious disease of cloven-hoofed animals characterised by the formation of vesicles in the mouth and on the feet and tongue. FMD has been reported to cause abortions in sheep. Our recent study demonstrated that FMD virus can cause transplacental transmission in sheep at 45 and 75 days gestation, causing foetal death. In the present study, 16 pregnant sheep at 90 days gestation were infected with FMD virus type O UKG 34/2001. Two were euthanased at 4, 7, 14, 21, 28, 35, and 42 days post inoculation (dpi). Quantitative Reverse Transcription polymerase chain reaction (qRT-PCR) was used to detect and characterise the distribution of viral RNA in the foetal tissues taken at post mortem. 21 foetuses underwent post mortem. No histopathological lesions were detected in the foetuses or placentae. No viral RNA was detected in foetal tissues collected at 4 dpi (n=3), 7 (n=2), 21(n=3), 28(n=3), 35 (n=3), dpi. Viral RNA was detected in one of the 2 foetuses at 14 dpi, and one of the 3 foetuses at 42 dpi. Both foetuses were found to have died *in utero*. The presence of viral RNA in foetal tissues at 42 dpi demonstrates the potential for FMDV-induced abortion to cause further disease transmission. Further studies are planned to carry out virus isolation on the tissues taken from these RNA positive foetuses, analyse specific immune response in blood collected from all 21 foetuses and characterise the pathogenesis in detail.

MOLECULAR EPIDEMIOLOGY OF AFRICAN SWINE FEVER AND PESTE DES PETITS RUMINANTS

O. Kwiatek¹, V. Michaud¹, C. Minet¹, J. Ravaomanana¹, A. Diallo², G. Libeau¹, E. Albina¹

¹ CIRAD, Département BIOS, UPR Contrôle des Maladies, Montpellier, France

² Animal Production Unit, FAO/AIEA Agriculture and Biotechnology Laboratory, Seibersdorf, Austria

Key words: African swine fever, peste des petits ruminants, phylogenetic analysis.

1. Introduction and Objectives

African Swine Fever (ASF) and Peste des Petits Ruminants (PPR) are two highly contagious and fatal diseases of domestic pigs and small ruminants, respectively. For the control and eradication of these diseases, it is helpful to find out the origin of the viruses and their mode of propagation. One of the options is the molecular tracing of the isolates based on partial genome sequencing and phylogenetic analysis.

2. Material and methods

For PPR, samples were collected from outbreaks and detection of PPRV RNA was performed by RT-PCR as described previously [1]. Specific PPR primers situated at the 3' end of the N gene was used and gave amplification product of 351 bp. These products were directly used for sequencing or amplified with blunted-end Cloning kit (Roche). Concerning ASFV, viral DNA was extracted from pig spleens collected during the ASF outbreak in Madagascar from 1998 to 2003. Five target genes were then amplified (VP72, p54, J9L, p22 and p32) and the sequencing was performed after cloning in pCR2.1 (Invitrogen).

3. Results

The occurrence of PPR outbreaks in three districts of Tajikistan allowed us to genetically characterize the causal strain. Partial sequence of its N protein gene was compared with 43 other strains isolated since 1968 in Africa, the Middle-East and Asia. The study demonstrated the value of the partial sequence of the N gene for the comparison of isolates obtained over an extended period of time and from various geographical origins. ASFV molecular epidemiology was performed on five different genes. All of them allowed the segregation of the isolates in large regional groups, but none of them were able to discriminate at a local level. Two genes (p22 and p32) were however found to be the most variable. They were concatenated to increase distinction between local isolates.

4. Discussion and Conclusions

Genotypic classification based on the N-protein gene permit to distinct four PPR lineages: viruses of lineage I and II were distributed in west and central part of Africa, lineage II was located in eastern-Africa, south of Middle-East and both sides of the red sea, and lineage IV was located in the Arabian Peninsula, the Middle-East and India. Phylogenetic analysis of VP72 discriminated the isolates in 12 different groups like in previous studies [2, 3]. Analysis of p54 and J9L genes were not more informative while p22 and p32 were more variable. Their concatenation allowed for the first time to pull-off the isolates within the Malagasy group but without a clear temporal and geographical discrimination.

5. Acknowledgements

This study was partially granted by the EU ASF control project and EPIZONE network of excellence.

6. References

1. Couacy-Hymann, E., Roger, F., Hurard, C., Guillou, J.P., Libeau, G. and Diallo, A. Rapid and sensitive detection of peste des petits ruminants virus by a polymerase chain reaction assay. *Journal of Virological Methods* 2002, 100, 17-25.
2. Bastos AD, Penrith ML, Cruciere C, Edrich JL, Hutchings G, Roger F, Couacy-Hymann E, R Thomson G. Genotyping field strains of African swine fever virus by partial p72 gene characterisation. *Arch Virol.* 2003 148(4): 693-706.
3. Lubisi BA, Bastos AD, Dwarka RM, Vosloo W. Molecular epidemiology of African swine fever in East Africa. *Arch Virol.* 2005 150(12): 2439-2452.

LONG-TERM STORAGE OF ANIMAL BLOOD ON FILTER PAPERS FOR DIRECT DETECTION AND GENOTYPING OF VIRUSES

V. Michaud¹, P. Gil¹, O. Kwiatek¹, S. Prome¹, L. Dixon², L. Romero³, M-F. Le Potier⁴, M. Arias³, E. Couacy-Hymann⁵, F. Roger¹, G. Libeau¹, E. Albina¹

¹ CIRAD, Département BIOS, UPR Contrôle des Maladies, Montpellier, France

² Institute for Animal Health, Pirbright, United Kingdom

³ Centro de Investigación en Sanidad Animal, Valdeolmos, Spain

⁴ AFSSA, Laboratoire d'études et de recherches avicoles et porcines, Ploufragan, France

⁵ Laboratoire Central de Pathologie Animale, Bingerville, Côte-d'Ivoire

Key words: Filter paper, PCR, African swine fever, peste des petits ruminants, phylogenetic analysis

1. Introduction and Objectives

Rapid diagnosis of infections is essential for the control of diseases. Not only the detection of the infection but also genotyping of the causative agent is often needed for efficient disease surveillance and control programmes. Filter papers have been shown to be suitable for the conservation of either DNA or RNA viruses for extended periods of time (up to 4 to 11 years) at moderate or tropical temperatures [1, 2]. Here, we show that conventional filter papers can be used for long-term storage of a DNA and a RNA virus and then direct detection by PCR without previous extraction of nucleic acids. In addition, these supports can be used for molecular typing.

2. Material and methods

Pigs and goats experimentally infected with African swine fever virus and peste des petits ruminants virus, respectively, were blood-sampled on EDTA-tubes and filter papers (FP). Control animals were also sampled. FP were dried and stored for various periods at room temperature or at 32-37°C. Small pieces of FP (5 mm²) were directly used in the PCR tests. Conventional extraction methods for nucleic acids were used on the EDTA blood. Both systems were compared in terms of sensitivity.

3. Results

Infected blood was collected on FP, dried and stored at different temperatures (22, 32 and 37°C) for various period of time (up to 9 months). Two animal viruses, African swine fever, a large double-stranded DNA virus and Peste des Petits Ruminants, a negative single-stranded RNA virus were used to validate the method. FP with dried blood containing virus or control plasmid DNA, were added directly to the PCR tube for conventional PCR. Nucleic acid from both viruses could still be detected after 3 months at 32°C. Moreover, the DNA virus could be detected at least 9 months after conservation at 37°C. PCR products obtained from FP were sequenced and phylogenetic analysis carried out. The results were consistent with published sequences, demonstrating that this method can be used for virus genotyping.

4. Discussion and Conclusions

In tropical countries, the diagnosis of viral infections of humans or animals is often hampered by the necessity to maintain a cold chain for the sample preservation up to the laboratory. Here, we describe the use of filter papers for rapid sample collection, and the molecular detection and genotyping of viruses when stored over long periods at elevated temperatures. The new protocol proposed in this study is rapid, does not need previous extraction of nucleic acids, limits the risk of cross-contamination between samples and simplifies shipment to the laboratory without the need for cold chain.

5. Acknowledgements

This study was partially granted by a EU ASF control project, the EU Pan-African programme for the Control of Epizootics and the EU EPIZONE network of Excellence.

6. References

1. Li, C.C., Beck, I.A., Seidel, K.D., Frenkel, L.M., 2004. Persistence of Human immunodeficiency virus type 1 subtype B DNA in dried-blood samples on FTA filter paper. *J. Clin. Microbiol.* 42, 3847-3849.
2. Chaisomchit, S., Wichajarn, R., Janejai, N., Chareonsriwatana, W., 2005. Stability of genomic DNA in dried blood spots stored on filter paper. *Southeast Asian J. Trop. Med. Public. Health* 36, 270-273.

**Abstracts poster presentations:
Theme 5 Intervention strategies**

CHIMERIC CALICIVIRUS-LIKE PARTICLES DISPLAYING B-CELL AND T-CELL EPITOPES FROM FOOT AND MOUTH DISEASE VIRUS

I. Angulo¹, C. Cubillos¹, H. Almanza¹, M. Morales¹, J. R. Castón², F. Sobrino³, E. Blanco¹,
J. Bárcena¹

¹ Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos, Madrid, Spain.

² Dpto. Estructura de Macromoléculas, (CNB-CSIC), Madrid, Spain.

³ Centro de Biología Molecular Severo Ochoa (CBMSO-CSIC), Madrid, Spain.

Key words: Virus-like particles VLPs, Calicivirus, RHDV, FMDV, T-cell and B-cell epitope.

1. Introduction and Objectives

The capsid protein (VP60) of rabbit hemorrhagic disease virus (RHDV) has been expressed in baculovirus and shown to form virus-like particles (VLPs) (1). The aim of the present study was the production and characterization of chimeric RHDV VLPs displaying B- and T-cell epitopes from foot and mouth disease virus (FMDV). To this end, we have prepared several VP60 insertion mutants harbouring peptides containing the main FMDV B-cell antigenic site located at the G-H loop of VP1 protein (2), and/or a major T-helper epitope which is located in the non-structural protein 3A (3).

2. Material and methods

Recombinant baculoviruses were produced using the BacPAK baculovirus expression system (Clontech). Purification of VLPs was performed as previously described (1). The antigenicity of the chimeric constructs was analysed by ELISA and Western blot using monoclonal antibodies (MAbs) directed against VP60 protein and the FMDV epitopes inserted.

3. Results

Three sets of chimeric VP60 proteins harbouring epitopes from FMDV were constructed. The first group of mutants had the FMDV B-cell epitope inserted at the C-terminal end of the VP60 protein. The second group harboured the FMDV T-cell epitope at the N-terminal end of VP60 protein and the B-cell epitope at the C-terminal end. The third group harboured the B-cell and T-cell epitopes at the C-terminal end of VP60 protein. Electrophoretic analysis of infected insect cell lysates showed the expression of grossly similar levels of the different VP60 constructs. In western blot, a MAb against RHDV capsid protein specifically detected VP60 protein, as well as the extension mutants. The chimeric constructs were also recognized by a MAb directed against the FMDV B-cell epitope. To determine if the different chimeric proteins were able to assemble into particulate material, infected insect cell cultures were subjected to CsCl-gradient centrifugation, and characterized by electron microscopy. The results obtained indicated that all the chimeric VP60 proteins prepared assembled into VLPs indistinguishable from wild-type VP60 capsids.

4. Discussion and Conclusions

We have prepared different sets of VP60 insertion mutants harbouring peptides containing the main FMDV B-cell antigenic site and a major T-helper epitope from FMDV. All the chimeric constructs were shown to self-assemble into VLPs, indicating that the RHDV capsid protein can accommodate simultaneous insertions of foreign epitopes at both terminal ends. The VP60 protein has been shown to be very efficient in inducing immune response (4). The immunogenic properties of VP60 protein together with the ability of RHDV VLPs to accommodate epitopes at either end of the molecule may lead to novel platforms for vaccine development and delivery. Work is in progress to assess the immunogenic potential of these chimeric VLPs.

5. Acknowledgements

This work was supported by grant BIO02-04091-C03-03 from the Ministerio de Educación y Ciencia and by the EPIZONE European Network of Excellence.

6. References

1. Bárcena et al. (2004) *Virology* **322**, 118-134.
2. Lea et al. (1994) *Structure* **2**, 123-139.
3. Blanco et al. (2001) *J. Virol.* **75**, 3164-3174.
4. Plana-Duran et al. (1996) *Arch Virol.* **141**, 1423-1436.

DNA VACCINES BASED ON FMDV-MINIGENES FUSED TO DIFFERENT SIGNAL PEPTIDES: ANTIGEN EXPRESSION AND *IN VIVO* IMMUNE RESPONSES DO NOT CORRELATE

B. Borrego¹, J. Argilagué², E. Pérez-Martín², M. Esguevillas¹, M. García-Briones³, B. Álvarez³, A. Ezquerro³, F. Sobrino^{1,4} & F. Rodríguez²

¹CISA-INIA, Valdeolmos, Madrid; ²CRISA, UAB, Bellaterra, Barcelona
³Dpto de Biotecnología INIA Madrid; ⁴CBMSO, UAM Cantoblanco, Madrid.

Key words: Foot-and-mouth disease virus, DNA vaccines, minigenes, signal peptide

1. Introduction

DNA vaccination has become a promising alternative to conventional vaccines. In addition to advantages related to their production, the feasibility of manipulating the DNA constructs allows application of different strategies for optimisation of the immune responses induced. We are interested in using this approach for developing novel vaccines against Foot-and-Mouth Disease Virus (FMDV). FMD is one of the most devastating diseases for animal health, and development of novel, safer marker vaccines is essential to avoid the problems associated to the inactivated virus vaccines currently in use. Using DNA vaccination as a tool, we have previously demonstrated the adjuvant properties of a signal peptide from the human prion protein, when fused to B and T cell epitopes from FMDV in a DNA vaccine, both in mice and in pigs (1, 2). In this work we analyzed the immunological potential of new DNA constructs encoding different combinations of same viral epitopes fused to signal peptides from two porcine molecules.

2. Materials & Methods

pCMV plasmids were constructed encoding in tandem different combinations of the FMDV Cs8c1 antigenic sites B (aa 133-156 of VP1), T3A (11-40 of the 3A) and TVP4 (20-34 of VP4), fused to the signal peptides from the porcine chemokine CCL20, or from CD163, a surface antigen of porcine monocytes. Expression in transfected cells was analyzed by immunodot, flow cytometry, immunofluorescence and immunochemistry. Specific FMDV-antibodies in serum from DNA-immunized mice were detected by a plaque-reduction neutralization assay. The specific cellular response was analyzed by IntraCellular Cytokine Staining of splenocytes after *in vitro* stimulation with FMDV peptides.

3. Results & Discussion

Upon transfection with the plasmids encoding for B- and T- FMDV epitopes fused to different signal peptides, clear differences were observed both at the expression level and regarding the localization of the FMDV antigen. Fusion to the CCL20 sp provided the strongest expression levels. As expected for constructions including the CCL20 sp, antigens were found to reach the cell membrane. One of these plasmids, pCMV-CCL20sp-BTT, was able to induce *in vivo* neutralization titers that may correspond with protection against viral infection (1). Interestingly, elimination of the sequence encoding for the VP4 peptide allowed efficient secretion to the milieu. However, these constructions failed in inducing antibodies in mice even though their high expression levels *in vitro*. No specific cellular responses could be detected.

4. conclusions

We have previously shown that DNA vaccines based on FMDV epitopes fused to a signal peptide can induce protective immune responses against FMDV (1, 2). Here we have further explored this successful strategy. Unfortunately, the high expression levels *in vitro* provided by the new plasmids based on spCCL20 did not correlate with immunogenicity in adult mice. Further work must be done to understand the immunological consequences of the FMDV peptide localization and to determine the protective capacity of these constructions in the natural hosts of FMDV.

5. References

- (1) Borrego et al. *Vaccine* 2006, 24(18):3889-99.
- (2) Ganges et al. *Proceedings of the 7th International Congress of the ESVV, Portugal, Sept 2006*

INVESTIGATION OF THE ROLE OF THE AFRICAN SWINE FEVER VIRUS A238L PROTEIN IN MODULATING HOST GENE TRANSCRIPTION USING PORCINE MICROARRAYS

R. Silk, F. Zhang, C. Abrams, L. Dixon

Institute for Animal Health Pirbright Lab. Pirbright, Woking Surrey, UK

Key words: ASFV, immune evasion, microarray

1. Introduction and Objectives

The African swine fever virus (ASFV) A238L protein acts to evade the host's defences by suppressing transcriptional activation of macrophage immunomodulatory genes. This is achieved by several mechanisms, including inhibiting activation of the host NF- κ B transcription factor (Powell et al., 1996, Revilla et al., 1998), inhibiting calcineurin phosphatase activity (Miskin et al., 1998, 2000) and inhibiting the activity of the p300 transcriptional co-activator (Granja et al., 2004, 2006). In order to define the spectrum of host genes whose transcription is inhibited by expression of A238L we have used a porcine microarray to compare expression profiles in stimulated cells expressing A238L with that in control cells.

2. Material and methods

A recombinant baculovirus containing the ASFV A238L gene under the control of eucaryotic gene promoter was used to transfect a porcine macrophage cell line (IPAM). Expression of the A238L protein was assayed by Western blotting and by confocal microscopy using an anti-PK antibody which recognizes the PK epitope tag fused at the 3' end of the gene. Control cells were treated with recombinant baculovirus not carrying the A238L gene. At 12 hours post-treatment, both test and control IPAM cells were stimulated with 100 nM PMA, 100 ug/ml LPS and 4 uM of Ionomycin. Total RNA harvested at 1 hr and 4 h post-treatment and mRNA was amplified and labelled with either Cy3 or Cy5 dyes before hybridisation to a porcine oligonucleotide array (Operon) containing 14,000 targets. Three independent experiments were carried out. Data was collected and analysed using the BlueFuse and TIGR software. Expression profiles between stimulated cells treated either with baculovirus expressing A238L or control baculovirus were compared indirectly via a pool of RNA collected from non-stimulated IPAM cells.

3. Results

A high percentage of IPAM cells expressed the A238L protein (~90%) following treatment with the baculovirus vector and expression was detected from 2 hours post-treatment. 1 and 4 hr data sets were analyzed separately using paired t-tests ($p < 0.05$) to identify genes differentially regulated by A238L. A total of 449 unique genes were shown to be significantly altered by A238L at either or both 1 and 4 hrs post stimulation compared to control cells. 280 genes were differentially regulated at 1 hr alone, 155 of which were down regulated by A238L and 125 were up regulated. 12 genes were differentially regulated at both time points and 157 genes were differentially regulated at 4 hrs alone, 73 of which were down regulated and 84 were up regulated. Several genes down regulated at 1 hr post stimulation were associated with in the Mitogen Activated Protein Kinase (MAPK) signalling pathway. These included the Dual Specific phosphatases 1 and 6 (DUSP 1 & 6), Growth Arrest and DNA-Damage-Inducible, Beta (GADD45beta) and the transcription factor c-jun.

4. Discussion and Conclusions

Global transcription profiling using the porcine oligonucleotide array has identified a significant number of genes that are regulated by A238L expression. Preliminary results have shown that A238L down regulates the expression of several genes associated with the JNK and p38 MAPK signalling pathway, raising the possibility that A238L may try and regulate this pathway to facilitate ASFV infection in host cells.

5. References.

- 1 Powell P et al., 1996 *J. Viro.*
- 2 Revilla Y et al., 1998 *J Biol Chem* 273, 5405-5411
- 3 Miskin J et al., 1998 *Science* 281, 562-565
- 4 Miskin J et al., 2000 *J. Virol* 74, 9412-9420.
- GranjaA et al., 2006 *J. Immunol* 176, 457-462

TISSUE DISTRIBUTION OF A PSEUDORABIES VIRUS PLASMID DNA VACCINE AFTER INTRAMUSCULAR INJECTION IN SWINE

D. Dory, R. Gravier, A. Jestin

Viral Genetics and Biosafety Unit, French Food Safety Agency (Afssa), Ploufragan, France

Key words: Real-time PCR, DNA vaccination, PRV

1.Introduction and Objectives

Progresses in enhancing DNA vaccination efficiency against Pseudorabies virus (PRV) infection have been obtained in swine [1-4]. The model consists of a single intramuscular (IM) injection of 3 plasmids encoding individually glycoproteins gB, gC and gD. The objective of this work was to determine the tissue distribution of the 3 plasmids following DNA vaccination.

2. Material and methods

24 pigs received by a single i.m. injection in the neck 2×10^{13} copies of each plasmid in 2 ml endotoxin free PBS. Groups of 3 pigs were euthanized and bled 5, 12, 30 and 90 minutes, 24 hours, 1, 2.5 and 4 weeks after plasmids injection. Injected and opposite muscles, draining and opposite prescapular lymph nodes, liver, spleen, kidney, lung and ovaries were collected. Plasmids were quantified in each organ by real time qPCR after extraction of total DNA.

3.Results

The 3 plasmids were found in the injected muscles at least until day 28 following injection. All the tested organs, including ovaries, were found positives. For example, plasmids were detected until day 7 in ovaries and day 17 in the draining prescapular lymphnodes. Differences between the 3 plasmids were observed.

4.Discussion and Conclusion

This is the first study concerning the biodistribution of plasmids following i.m. in pigs. Plasmids were found until day 28 in the injected muscle and for a shorter period in the other organs tested, including ovaries. Differences in persistence were observed between the 3 plasmids.

5.Acknowledgements

The authors are grateful to the staff of the healthy pigs production and experimentation section (Afssa, Ploufragan, France) for expert manipulation of pigs.

6.References

- 1) Dufour, V., Chevallier, S., Cariolet, R. et al. Induction of porcine cytokine mRNA expression after DNA immunization and pseudorabies virus infection. *J Interferon Cytokine Res* 2000, **20**(10), 885-890.
- 2) van Rooij, E.M., Glansbeek, H.L., Hilgers, L.A. et al. Protective antiviral immune responses to pseudorabies virus induced by DNA vaccination using dimethyldioctadecylammonium bromide as an adjuvant. *J Virol* 2002, **76**(20), 10540-10545.
- 3) Dory, D., Torché, A.M., Béven, V. et al. Effective protection of pigs against lethal Pseudorabies virus infection after a single injection of low-dose Sindbis-derived plasmids encoding PrV gB, gC and gD glycoproteins. *Vaccine* 2005, **23**(26), 3483-3491.
- 4) Dory, D., Béven, V., Torché, A.M., Bougeard, S., Cariolet, R. & Jestin, A. CpG motif in ATCGAT hexamer improves DNA-vaccine efficiency against lethal Pseudorabies virus infection in pigs. *Vaccine* 2005, **23**(36), 4532-4540.

ANTIGENIC AND GENETIC ANALYSIS OF SWINE INFLUENZA VIRUSES CIRCULATING IN SPAIN. CONSTRUCTION OF AN ATTENUATED H1N2 STRAIN

G. del Real, M. Martin del Burgo

Centro de Investigación en Sanidad Animal (CISA), INIA, Valdeolmos, Madrid, Spain

Key words: Swine Influenza, Epizootiology, vaccine

1.Introduction and Objectives

Swine Influenza (SI) is one of the most prevalent respiratory diseases in pigs. SI is also a zoonosis for which pigs can act as a reservoir and as intermediate host for genetic reassortment between human and avian viruses. Three antigenically distinct subtypes of SIV, H1N1, H1N2 and H3N2, are now circulating in the European swine population. Common vaccines against the flu are monospecific; that is, they are only effective against those viruses that are similar in antigenic composition. The recent emergence of the new H1N2 subtype in conjunction with the relative age of current porcine vaccines nonetheless makes it necessary to evaluate vaccine effectiveness. Current vaccines are composed of inactivated virus that stimulates a humoral response to surface antigens. Attenuated live vaccines can confer heterosubtypic protection, due to the induction of a cellular immune response to the conserved internal proteins of the virus. Due to the increased antigenic and genetic diversity of viruses within the swine reservoir, a number of aspects related to disease control need to be clarified. The objective of this work is to address those aspects including cross-protection, vaccine composition and efficacy and identification of virulence and transmission determinants. Another objective is the construction of an attenuated H1N2 strain, based on the truncation of the NS1 gene, as a candidate vaccine.

DIFERENTIAL EXPRESSION OF HOST GENES DURING EARLY AFRICAN SWINE FEVER VIRUS INFECTION

R. Herbert, F. Zhang, C. Abrams and L. Dixon

Institute for Animal Health, Ash road, Pirbright, Surrey, England

Key words: ASFV, Microarray

1.Introduction and Objectives

ASFV causes a severe and often fatal haemorrhagic disease in domestic swine with massive economic consequences. ASFV infects macrophage cells and the interaction of the virus with these cells is believed to play a crucial role in pathogenesis (1,3) . ASFV gene transcription can be separated into early and late genes. Early gene transcription begins immediately after infection. A 4 hour early time point has been chosen to investigate host gene expression in infected and non-infected macrophages. UV inactivated virus has also been used to define the host genes diferentially regulated on binding and entry of the virus compared with those activated at early times following infection. These results have highlighted a large number of genes and pathways of interest. One of the genes which has been investigated in more detail is galectin-3. The main objectives of this work was to identify host pathways that are activated or repressed by the virus. A second objective was to identify those pathways altered by virus binding and entry.

2.Material and methods

A porcine oligo array containing 14,000 genes was used to investigate porcine macrophage gene expression during ASFV infection by microarray. Host gene expression was measured 4 hours after infection with ASFV Malawi isolate, UV inactivated virus and in mock-infected cells.

3.Results

Preliminary analysis of the microarray results shows that approximately 400 host genes are differentially regulated between Malwai virus infection compared with a mock infection. Many of these are also differentially regulated during infection with UV inactivated virus, however, there are a number of gene which are altered during infection with UV inactivated virus and not during a normal infection.

4.Discussion and Conclusions

Using microarrays we have identified many genes which are differenentially expressed during ASFV infection and a number of pathways which regulated by virus infection which might either facilitate a productive infection or interfere with host defence responses. One gene, galectin-3, which was shown to be up-regulated during ASFV infection. This multifunctional protein (2) has recently been linked to immune cell regulation and may play a role in promoting viral pathogenicity by contributing to lymphocyte apoptosis a key feature of virulent ASFV infection.

5.Acknowledgements

L.Dixon, Charles, Abrams, Fuquan, Zhang, Lynette Goatley,

6.References

1. Gomez del Moral, M., E. Ortuno, et al. (1999). "African swine fever virus infection induces tumor necrosis factor alpha production: implications in pathogenesis." *J Virol* 73(3): 2173-80.
2. Rabinovich, G. A., L. G. Baum, et al. (2002). "Galectins and their ligands: amplifiers, silencers or tuners of the inflammatory response?" *Trends Immunol* 23(6): 313-20.
3. Whittall, J. T. and R. M. Parkhouse (1997). "Changes in swine macrophage phenotype after infection with African swine fever virus: cytokine production and responsiveness to interferon-gamma and lipopolysaccharide." *Immunology* 91(3): 444-9.

TRANSCRIPTIONAL RESPONSE IN THE SKIN OF PIGS AFTER INTRA-DERMAL VACCINATION WITH DOE FORMULATED FMDV VACCINE.

M. Hulst¹, S. Durand², A. Wit¹, A. Dekker², R. Zwart¹, N. Stockhofe¹ and P. Eble².

¹*Animal Sciences Group, Wageningen University and Research Centre, Lelystad; The Netherlands.*

²*Central Institute for Animal Disease Control, CIDC-Lelystad, Lelystad; The Netherlands.*

Key words: FMDV, Intra-dermal vaccination, transcriptional response skin.

1.Objectives

The transcriptional response in the skin of pigs after intra-dermal vaccination with DOE formulated FMDV vaccine was studied using cDNA microarrays.

2.Material and methods

Binary ethyleneimine inactivated FMDV strain O Taiwan formulated in a double water-oil emulsion (DOE) was used to vaccinate pigs intradermally (ID) using the IDAL vaccinator (kindly supplied by Intervet). Using high pressure this vaccinator injects vaccine in the skin without the use of needles, causing minimal damage to the skin and subcutaneous tissue. Three groups of eight pigs were vaccinated ID in the left side of the neck with a dose of 1x 0.2 ml, a dose of 4x 0.2 ml, or with 1x 0.2 ml PBS (control group). Three additional groups of eight pigs were vaccinated intramuscularly (IM) in the left side of the neck with a dose of 1 x 0.2 ml, a dose of 4 x 0.2 ml, or a dose of 1 x 2 ml. One, three, and seven days post vaccination (dpv), one pig per group was sacrificed to collect skin tissue from the vaccination spot in the left side of the neck and untreated skin tissue from the right side of the neck. At 28 dpv the remaining pigs (n=5) in all groups were challenged intradermally in the bulbus of the heel with 10 000 TCID₅₀ of O Taiwan FMDV challenge virus to monitor the efficacy of vaccination. Using a home-made cDNA microarray prepared from porcine spleen and jejunum tissue (complemented with relevant immune-related genes) mRNA expression levels in the vaccinated skin were compared to levels in untreated skin (isogenic) at 1, 3 and 7 dpv for ID, IM, and PBS vaccinated pigs.

3.Result and Discussion

Except for 3 pigs in the 1 x 0.2 ml IM group all pigs in other IM and ID groups were completely protected against this challenge, indicating that ID vaccination with a dose of 1 x 0.2 ml was more effective than a similar dose applied IM. From the isogenic microarray comparisons several genes could be identified for which mRNA expression was regulated specifically by the ID applied vaccine. QRT-PCR of five selected genes confirmed the expression data of the microarray experiments. Regulated genes were functionally clustered in MHC I and II antigen presentation genes, markers for antigen presenting cells, dsRNA mediated antiviral response genes, and genes involved in repair of damaged tissue. Histological examination of tissue sections prepared from skin samples showed that the dermis of ID vaccinated pigs contained significant more antigen presenting cells (CD1+) than that of ID-PBS vaccinated pigs.

CONSTRUCTION OF A QUADRUPLE RECOMBINANT BACULOVIRUS COEXPRESSING H5, N3, M, and M2 PROTEINS OF A H5N3 LPAI VIRUS

A.Prel, G. Le Gall-Reculé, V.Jestin

AFSSA, Swine and Poultry Research Laboratory, Avian And Rabbit Virology Immunology and Parasitology Unit, Ploufragan, France

Key words: Avian Influenza, H5N3 subtype, low pathogenic, recombinant baculovirus

1.Introduction and Objectives

Domestic ducks can play pivotal roles in the transmission of H5 low pathogenic (LP) and highly pathogenic (HP) avian influenza viruses, and a few inactivated and recombinant commercial or experimental vaccines have been assessed in these species essentially for the prevention of H5N1 HP. No data are reported with regards to the protection afforded by inactivated vaccines against H5 LPAI infection in ducks. To date, there is no recombinant vaccine for ducks. The combination of inactivated and recombinant vaccines as a prime-boost vaccination has been shown efficient. We think that substituting inactivated vaccines by subunit vaccines might be more advantageous. In a precedent study, we attempted to develop a VLP H5N3 influenza vaccine composed of the three structural proteins H5, N3 derived from a recent French LPAI virus strain and M derived from an Italian LPAI virus strain (2,3). We generated a triple recombinant baculovirus (rB) and we demonstrated that the three proteins were successfully expressed in rB-infected cells and displayed the expected biological activity. Although we showed that HA, NA and M were co-released in supernatant of rB-infected cells, results did not display the formation of VLPs. However, we assessed the protection afforded by lysates of rB-infected cells in ducks following a homologous LPAI virus challenge and showed a significant decrease of cloacal shedding and a delayed peak of tracheal shedding. To improve the expression of VLPs, we changed the molecular construction to avoid eventual competition between HA and NA genes. Since the M2 protein was shown to play an important role in influenza virus assembly we generated a quadruple recombinant baculovirus by adding the M2 matrix gene.

2.Material and methods

The expression system "Bac to Bac baculovirus expression system" (Invitrogen) was used to produce a quadruple recombinant baculovirus. The H5 gene was cloned downstream of the polyhedrin (PH) promoter and the M gene was cloned downstream of the AcMNPV P10 promoter within "pfastBac Dual vector". This resulted in pMHA plasmid (2). In parallel, we constructed two single recombinant plasmids: pNA with NA gene cloned downstream of the p10 promoter and pM2 with M2 gene cloned downstream of the PH promoter within pfastBac Dual and pfastBac1 vectors, respectively. Then, using a subcloning strategy, the "p10 promoter - N3 gene - HSV tk termination sequence" and the "PH promoter - M2 gene - SV40 termination sequence" were inserted into pMH5 to generate pMH5N3M2. Achievement of pMH5N3M2 plasmid was checked by nucleotide sequencing. Quadruple recombinant bacmids were produced according to manufacturer's instructions. The obtained construct was transfected into Sf9 insect cells to generate a quadruple recombinant baculovirus (bMH5N3M2). To protein expression, Sf9 cells were infected for 72 hours with bMH5N3M2.

3.Results

First, we had checked integrity of inserted cassettes and the absence of any mutation by nucleotide sequencing. At this date we succeeded to construct a quadruple recombinant plasmid pMH5N3M2 compound of four structural H5, N3, M and M2 proteins and demonstrated the expression of H5 and M1.

4.Discussion and Conclusions

We attempt to develop a VLP H5N3 influenza vaccine composed of four proteins derived from a French LPAI virus strain. To determine whether avian influenza VLPs will be formed by self-assembly from four structural proteins HA, NA, M1, and M2, Sf9 cells will be infected with quadruple recombinant baculoviruses. Then, M2 and NA proteins expression will be checked by western blot and by neuraminidase inhibition assay. Electron microscopy will be implemented to look for VLPs after sucrose density gradient concentration and purification.

5.Acknowledgements

We thank Béatrice Grasland and Anne-Cécile Nignol (GVB unit, AFSSA Ploufragan) for insect cell culture.

6.References

1. McCown, M. F., and A. Pekosz. 2006. *J Virol* 80:8178-89.
2. Prel, A., G. Le Gall-Reculé, M. Cherbonnel, B. Grasland, M. Amelot, and V. Jestin. 2006. *Avian Diseases* 50 (In Press).
3. Prel, A., G. Le Gall-Reculé, and V. Jestin. 2006. Oral Communication WP5.1 Kick off meeting Epizone, Pirbright (UK), October 5- 6th 2006.

GENERATION OF VACCINES BASED ON RECOMBINANT VACCINIA VIRUS-EXPRESSED BLUETONGUE VIRUS ANTIGENS

E. Calvo, J. Ortego

CISA-INIA, Valdeolmos, Madrid, Spain

Key words: Bluetongue virus, Marker Vaccine, MVA, Vaccinia

1. Introduction and Objectives

Bluetongue virus (BTV) causes an infectious, non-contagious, Office International Epizooties (OIE) listed disease of wild and domestic ruminants, especially sheep. It is transmitted by *Culicoides* biting midges. Bluetongue vaccines play an important role in the control of the disease; however, there are concerns over the use of current polyvalent attenuated vaccines. The protective efficacy of poxvirus-vectored vaccines is well known for other diseases. The objective of this work is the generation of recombinant modified vaccinia Ankara (MVA) vectors expressing BTV-4 outer capsid proteins.

2. Material and Methods

VP2 and VP5 genes of BTV-4/Menorca were synthesized, cloned and sequenced downstream of the p7.5 vaccinia promoter into the vaccinia transfer plasmid pSC11, that allows the screening using LacZ marker gene. Recombinant MVA viruses were generated by homologous recombination at the thymidine kinase locus.

3. Results

Recombinant MVA viruses expressing VP2 or VP5 proteins from BTV-4 were generated and plaque purified. VP2 and VP5 protein expression was confirmed by immunoblot or detection of mRNA by RT-PCR.

4. Discussion and Conclusions

Full-length cDNA copies of genome segment 2 and -6 (which encodes VP2 and VP5, respectively) from the strain Menorca of BTV-4 were synthesized, cloned and sequenced. Recombinant MVA viruses expressing VP2 and VP5 proteins from BTV-4 were generated. The immune response after vaccination with rMVA expressing VP2 or VP5 will be evaluated in small animal models based on the generation of neutralizing antibodies.

5. Acknowledgements

We thank Dr. Peter Mertens and Dr. Francisco Rodriguez for helpful discussions throughout the study.

IN VIVO COMPARATIVE STUDY OF IMMUNOPATHOGENY RELATED ON THE VIRULENCE OF THE CSFV STRAINS: PRELIMINARY SETTINGS IN PREPARATION FOR A TRANSCRIPTOMIC ANALYSIS

P. Renson¹, R. Cariolet², M. Le Dimna¹, E. Hutet¹, M. Le Potier¹

Agence Française de Sécurité Sanitaire des Aliments (AFSSA)

¹ *Unité Virologie et Immunologie Porcines et* ² *Service Production de Porcs Assainis et d'Expérimentations Zoopôle Les Croix, Ploufragan, France*

Keywords: CSFV, Virulence, Immunopathogeny, Leucopenia, Apoptosis

1. Introduction and Objectives

Classical Swine Fever Virus (CSFV) is a member of the *Flaviviridae* family which causes severe leucopenia related as an apoptotic phenomenon [1]. This leucopenia is observed whatever the virulence of the strain but the mechanisms implicated are still unknown [2].

This is the first step of a molecular study based on a microarray technology intending to understand this immunopathogenic mechanisms and particularly those involved in the apoptosis induction. Indeed, we had to describe the leucopenic phenomenon kinetics that occurs during an infection with a Highly Virulent (HV) and a Moderately Virulent (MV) strain to have physiological basis dataset to relate to the molecular modulations that we would further assessed.

2. Material and methods

Eight SPF pigs, 7-week-old aged, were oronasally infected with 10⁶ TCID₅₀ CSFV Eystrup HV strain in a first experiment and CSFV Paderborn MV strain in a second. Blood samples were collected before infection and 24, 48 and 72h post-inoculation (pi). Blood cells counts were determined with a MS9 Coulter Counter MS9 (Beckman Coulter) and virus infection was assessed in real time RT-PCR (QUIAGEN RNeasy and LSI TaqVet CSF kits). Then, PMBCs were purified, followed by a red cells lysis in Versalyse (Beckman Coulter) for a flow cytometry analysis, performed using a triple-labelling with FITC-mAbs anti-porcine CD3 (PPT3 clone; SBA), CD21 (BB6-11C9.6 clone; SBA) or SWC3 (74-22-15 clone; SBA) / Biotin-Annexin V (Beckman coulter) stained by RPE-Streptavidin (SBA) / 7-AminoActinomycin D (SBA). Datas were acquired on a FACSort flow cytometer using the CellQuest Software (Beckton Dickinson) and analysed using WinList 5.0 (Verity Software House). The remaining PBMCs were frozen in Trizol for an awaiting transcriptomic analysis using porcine microarrays in a follow-up study.

3. Results

The Eystrup strain infection, detected in blood from 24 or 48h pi, shows a progressive decrease of lymphocytes and granulocytes counts from the first 24h pi. A 16% raise of dying PBMCs (in early and late apoptosis or necrosis stages) appears at 48h pi and then it declines at 72h pi. The same results are obtained on [CD3+] cells and [SWC3+] cells (16% and 17% respectively). With [CD21+] cells, identified as B cells subset, the dying cells level increase starts at 24h pi and reaches 53% at 72h pi.

The second experiment with the Paderborn strain is currently running.

4. Discussion and Conclusion

This results show that the interesting timing to study the immunopathogenic mechanisms by microarrays should be before 48h pi with a HV CSFV strain and we will know if it is delayed with a MV strain soon.

5. Acknowledgements

We thank F. Marot for her technical help and B. Jan and A. Kéranflec'h for animals care and blood sampling. This work was supported by grants from the Conseil Régional de Bretagne (211-B2-9/2005/ARED /PPCVIRUL) and the NoE EPIZONE (FP6).

6. References

- [1] Summerfield et al. (1998) "Lymphocyte apoptosis during classical swine fever: implication of activation-induced cell death". *J Virol.* 72(3): 1853-61.
- [2] Summerfield et al. (2001) "Depletion of CD4(+) and CD8(high+) T-cells before the onset of viraemia during classical swine fever". *Vet Immunol Immunopathol.* 78(1):3-19.

INTERFERON ACTIVITY IN THE PLASMA, CULTURE SUPERNATANTS OF PBMC AND LEVEL OF IFN γ -mRNA IN PBMC FROM SHEEP INFECTED WITH BOVINE IMMUNODEFICIENCY VIRUS (BIV)

M. Rola¹, M. Materniak¹, M. Kandefer-Szerszen², A. Szuster-Ciesielska², J. Kuzmak¹

¹National Veterinary Research Institute, Department of Biochemistry, Pulawy, Poland;

²University of Maria Curie-Skłodowska, Department of Virology and Immunology, Lublin, Poland

Key words: BIV, interferon, sheep

1. Introduction and Objectives

Bovine immunodeficiency virus induces immune dysfunction in experimentally infected animals. Similar to HIV and FIV, the pathogenesis of BIV can be associated with dysregulation of the cytokine network. This study evaluated the effect of BIV infection on interferon (IFN) activity in plasma samples, in supernatants from PBMC and level of IFN γ -mRNA in PBMC of five BIV-infected sheep and two controls.

2. Material and methods

Five sheep were inoculated with FBL/BIV cells and two inoculated with non-infected FBL cells served as controls. Collection of plasma samples and isolation of PBMC by the density gradient technique were carried out at monthly intervals up to 44 weeks after infection. IFN concentration in blood plasma and supernatants was determined by measuring the degree of IFN-induced inhibition of the cytopathic effect caused by VSV in bovine fetus fibroblast. Total cellular RNA was extracted from PBMC and reverse transcribed. After that PCR was carried out with primers specific for ovine IFN γ and results were analyzed by Gel-Doc system.

3. Results

BIV infection caused significant temporal increase in IFN activity in both plasma samples and PBMC supernatants. In supernatants IFN activity peaked at 6 to 12 weeks after infection (a.i.) and did not appear to alter significantly up to the end of experiment. In plasma samples the maximum activity of IFN was noted between 8 and 16 weeks a.i., however IFN levels were substantially decreased with respect to those observed in the supernatants. While this biological method measured whole IFN-activity (IFN α, β, γ), the molecular approach allows for monitoring particularly IFN γ . Our results indicated some correlation with the results obtained for plasma and supernatants samples. Namely the peak of IFN γ level was also observed at 12 weeks a.i. Interestingly and contrary to plasma and supernatants results, beginning from 32 weeks to 40 weeks a.i. the level of IFN γ mRNA increased and stayed at high level during this period.

4. Discussion and Conclusions

A cytokine response dominated by gamma interferon has been shown to be important in the clearance of intracellular pathogens including viruses. The early cytokine response in BIV-infected sheep was similar to those found in HIV and FIV studies (1, 3). It is worth to notice that elevated levels of IFN in both plasma samples, supernatants and IFN γ mRNA in PBMC coincide with the increased number of T-lymphocytes (2). This confirms the role of IFN secreted by activated T-cells in response to BIV infection. Moreover the kinetics of IFN response correlated with the increased level of BIV antibodies particularly at 12 weeks after infection. However because of the elevated level of IFN γ from 32 up to 44 weeks after infection it can be also suggested that IFN induction might be influenced not only by the BIV infection but also by disease progression. Understanding of the cytokine expression in BIV-infected animals can be critical to explore the issue of low cattle responsiveness to vaccines.

5. References

1. Avery P.R., Hoover E.A. Gamma interferon/interleukin 10 balance in tissue lymphocytes correlates with down modulation of mucosal FIV infection *J Virol* 2004, 78, 4011-4019
2. Kozaczynska B, Bicka L, Kuzmak J, Winnicka A. Phenotypic analysis of PBL in sheep experimentally infected with BIV *Med Wet* 2001, 57, 129-134
3. Than S, Hu R, Oyaizu N, Romano J, Wang X, Sheikh S, Pahwa S. Cytokine pattern in relation to disease progression in HIV-infected children *J Infect Dis* 1997, 175, 47-56

COINJECTION OF RAINBOW TROUT INTERLEUKIN 8 WITH THE GLYCOPROTEIN GENE FROM VIRAL HEMORRHAGIC SEPTICEMIA VIRUS (VHSV) MODULATES THE CC CHEMOKINE RESPONSE

E. Sanchez¹, J. Coll² and C. Tafalla¹

¹ Centro de Investigación en Sanidad Animal (CISA-INIA). Valdeolmos (Madrid). Spain.

² INIA, Biotecnología. Madrid, Spain.

Key words: inducible CC chemokines, interleukin 8 (IL-8), viral hemorrhagic septicaemia virus (VHSV), DNA vaccine, rainbow trout

1. Introduction and Objectives

In mammals, chemokines are among the adjuvants more widely used for DNA vaccination against viruses. In a previous work, we had demonstrated that a plasmid coding for the CXC rainbow trout chemokine interleukin 8 (IL-8, pIL8+) when co-administered with a DNA vaccine against viral hemorrhagic septicemia virus (VHSV) (pMCV1.4-G) was able to modulate the expression of pro-inflammatory cytokines induced (1). In the current study, we have also studied the effect of pIL8+ co-administration on the expression of other chemokines, focusing on inducible CC chemokines. This study not only continues the evaluation of effectiveness of pIL8+ as an adjuvant, but will also help establish the relations among the different chemokine groups.

2. Material and methods

Five groups of rainbow trout (*Oncorhynchus mykiss*) were intramuscularly injected with one of the following treatments: (Group 1) 100 µl of phosphate buffered saline (PBS) (Group 2) 100 µl of PBS containing 0.5 µg pMCV1.4-G plasmid DNA per fish, (Group 3) 100 µl of PBS containing 0.5 µg pMCV1.4-G and 0.5 µg pIL8- (negative control) per fish, (Group 4) 100 µl of PBS containing 0.5 µg pMCV1.4-G and 0.5 µg pIL8+ per fish or (Group 5) 100 µl of PBS with 0.5 µg pIL8+ per fish. At days 3 and 7 post-injection, five trout from each group were killed and cDNA obtained from spleen, head kidney and muscle as described before (1). The levels of expression of different CC inducible chemokines (CK5A, CK5B, CK6, CK7A and CK7B) were evaluated through semi-quantitative RT-PCR as described before (1) using primers previously described by Laing and Secombes (2).

In order to confirm the effects of rainbow trout IL-8 on the expression of inducible CC chemokines, trout head kidney leucocytes were incubated with 400 µl of supernatants from EPC cells transformed with either pIL8- or pIL8+. After 6h of incubation at 20°C, the levels of expression of the different chemokines was evaluated as described above.

3. Results

All chemokines were induced in the head kidney of fish injected with the DNA vaccine, and the co-administration of pIL8+ together with the vaccine modulated the expression of all CC chemokines studied.

Using supernatants from EPC cells transfected with pIL8+, we demonstrated that IL-8 induces the expression of CK5A, CK6, CK7A and CK7B in head kidney cells. Only a slight induction was seen however on the expression of CK5B.

4. Discussion and Conclusions

We have determined for the first time in fish that when IL-8 is used as a molecular adjuvant, the expression of CC chemokines is modulated, probably through a direct effect in the case of CK5A, CK6 and CK7, and through an indirect pathway in the case of CK5B.

5. Acknowledgements

This work was supported by grant AGL2004-07404-C02-02 from the Ministerio de Educación y Ciencia and by the EPIZONE European Network of Excellence.

6. References

(1) Jimenez N, Coll J, Salguero FJ, Tafalla C. Co-injection of interleukin 8 with the glycoprotein gene from viral haemorrhagic septicemia virus (VHSV) modulates the cytokine response in rainbow trout (*Oncorhynchus mykiss*). *Vaccine* 2006;24: 5615-26.

(2) Laing KJ, Secombes CJ. Trout CC chemokines: comparison of their sequences and expression patterns. *Mol Immunol* 2004;41: 793-808.

DIFFERENT EXPRESSION PATTERNS REVEALED BY MICROARRAY ANALYSES OF PORCINE ALVEOLAR MACROPHAGES INFECTED WITH AFRICAN SWINE FEVER VIRUSES OF DIFFERENT VIRULENCE

F. Zhang¹, P. Hopwood², C. Abrams¹, A. Downing³, F. Murray³, R. Talbot³, A. Archibald³, S. Lowden², and L K. Dixon¹

¹Institute for Animal Health Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, UK;

²ARK-Genomics Roslin Institute, Roslin, Midlothian, UK;

³Royal Dick Veterinary College, University of Edinburgh, Summerhall, Edinburgh, UK

Key words: microarray, ASFV

1. Introduction and Objectives

African swine fever virus (ASFV) is a large, icosahedral, double-stranded DNA virus which replicates in the cytoplasm of infected cells, mainly macrophages, and has been classified as the only member of a new virus family, the *Asfarviridae*(1). By using a house-made cDNA microarray containing 2,880 genes, we have investigated changes in macrophage gene transcription that occur during in vitro infection with a virulent isolate of ASFV (Malawi LIL20/1). This identified about 120 temporally altered genes at an early or/and a late time point post-infection (2). In the present study we used the same array to compare Malawi LIL20/1 isolate with a low-virulence isolate, OUR T88/3 at 4 hr and 16 hr post infection of porcine alveolar macrophages, in an attempt to narrow down the candidate host genes and pathways involved in ASFV pathogenicity.

2. Material and methods

The porcine immune array (A-MEXP-494) was used in this study. Total RNA was extracted from mock-infected and infected cells and linearly amplified before labelled with Cy3 and Cy5. Samples for hybridisation were allocated according to a double triangular design with dye-swap. Briefly, samples from Malawi LIL20/1 or OUR T88/3 infected cells at 4 hr and 16 hr post infection were compared with those from mock-infected cells; in addition, at both time points, Malawi LIL20/1 and OUR T88/3 samples were compared directly. Hybridisation signals were extracted from the scan images by using BlueFuse software, and the raw data were normalised and further analysed in TM4 package (3).

3. Results

The data from these experiments showed that macrophages infected with the low virulence isolate shared a similar transcription profile to those infected with the high virulence isolate at 4 hr post-infection. In contrast with the return to base transcription level of most of these genes at 16 hours post-infection in macrophages infected with the high virulence isolate, transcription levels of many of these genes remained elevated at 16 hours post-infection with the low virulence isolate. Of obvious potential importance is the sustained increased transcription of proinflammatory cytokine genes. Other interesting genes with different expression profiles between these two isolates include immune proteasome genes, MHC-1, CD14, SP1, HnRNP A3, etc.

4. Discussion and Conclusions

We have identified some genes showing different expression profiles in response to Malawi LIL20/1 and OUR T88/3 infection. Further investigation on those genes will help to understand the pathogenicity and immune evasion strategies of ASFV.

5. Acknowledgements

This project was funded by BBSRC.

6. References

1. Dixon, L. K, et al. 2005. *Asfarviridae*, p. 135-143. In C. M. Fauquet, et al (ed.), *Virus taxonomy: classification and nomenclature of viruses. Seventh report of the International Committee on Taxonomy of Viruses*.
2. Zhang F, et al. *J Virol*. 2006 Nov;80(21):10514-21.
3. Saeed Al, et al. *Biotechniques*. 2003 Feb;34(2):374-8.

INTELLIGENT DESIGN OF A VACCINE FOR AFRICAN SWINE FEVER VIRUS.

D. Chapman¹, L. Goatley¹, H. Takamatsu², L. Dixon¹

¹*institute for Animal Health, Department of Microbiology, Pirbright Laboratory, Ash Lane, Pirbright, UK;*

²*institute for Animal Health, Department of Immunology, Pirbright Laboratory, Ash Lane, Pirbright, UK*

Key words: Genomes, ASFV vaccine

1. Introduction and Objectives

African swine fever virus (ASFV) is a large DNA virus, which replicates in the cytoplasm of infected cells. It contains a linear double-stranded DNA genome varying between 170 and 193 kbp. In its natural hosts, warthogs, bushpigs and soft ticks of the *Ornithodoros spp.*, ASFV causes a persistent but asymptomatic infection. In domestic swine it causes an acute haemorrhagic infection. ASF causes major economic losses and is endemic in many African countries as well as in Sardinia (1). Currently no vaccine or treatment is available and control of an outbreak relies on quarantine and slaughter. A previous attempt in Portugal using a serially passaged strain of ASFV as a vaccine led to several problems, of 550,000 vaccinated animals 128,684 developed postvaccinal reactions. These included pneumonia, skin ulcers, abortions, locomotor disturbances, disturbances in lactation and death (2). The objective of this project was to use genomics and previous work to design a recombinant virus capable of use as a vaccine against West African strains of ASFV.

2. Material and methods

The entire genomes of the virulent ASFV strain Benin 97/1 and the non-virulent OURT 88/3 were sequenced by Qiagen (Germany). Several constructs based on a pcDNA3 back bone were constructed, then used in transient dominant recombination to produce recombinant Benin 97/1 virus.

3. Results

Sequencing of the low and high virulence isolates of ASFV in conjunction with the already available tissue culture adapted strain has led to insights into regions of the genome important in virus pathogenicity. This information along with previous work has enabled us to highlight regions of interest for deletion in the production of a vaccine strain. Vectors suitable for deleting said regions, using transient dominant recombination, have been produced. One recombinant virus has been produced and is currently being studied.

4. Discussion and Conclusions

Previously vaccines have been produced by serially passaging field isolates to produce attenuated viruses. This type of vaccine has several problems as the nature of the attenuation is not fully known and the potential for revertants hard to calculate. It is therefore essential use intelligent design to ensure that vaccines produced are safe and efficacious. This project is striving to produce such a vaccine against ASFV.

5. Acknowledgements

We would like to acknowledge the invaluable help of Vasily Tcherepanov and Chris Upton in fully analysing the sequenced genomes.

6. References

1. Dixon L, K., V. Costa J, M. Escribano J, L. Rock D, E. Vinuela, and J. Wilkinson P. (2000). *Asfarviridae*, p. 159-165. In F. C.M.B.D.H.L.O. Regenmortel M.H.V. Van (ed.), London Academic Press;
2. Manso Ribiero, J., Nunes Petisca, J.L., Lopes Frazao, F. and Sobral, (1963) *M. Bull. Off. Init. Epiz.* **60**: 921-937.

DEVELOPMENT OF DIVA VACCINE AGAINST PESTE DES PETITS RUMINANTS BY REVERSE GENETICS

C. Minet, P. Gil, G. Libeau, E. Albina

CIRAD, Département BIOS, UPR Contrôle des Maladies, Montpellier, France

Key words: Morbillivirus, Peste des Petits Ruminants, marker vaccine, reverse genetic

1. Introduction and objectives

Peste des Petits Ruminants (PPR) is a contagious viral disease of goats, sheep and wildlife in sub-Saharan African countries, Middle East and South-West of Asia. It is caused by a single strand negative RNA virus, belonging to the *Paramyxoviridae* family and *Morbillivirus* genus. Current vaccines consist of viral strains attenuated by several passages on cell cultures. These vaccines protect animals against PPR but do not permit the distinction between vaccinated and infected animals. Development of reverse genetic to manipulate negative strand RNA genome allows generating an infectious marked clone of PPR vaccine strain.

2. Materials and methods

A rescue system with a minigenome containing the eGFP reporter gene was first generated. This gene was placed between the leader and trailer of PPRV in antisens position between the T7 polymerase promoter and terminator. This minigenome was co-transfected on 293-T7 cells (293 cells which expressed the T7 RNA polymerase) with 3 plasmids containing the N, P and L gene of PPRV to reconstitute the viral ribonucleoprotein. Four clones covering the full-length genome of PPRV were assembled in antisens orientation between T7 promoter and terminator into a modified pBluescript. This full length genome was co-transfected as previously described for the minigenome. Cells were harvested three days later, co-cultured on Vero cells and passaged until CPE developed.

3. Results

The rescue system using the eGFP minigenome and 293-T7 cells generated fluorescent cells 3 days after transfection. These results were confirmed by detection of eGFP mRNA in transfected cells. The full length genome of PPRV vaccine strain was assembled and the sequence checked (this sequence was deposited in GenBank n°X74443). Generation of the first infectious clone of PPRV by reverse genetic is ongoing.

4. Discussion and Conclusions

The objective was to develop the reverse genetic for PPRV and to generate a first clone of PPRV. The rescue system based on the use of a minigenome expressing the eGFP was validated. Attempts to generate an infectious clone of PPRV from the full-length genome are ongoing. The final objective is to insert a mark in this genome and to develop appropriate companion diagnosis tests.

5. Acknowledgements

F. Tangy (Institut Pasteur, Paris, France), T. Barrett (Institute of Animal Health, Pirbright, Great Britain) and M. Skinner (Institute of Animal Health, Compton, Great Britain). This study is partially granted by the UE Markvac project and EPIZONE network of excellence.

6. References

- 1-Baron, MD et al.: Expression in cattle of epitopes of a heterologous virus using a recombinant Rinderpest virus. *J. Gen. Virol.*, 1999, 80, 2031-2039
- 2-Conzelmann KK: Genetic manipulation of non-segmented negative strand RNA viruses. *J. Gen. Virol.*, 1996, 77, 381-389.
- 3-Das SC, Baron MD, Skinner MA, Barrett T. Improved technique for transient expression and negative strand virus rescue using fowlpox T7 recombinant virus in mammalian cells. *J. Gen. Virol.*, 2000, 89, 119-127
- 4-Radecke F, Spielhofer P, Schneider H, Kaelin K, Huber M, Dötsch C, Christiansen G, Billeter MA. Rescue of measles viruses from cloned cDNA. *EMBO journal*, 1995, 14 (23), 5773-5784.

CONTROL OF MORBILLIVIRUS REPLICATION BY RNAI

R.S. Almeida, D. Keita, G. Libeau, E. Albina

CIRAD, Département BIOS, UPR Contrôle des Maladies, Montpellier, France

Key words: Morbillivirus, RNA interference, Adenovirus Vector, Control

1. Introduction and Objectives

RNA interference (RNAi) is a natural process whereby introduction of double-stranded RNA into cells results in post-transcriptional gene silencing. Specific inhibition of virus genes by RNAi can be triggered by small interfering RNA (siRNA) as was obtained previously [1, 2, 3, 4]. In this study, we report on the identification of three conserved loci in nucleoprotein gene of PPRV, RPV and MV that can be targeted for efficient *in vitro* RNA interference. A recombinant human type 5 adenovirus (Ad-5) encoding one of the functional sequences to N gene of PPRV was constructed to be tested *in vivo*.

2. Material and methods

Conserved regions were identified on N gene of all morbilliviruses and siRNAs covering these loci were synthesized. Vero cells were transfected with siRNAs using usual methodology and 24 hours after, the cells were infected with PPRV, RPV or MV. Four days later, the siRNA silencing effect was evaluated by virus titration, flow cytometry and real time PCR. The adenovirus vector containing a functional shRNA sequence (NPPRV1) was constructed using a commercial system (Invitrogen™) and it was transduced in Vero cell cultures. A kinetic analysis of PPRV multiplication in these cell cultures was carried out through virus titration of cell suspension collected after different hours post-infection.

3. Results

One siRNA targeting the conserved locus 1 (position 480-498) of PPRV, RPV and MV inhibited the virus multiplication by over 90% as assessed by flow cytometry. The production of PPRV N transcripts and PPRV progeny was efficiently shut down 100- and 10,000-fold, respectively. Additionally, we demonstrated that two other loci of N gene between PPRV and RPV (loci 6 and 7, positions 741-759 and 899-917) clearly inhibited the virus replication as assessed by a decrease of viral protein over 85% and a reduction of 100- to 1.000-fold in PPRV and RPV titers, respectively. The active loci were mapped by testing sequential siRNA overlapping by one nucleotide. Results demonstrated that activity was lost after 1 or 2 base shift within the locus in both 5'- and 3'- directions. Results of the silencing capacity of the adenovirus vector (Ad-shRNAN1) in infected cell culture demonstrated that it clearly inhibited the PPRV replication characterized by a decrease in virus titres higher than 2 logs.

4. Discussion and Conclusions

We were able to identify one locus in the N gene of PPRV, RPV and MV and two other loci in the same gene of PPRV and RPV which showed very strong *in vitro* antiviral effect when inhibited by siRNA. All these siRNAs may be used alone or in association for targeting multiple viral regions to prevent the emergence of escape mutants. Currently, we are studying delivery methods of siRNAs to be applied *in vivo* and the adenovirus vector produced in this study is one of the options.

5. Acknowledgements

This work was partially supported by EU Pan-African program for the Control of Epizootics, International Atomic Energy Agency, Marie Curie International Fellowship of *Community's* 6th Framework Program and the French Ministry of Foreign Affairs.

6. References

1. Chen, W., Liu, M., Jiao, Y., Yan, W., Wei, X., Chen, J., Fei, L., Liu, Y., Zuo, X., Yang, F., Lu, Y., Zheng, Z., 2006. *J. Virol.*, 80, 3559-3566.
2. Ge, Q., Eisen, H.N., Chen, J., 2004. *Virus Res.* 102, 37-42.
3. Novina, C.D., Murray, M.F., Dykxhoorn, D.M., Beresford, P.J., Riess, J., Lee, S.K., Collman, R.G., Lieberman, J., Shankar, P., Sharp, P.A., 2002. *Nat. Med.*, 8, 681-686.
4. Giladi, H., Ketzinel-Gilad, M., Rivkin, L., Felig, Y., Nussbaum, O., Galun, E., 2003. *Mol. Ther.*, 8, 769-776.

RECOMBINANT CAPRIPPOXVIRUSES EXPRESSING PROTEINS OF BLUETONGUE VIRUS: EVALUATION OF IMMUNE RESPONSE AND PROTECTION IN SMALL RUMINANTS

A. Perrin¹, E. Albina¹, P. Russo³, S. Zientara², C. Cêtre-Sossah¹

¹ CIRAD, Département BIOS, UPR Contrôle des Maladies, Montpellier, France

² AFSSA, UMR 1161, Maisons-Alfort, France;

³ AFSSA, Unité pathologie des petits ruminants, Sophia Antipolis, France

Key words: Bluetongue, Capripoxvirus, Vaccine

1. Introduction and objectives

Bluetongue is an infectious, arthropod-borne viral disease of sheep, domestic and wild ruminants caused by Bluetongue Virus (BTV) and transmitted by *Culicoides*. Since 1998, outbreaks of BT involving 6 distinct serotypes have occurred in Europe. The only BTV vaccines currently available are serotype-specific. Our objective was to generate protective immune responses against conserved antigens of BTV using recombinant capripoxviruses.

2. Material and methods

Recombinant capripoxviruses expressing NS1, NS3, VP7 and VP2 genes of the Corsican strain BTV-2 (Cpox-BTV) were produced as previously described [1]. Two groups of 4 Saanen goats and 21 Pré-Alpes sheep were individually inoculated subcutaneously (SC) with either a suspension of 2.10^6 TCID₅₀ of an irrelevant recombinant capripoxvirus vaccine (control group) or with 2.10^6 TCID₅₀ of each BTV-Cpox recombinant vaccine (BT-Cpox group). Three weeks after inoculation, all animals were challenged SC with either 10^4 TCID₅₀ of the BTV-2 attenuated vaccine strain for goats or virulent BTV-2 strain for sheep. Sheep were observed daily after challenge and whole blood and serum were collected weekly.

3. Results

In goats and sheep, seroconversions to NS3, VP2 and VP7 were observed at the time of BTV challenge. A boosting effect after BTV challenge was observed in all animals pre-immunized with the Cpox-BTV vaccine. Seroconversion to capripoxvirus was observed in all animals. A BTV specific proliferation and blastogenesis were observed 1 week post-challenge in 3/4 goats in the Cpox-BTV group. The protective effect against a BTV virulent challenge, induced by BTV-Cpox, was assessed in sheep. One sheep out of 11 died in the BTV-Cpox group whereas 3 animals out of 10 died in the control group. Most of sheep showed clinical signs, however, a significant difference in clinical scores was observed between animals of the Cpox-BTV group and those in the control group. BTV genome was detected in the blood of all control sheep after challenge but in 10/11 sheep immunised with BTV-Cpox. In contrast, BTV genome was not detected in BTV-Cpox immunized goats but in all goats of the control group.

4. Discussion and Conclusions

Both humoral and cellular immune responses were stimulated by BTV-Cpox. These results demonstrated the capacity of Bluetongue recombinants capripoxviruses, used as vaccines, to elicit an immune response in sheep and goats against both the capripoxvirus itself and the expressed BTV proteins. However, only a partial protection could be obtained in sheep since several animals developed clinical signs. This may result from various reasons like insufficient levels of protein expression, inappropriate route of inoculation and inadequate immune responses.

5. Acknowledgements

This research was supported in part by the Région Languedoc Roussillon and EPIZONE network of excellence.

6. References

1. Romero, CH, Barrett, T, Evans et al., *Vaccine*, 1993; 11: 737-74

CONTROL OF AFRICAN SWINE FEVER VIRUS BY siRNA

D. Keita, E. Albina

CIRAD, Département BIOS, UPR Contrôle des Maladies, Montpellier, France

Key words: Filter paper, PCR, African swine fever, peste des petits ruminants, phylogenetic analysis

1. Introduction and Objectives

African Swine Fever (ASF) is a highly contagious, viral disease of pigs caused by an *Asfivirus*. It can cause up to 100% of mortality in domestic pigs and European wild boars, although varying degrees of virulence have been shown. In contrast, the infection is unapparent in African wild suids (warthogs, bush pigs, giant forest hogs). There is no treatment or vaccine to control this severe disease. To address this issue, we are currently working on the development of an attenuated vaccine and new generation of biological antivirals. For the generation of attenuated ASFV vaccine strains, we envisage to delete the 10 Kb region located 23638-33336 in the genome, which includes nine genes that are thought to determine the virulence of ASFV. Of these 9 genes, 4 (A104R, A118R, A151R and A240L) have unknown function in the virus replication cycle. To establish the importance of these genes, we have used the RNA interference approach. RNA interference is a natural biological process initially described in plants [1]. It is a natural cell mechanism by which small interfering RNAs (siRNAs) of about 20 nucleotides operate to down regulate the expression of a gene by targeting and cleaving its mRNA (post-transcriptional gene silencing).

2. Material and methods

The 4 ASFV genes of interest were cloned from a Spanish strain isolated in 1971 (Ba71v) and sequenced. The sequences were checked and compared with the reference sequence available in GenBank (accession number ASU18466) before to be sent to Ambion for siRNA design. Seventeen 17 siRNAs were received from Ambion of which, 3 were directed against each of the 4 genes of interest and 5 were directed against B646L encoding the essential viral capsid protein (control of siRNA efficacy). Vero cells were transfected with each of these siRNA and 24 hours later they were infected with the Ba71v virus. Cytopathic effect (CPE), virus titres and virus mRNA production were then checked at different times post-infection.

3. Results

Two of the siRNA targeting the A151R gene were shown to neutralise consistently the CPE of Ba71v virus on Vero cells. In addition, the virus titre was reduced with these two siRNA. As expected, one siRNA against p72 was also able to inhibit the CPE, but surprisingly, the effect was less strong than with the two other siRNA targeting A151R. In contrast, all other siRNA did not have any effect.

4. Discussion and Conclusions

The results show that A151R which is still of unknown function in the virus cycle, is however an essential gene for its replication. The other genes investigated are non essential. For the approach of virus attenuation by deletion of the 10 Kb region located 23638-33336, it will be necessary to complement first the genome with an extra-copy of this A151R gene. Considering the marked effect of siRNA against A151R against the virus replication, it is also foreseen to explore the feasibility of using these molecules as an alternative tool to control the infection in pigs as previously reported for foot and mouth disease virus [2].

5. Acknowledgements

This study was partially granted by the Wellcome trust foundation and the EU EPIZONE network of excellence.

6. References

1. Fire, A., Xu, S.Q., Montgomery, M.K., Kostas, S. A., Driver, S.E., Mello, C.C., 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* 391, 806-811.
2. Chen, W., Liu, M., Jiao, Y., Yan, W., Wei, X., Chen, J., Fei, L., Liu, Y., Zuo, X., Yang, F., Lu, Y., Zheng, Z., 2006. *J. Virol.*, 80, 3559-3566.

LYSSAVIRUS TRAFFICKING USING CO-CULTURE TECHNIQUES

C. J. Finnegan, S. M. Brookes, A. R. Fooks

*Rabies and Wildlife Zoonoses Group, Department of Virology, Veterinary Laboratories Agency (Weybridge), New Haw, Addlestone, Surrey, United Kingdom.
WHO Collaborative Centre, OIE Reference Laboratory*

Key words: Co-culture, Lyssavirus, Trafficking

1.Introduction and Objectives

Rabies viruses usually enter the periphery of a new host directly into muscle tissue by a bite from an infectious animal. Virus then invades unmyelinated nerve terminals before migrating to neuronal cell bodies. When replication in the cell body of a neuron is complete, infection continues via retrograde axonal transport and synaptic spread to the central nervous system (CNS). Once viruses reach the brain they later disseminate to other organs. Experiments were set up to mimic the initial events of infection of muscle and subsequent transport of viruses to and through axons.

2.Material and methods

Dissociated dorsal route ganglion (DRG) cells from rats of gestation E14 and E15 were grown for two days to establish a neuronal network. Rat hind muscle tissue (explant) of gestation E20 was infected with virus (either rabies virus (RABV), European Bat Lyssavirus type 1 (EBLV-1) or European Bat Lyssavirus type 2 (EBLV-2)), together with negative controls and placed within the dishes containing axon growth to form co-cultures.

At four days post infection, the cultures were fixed before incubating with anti-RABV FITC conjugate and DAPI. Cultures were then visualised under U.V light.

3.Results

Fluorescence was detected within the axons directly connected to the infected muscle explants. RABV, EBLV-1 and EBLV-2 were all detected in the axons.

4.Discussion and Conclusions

RABV, EBLV-1 and EBLV-2 all travel from infected muscle by first invading nerve terminals before progressing onwards to axonal cell bodies leading to invasion of the CNS.

5.Acknowledgements

Prof. Robert Campenot's group, University of Alberta, Canada, for the training received in co-culture techniques.

Training funded by the President's Fund, Society for General Microbiology, UK.

This research was funded by the Department for Environment Food and Rural Affairs (Defra) ROAMES SE0413 and SE0418.

6.References

Campanot, R.B. (1992) Compartmented culture analysis of nerve growth. In Cell-Cell Interactions: A Practical Approach. Stevenson, B., Paul, D. and Gallin, W. eds. IRL Press, Oxford. pp275-298.

DISTRIBUTION OF RABIES AND RABIES BAT VARIANTS WITHIN THE MOUSE BRAIN

D.M. Healy^{1, 2}, S.M. Brookes¹, A. Nunez¹, D. Hicks¹, S.L. Cosby² and A.R. Fooks¹.

¹Veterinary Laboratories Agency, New Haw, Surrey, UK;
²Queen's University Belfast, Belfast, Northern Ireland, UK.

Key words: Lyssavirus, Brain, Distribution

1. Introduction and Objectives

Rabies encephalitis is caused by neurotropic RNA viruses in the family *Rhabdoviridae*, genus *Lyssavirus*, which includes the European Bat Lyssavirus variants type 1 and 2 (EBLV-1, -2). Transmission occurs mainly via animal bite, and once the virus is deposited in peripheral wounds, centripetal spread occurs towards the CNS. The clinical presentation of rabies virus can vary both within and across species, presenting as either the "furious" or "paralytic" forms, both of which are invariably fatal. The objective of this on-going study is to assess any clinical or distributional differences between the different lyssavirus genotypes.

2. Materials and Methods

The localisation of three different lyssavirus genotypes, [genotype 1 (RABV & wtRABV), genotype 5 (EBLV-1) and genotype 6 (EBLV-2)], throughout the brain of the Swiss OF-1 mouse was examined over a time course following peripheral inoculation. Clinical signs and weight loss were also monitored.

3. Results

The onset and appearance of clinical signs varied across the different genotypes, RABV<wtRABV<EBLV-1<EBLV-2, (generally days 5.5, 9.5, 9.9 and 19 respectively), as did average weight loss RABV<wtRABV<EBLV-2<EBLV-1, (11.8%, 12.8%, 16.6% and 17.6% respectively). Differences in the distribution of the various genotypes throughout the mouse brain were also observed. There are clear differences between the distribution of the fixed strain (RABV) and the wildtype strains of lyssavirus, especially noticeable within the cortex and thalamus. There is also intense staining of the cerebellum for both RABV and EBLV-1, but there is substantially less staining in this region for both wtRABV and EBLV-2.

4. Discussion and Conclusions

There are clear differences in the appearance and onset of clinical signs (including weight loss) between the different lyssavirus genotypes. The histopathological studies also suggest that there are differences between the distribution patterns of RABV and EBLVs in the mouse brain over time. These variations in distribution between the genotypes may be linked to the observed differences in clinical signs.

**Abstracts poster presentations:
Theme 6 Surveillance and Epidemiology**

DIFFERENT EXPRESSION PATTERNS REVEALED BY MICROARRAY ANALYSES OF PORCINE ALVEOLAR MACROPHAGES INFECTED WITH AFRICAN SWINE FEVER VIRUSES OF DIFFERENT VIRULENCE

F. Zhang¹, P. Hopwood², C. Abrams¹, A. Downing³, F. Murray³, R. Talbot³, A. Archibald³, S. Lowden², and L K. Dixon¹

¹*Institute for Animal Health Pirbright Laboratory, Ash Road, Pirbright, Woking, Surrey, UK;*

²*ARK-GenomicsRoslin Institute, Roslin, Midlothian, UK;*

³*Royal Dick Veterinary College, University of Edinburgh, Summerhall, Edinburgh, UK*

Key words: microarray, ASFV

1. Introduction and Objectives

African swine fever virus (ASFV) is a large, icosahedral, double-stranded DNA virus which replicates in the cytoplasm of infected cells, mainly macrophages, and has been classified as the only member of a new virus family, the *Asfarviridae*(1). By using a house-made cDNA microarray containing 2,880 genes, we have investigated changes in macrophage gene transcription that occur during in vitro infection with a virulent isolate of ASFV (Malawi LIL20/1). This identified about 120 temporally altered genes at an early or/and a late time point post-infection (2). In the present study we used the same array to compare Malawi LIL20/1 isolate with a low-virulence isolate, OUR T88/3 at 4 hr and 16 hr post infection of porcine alveolar macrophages, in an attempt to narrow down the candidate host genes and pathways involved in ASFV pathogenicity.

2. Material and methods

The porcine immune array (A-MEXP-494) was used in this study. Total RNA was extracted from mock-infected and infected cells and linearly amplified before labelled with Cy3 and Cy5. Samples for hybridisation were allocated according to a double triangular design with dye-swap. Briefly, samples from Malawi LIL20/1 or OUR T88/3 infected cells at 4 hr and 16 hr post infection were compared with those from mock-infected cells; in addition, at both time points, Malawi LIL20/1 and OUR T88/3 samples were compared directly. Hybridisation signals were extracted from the scan images by using BlueFuse software, and the raw data were normalised and further analysed in TM4 package (3).

3. Results

The data from these experiments showed that macrophages infected with the low virulence isolate shared a similar transcription profile to those infected with the high virulence isolate at 4 hr post-infection. In contrast with the return to base transcription level of most of these genes at 16 hours post-infection in macrophages infected with the high virulence isolate, transcription levels of many of these genes remained elevated at 16 hours post-infection with the low virulence isolate. Of obvious potential importance is the sustained increased transcription of proinflammatory cytokine genes. Other interesting genes with different expression profiles between these two isolates include immune proteasome genes, MHC-1, CD14, SP1, HnRNP A3, etc.

4. Discussion and Conclusions

We have identified some genes showing different expression profiles in response to Malawi LIL20/1 and OUR T88/3 infection. Further investigation on those genes will help to understand the pathogenicity and immune evasion strategies of ASFV.

5. Acknowledgements

This project was funded by BBSRC.

6. References

4. Dixon, L. K, et al. 2005. *Asfarviridae*, p. 135-143. In C. M. Fauquet, et al (ed.), *Virus taxonomy: classification and nomenclature of viruses. Seventh report of the International Committee on Taxonomy of Viruses*.
5. Zhang F, et al. *J Virol*. 2006 Nov; 80(21):10514-21.
6. Saeed AI, et al. *Biotechniques*. 2003 Feb;34(2):374-8.

INTRODUCING THE NETWORK "RODENT-BORNE PATHOGENS" IN GERMANY

S. Essbauer¹, J. Schmidt², F.J. Conraths³, B. Ehlers⁴, R.G. Ulrich⁵

¹Bundeswehr Institute of Microbiology, Munich, Germany;

²Institute of Medical Virology, Frankfurt University Medical School, Frankfurt, Germany;

³Friedrich-Loeffler-Institut, Institute of Epidemiology, Wusterhausen, Germany;

⁴Robert Koch-Institut, Berlin, Germany;

⁵Friedrich-Loeffler-Institut, Institute for Novel and Emerging Infectious Diseases, Greifswald –Insel Riems, Germany

Key words: Hantavirus, Zoonosis, Reservoir Host, Monitoring, Outbreak

1. Introduction and Objectives

Rodents are important reservoirs for a large number of zoonotic pathogens. Rodents can be reservoirs of pathogens and can transmit them directly to humans (e.g. hantaviruses). Alternatively, rodents represent reservoirs for pathogens that are transmitted to humans by arthropods such as ticks (e.g. Tick-borne encephalitis virus and *Borrelia* species).

The knowledge on the geographical distribution of rodent-borne pathogens, their evolution and potential changes in their distribution as well as reasons for outbreaks of human infections in Germany is limited. Therefore a network "Rodent-borne pathogens" was initiated which allows a close collaboration of experts from different research fields. The network comprises a combination of studies dealing with rodent reservoirs themselves, i.e. their biology, genetics, distribution and population development, and studies dealing with pathogens of different nature (i.e. viruses, bacteria, parasites).

2. Material and methods

Surveys of rodent-borne pathogens were mainly focused on three aspects, (I) a longitudinal monitoring of rodent-borne pathogens, (II) investigations of rodent reservoirs in hantavirus outbreak regions during 2004/5, (III) search for new model viruses for human pathogens.

For the monitoring program rodents were trapped three times each year at defined sites in different Federal States of Germany. Outbreak investigations were focussed on regions in Federal States Bavaria, North Rhine-Westphalia and Lower Saxony where a highly increased number of human hantavirus infections was observed in 2004/5. Hantavirus serology was performed by ELISA using yeast-expressed recombinant proteins. For Hantavirus nucleic acid detection RT-PCR and nested RT-PCR formats were carried out. The detection of novel rodent herpesviruses was based on a nested pan-herpesvirus PCR. Amplification products were sequenced and the obtained sequences were phylogenetically analysed.

3. Results

(I) In a pilot monitoring study in the Federal State Brandenburg, a screening of about 1700 rodents trapped during 1994-2005 demonstrated for the first time a continuous presence of Tula hantavirus (TULV) in certain common vole (*Microtus arvalis*) and field vole (*Microtus agrestis*) populations throughout the study period.

(II) Our serological and molecular biology studies on bank voles (*Myodes glareolus*) from Lower Bavaria, North Rhine-Westphalia (Cologne) and Lower Saxony (Osnabrück) revealed a high prevalence of the bank vole-transmitted Puumala hantavirus (PUUV). Nucleotide sequence and subsequent phylogenetic analysis demonstrated significant differences between PUUV strains originating from the three regions.

(III) Investigations of rodent samples from Germany, the United Kingdom and Thailand revealed a large number of novel rodent-associated beta- and gammaherpesviruses, but failed to detect rodent alphaherpesviruses.

4. Discussion and Conclusions

The initiated studies will allow conclusions on the evolution of rodent-borne pathogens and changes in their distribution which might result in a risk assessment for human infections in the investigated regions. This may become very important in order to judge changes in the epidemiology of rodent-borne pathogens in the light of expected global climate changes in the future.

EMERGING OF A HIGHLY PATHOGENIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS IN CHINA

G-Z. Tong, Y-J. Zhou, X-F. Hao, H-J. Qiu, Z-J. Tian, T-Q. An

National Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Harbin, China

Key words: PRRS, PRRSV, Variant

1. Introduction and Objectives

From March of 2006, an infectious disease with unknown agents characterized by high fever and high mortality in all-aged pigs emerged in some pig farms in the mid-east of China and the disease rapidly spread to most provinces of China within a few months. A new variant of Porcine reproductive and respiratory syndrome (PRRS) virus with high lethality was isolated from diseased pigs. The objective of this study is to examine the virulence of the PRRSV mutant and to find out relationship between the emerged virus and the pandemic.

2. Material and methods

The samples were collected from 36 pig farms characterized by fever and high mortality in 10 provinces. ORF5 and part of NSP2 gene were amplified and sequenced. Virus isolation was performed on MARC-145 cells. After several passages of the sample on cell culture, DNA and RNA were extracted from the cells and PCR or RT-PCR were conducted for detecting PRRSV, HCV, PCV and PRV, respectively. IFA test was conducted using PRRSV NP-, M- and Gp5-specific monoclonal antibodies.

3. Results

The viruses isolated from recent epidemics in different provinces were identified as PRRSV by IFA test with monoclonal antibodies against NP-, M-, Gp5-protein of PRRSV. Sequence analysis of ORF5 showed that those viruses are highly homologous to each other, but varied greatly in comparison with the viruses isolated during 1996-2005. In addition, all those viruses from 12 provinces were characterized by two deletions in NSP2, 1 amino acid deletion at 485, and 29 amino acids deletion at 535-563. Preliminary animal infection test showed that the newly isolated PRRSV is highly pathogenic to pigs.

4. Discussion and Conclusions

Although the cause of the emerging pandemic disease of pigs with high mortality in 2006 is unknown, we found high coincidence between PRRSV isolation rate and disease pigs. The regression test in its natural animal revealed that the new isolated PRRSV was much more virulent than earlier PRRSV isolates. And sequences analysis demonstrated there was a great diversity with the previously isolated PRRSVs during 1996-2005. Further study is needed to answer the question: What role does the newly isolated PRRSV play on the 2006 outbreaks in many of swine farms in China?

5. Acknowledgements

The study was supported by grants from the National Basic Research Program (973) of China (No. 2005CB523200), National Scientific Supporting Program (No. 2006BAD06A03/01 /04) and NSFC (No.30470072).

6. References

1. Laroche R, Magar R. 1997. Evaluation of the presence of porcine reproductive and respiratory syndrome virus in packaged pig meat using virus isolation and polymerase chain reaction (PCR) method. *Vet. Microbiol.* 58, 1-8;
2. Horter DC, Pogranichniy RM, Chang CC, et al. 2002. Characterization of the carrier state in porcine reproductive and respiratory syndrome virus infection. *Vet Microbiol.* 86, 213-218.

CLASSICAL SWINE FEVER IN CHINA

**S-Q. Sun, X-T. Liu, S-H. Yin, Y-J. Shang, H. Tian, Y. Jin, H-X. Zheng,
Z-X. Liu, J-W. Ma, X-Q.g Han, H. Yin, X-P. Cai, Q-G. Xie**

Key Laboratory of Animal Virology of Ministry of Agriculture, State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China

Key words: Classical swine fever, vaccine, molecular epidemiology, diagnosis, reverse genetic

Classical swine fever (CSF) is a serious and contagious viral disease of pigs and wild boars with a widespread worldwide distribution. The disease was first recognized in China in the 1920s and for many years there have been major epizootics. Owing to a nation-wide policy of twice-yearly vaccinations of pigs (spring and autumn), CSF is well controlled in China, with large-scale outbreaks rarely seen. However, sporadic epizootics still occur every year and a mild, atypical form of the disease with a long duration, atypical clinical signs, and relatively low morbidity and mortality have been observed often since the late 1970s, even in a certain proportion of vaccinated pigs. More recently, infection in piglets under 3 months old has been seen more frequently than in young and adult pigs.

Live attenuated vaccines are being used as a safe and efficient prophylactic tool in China. In order to overcome the disadvantages of conventional vaccination, genetic engineering vaccines including DNA vaccine and subunit vaccine based on prokaryotic expression system, retrovirus vector and *pichia* yeast expression system, have been developing. All of these vaccines can induce specific antibody in rabbits and can protect rabbits from C-strain challenge, and the vaccine based on E2 protein solubly expressed in *E. coli* can protect swine from lethal Shimen strain challenge.

In order to survey and control disease efficiently, the molecular epidemiology method based on phylogenetic analysis and laboratory diagnostic method based on RT-PCR, PCR-ELISA and indirect ELISA using recombinant protein expressed in *E. coli*. have been developed. Phylogenetic analysis has revealed that CSF virus strains and isolates originating from different regions of China were segregated into two major groups (group 1 and 2) and subdivided into four subgroups (subgroup 1.1, 2.1, 2.2 and 2.3). Most of isolates belonged to group 2 and were further divided into three subgroups, subgroup 2.1, 2.2 and 2.3. Subgroup 2.1 was the largest subgroup whilst subgroup 2.3 was the smallest subgroup, the viruses in this group were restricted to south China. However, none group 3 and subgroup 1.2 and 1.3 viruses were found in China.

CSF virus, the causative agent of CSF, was difficult to work with, and many details concerning the virion remain obscure although many great progresses especially in molecular virology have developed in recent years. In order to study the functional role of the virus gene and the molecular markers of virulence, reverse genetics system including *in vitro* and *in vivo* transcription system based on T7 RNA polymerase and eukaryotic RNA polymerase I (pol I)-mediated transcription system have been developed.

PRRSV: IMPACT OF TRADE BARRIERS ON VIRUS EPIDEMIOLOGY

T. Stadejek¹, M. Oleksiewicz², A. Stankevicius³, D. Potapchuk⁴

¹*National Veterinary Research Institute, Pulawy, Poland;*

²*Novo Nordisk A/S, Måløv, Denmark;*

³*Lithuanian Veterinary Academy, Kaunas, Lithuania;*

⁴*National Academy of Sciences of Belarus, Minsk, Belarus*

Key words: PRRSV, molecular epidemiology

Porcine reproductive and respiratory syndrome virus (PRRSV) is a recently emerged pathogen. Based on reporting of disease symptoms, and retrospective serological surveys, the virus emerged in North America in 1979, in Asia in 1985 and in Europe in 1987. In Western Europe, the first clinical outbreaks were reported in November 1990 in Germany, with outbreaks in the Netherlands, Spain, UK, France, Belgium and Denmark occurring through 1991-1992. Thus, PRRSV emerged globally through a brief time window. Surprisingly, the viruses that appeared in Europe and North America were only distantly related (55-70% nucleotide identity). PRRSV was first isolated in the Netherlands, and that isolate (Lelystad virus) together with the first North American isolate (VR2332) now define the 2 recognized genotypes of PRRSV: European (EU-genotype, type I) and North American (US-genotype, type II). Originally, EU-genotype viruses were thought to be very homogenous and "Lelystad-like". More recent studies have demonstrated that some East European countries such as Lithuania or Belarus harbour exceptionally diverse EU-genotype strains. We suggested that EU-genotype PRRSV consists of distinct subtypes: sequences from West and Central Europe belong to a single genetic subtype, subtype 1. The highly diverse East European sequences belong to at least three further subtypes. There seems to be a sharp geographical demarcation of PRRSV diversity along the Polish eastern border. The subtype 1 viruses were only detected west of the border, and subtype 2, 3 and 4 viruses were only detected east of the border, in Lithuania, Belarus and the former USSR. We currently believe that political and trade barriers are most likely explanation of this pattern. The currently available sequences place the most recent common ancestor of the EU-genotype PRRSV strains between 1946-1967, ie, during the post-World War II development of Europe. In the former Soviet Union, this period involved massive geographical movement of pigs from Western Europe, from countries incorporated to the Soviet Union, and from different parts of Russia resulting in new genetic lines of pigs. It seems plausible that the post-war expansion of the former Soviet Union created an environment allowing a new virus to emerge, or an already emerged virus to spread. Also, because Poland and other East and Central European countries were much less influenced by these policies than for example Belarus and the Baltic states (eg Lithuania), it seems plausible that if new PRRSV subtypes arose during the post-war upheavals, they would be dominant in Lithuania, Belarus and probably other countries formerly being part of the Soviet Union, but not necessarily be present west of the eastern Polish border.

PROBE OF VARIATIONS OF 12 FIELD STRAINS OF CLASSICAL SWINE FEVER VIRUS E2 GENES IN CHINA

CQ. Qiu, HC. Guo, H. Hu

State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agriculture Sciences, Lanzhou Gansu 730046, China

Key words: *Mycobacterium bovis*, red deer, wild boar, molecular typing, diagnostic tests

1.Introduction and Objectives

Classical swine fever (CSF) is considered one of the most threatening diseases for pig industry. Although the C-strain vaccines against CSF have been extensively used atypical CSFs which caused serious loss in pig farming often occur in China. The E2 protein of classical swine fever virus (CSFV) is the most protective antigen. The E2 genes of 12 field strains of CSFV were sequenced and compared with that of the C strain in order to know whether or not the E2 genes of field strains mutated.

2.Material and methods

In 2003 and 2004 years, 12 field strains of CSFV isolated from the 8 provinces and confirmed by IF were used in the tests and E2 genes of the field isolates of CSFV were amplified by RT-PCR and nested-PCR.

3.Results

In 2003 year, comparison and analysis of the nucleotide sequences and the deduced amino acids sequences of 5 of the 12 field isolates of CSFV with CSFV C-strain were performed and the results showed that the homologies of E2 gene nucleotides and deduced amino acids sequences of the 5 field isolates with the C-strain ranged from 81.7% to 83.1% and from 87.3% to 88.6%, respectively. But the homologies of E2 gene nucleotides sequences of the five field isolates were very high reached 99.6% to 99.2%.

In 2004 year, Analysis of E2 gene of the remaining 7 strains of CSFV were amplified by RT-PCR and their nucleotide sequences were determined and the amino acid sequences were deduced. Compared with the corresponding region of C, Alfort and Brescia strain of CSFV, the nucleotide sequence homologies were 91.6%~94.5%, 89.2%~92.7% and 85.9%~89.3%, respectively, the deduced amino acid consistencies were 91.2%~95.8%, 88.9%~92.0% and 85.9%~89.3%, respectively. But the homologies among the 7 field strains were very high, their nucleotide sequence homologies and amino acid homologies were 95.8%~99.7%, 96.3%~99.1%, respectively.

4.Discussion and Conclusions

The analysis of the phylogenetic tree revealed that in comparison with the C strain, the field isolates' E2 gene had varied genetically to a certain extent and they were classified into the same subgroup.

5.Acknowledgement

The study was funded by the Ministry of Science and Technology (No.2000DIB10070, 2001DIA10006).

6.Reference

1. CC. Tu, et al. *Virus Res.* 2001, 81(1-2): 29-37;
2. Ishkawa K, et al. *Arch Virology*, 1995, 40: 1385- 91;
3. Widjojoamodjo M N, et al. *Vet Microbiol*, 1999, 66: 291-99; (4) Ruggli N, et al. *J Virol*, 1996, 70: 3478- 87.

USING ARC HYDRO DATA MODEL TO SUPPORT THE AQUATIC ANIMAL DISEASE SURVEILLANCE

N. Ferré¹, M. Bassan, F. Borghesan, L. Bortolotti, M. Dalla Pozza

¹*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (Padova), Italy*

Key words: Fresh water, Arc Hydro, Surveillance

1. Introduction and Objectives

Diseases surveillance and reporting is considered a fundamental part of the eradication and control strategy on aquatic animal health. As a result, there is a growing interest in developing management information systems (M.I.S) efficiently able to collect, store, process data arising from aquatic animal diseases monitoring programmes, aimed at studying the epidemiological evolution of diseases and reporting the results of these programmes. A geographic information system (GIS) represents an important part of this M.I.S, able to collect spatial data related to aquaculture farms and combine them with epidemiological and managerial information, useful for aquaculture management and veterinary surveillance. Stream, catchments and basin, organised according to the Arc Hydro data model, can be combined with fisheries location, epidemiological data and water resource data inventories to create a veterinary hydrologic information system. This information system can be used to support contingency planning, monitoring of diseases, provide sound aquatic animal health advice to farmer, for international reporting, confirmation of freedom from diseases and finally for an effective aquatic animal emergency diseases readiness.

2. Material and methods

The regional registry farm database and the hydrological network of Veneto region developed according with the Arc Hydro data model (ESRI).

3. Results

Aim of this paper is to describe the integration of the "new" feature fish-farm into the Arc Hydro data model. An hypothesis of the geometry of the spatial object, its spatial relationship, its topological relationship and the methods, properties and events associated with this new object are presented.

4. References

1. Cameron A. 2002. *Survey Toolbox for Aquatic Animal Diseases: A Practical Manual and software package.* Australian Centre for International Agricultural Research. Canberra, Aus;
2. ESRI. 2001. *ArcGIS Hydro Data Model (Draft, July 2001).* ESRI, Technical paper, Redlands, California;
3. Maidment, D.R. 2002. *Arc Hydro: GIS for water resources.* ESRI press. Redlands California;
4. OIE. 2006. *Manual of Diagnostic Test for Aquatic Animals.* OIE. Paris

SIGNIFICANT DIFFERENCE IN WITHIN- AND BETWEEN-PEN FOOT-AND-MOUTH DISEASE VIRUS TRANSMISSION AMONG PIGS

P. Eblé¹, A. de Koeijer², A. Bouma³, A. Dekker¹, A. Stegeman³

¹Central Institute for Animal Disease Control Lelystad, CIDC, Lelystad, The Netherlands;

²Division of Infectious Diseases, Animal Sciences Group Wageningen UR, Lelystad, The Netherlands

³Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Keywords: FMDV, pigs, transmission, modelling

1. Introduction and Objectives

Transmission parameters of Foot-and-Mouth Disease Virus (FMDV) are needed as input for epidemic models used for the development of control and surveillance programmes. Experimentally, usually within-pen virus transmission is studied. However, the contact structures between animals might influence the rate of transmission.

2. Material and methods

We quantified the within- and between-pen transmission of Foot-and-Mouth Disease virus (FMDV) in groups of non-vaccinated pigs by estimating the transmission rate β , i.e. the number of secondary infections caused by one infectious pig during one day, using an SIR (susceptible-infectious-removed) model. Within-pen transmission was studied in four groups of ten pigs in which 5 infected and 5 susceptible pigs had direct contact. Between-pen transmission was studied in one group of ten pigs in which 5 infected and 5 susceptible pigs had indirect contact only. Daily results of virus isolation of oropharyngeal fluid were used to quantify the transmission rate β_i using Generalised Linear Modelling (GLM) and a maximum likelihood method. In addition, we estimated the expected time to infection of the first pig within a pen T_w and in the indirect-contact pen T_b .

3. Results

The between-pen transmission rate β_b was estimated to be 0.59 per day [0.08– 4.2], which was significantly lower than the within-pen transmission rate β_w of 6.1 [3.8 – 10]. T_w was calculated to be 2.1h and T_b 17h.

4. Discussion and Conclusions

Our results show that the transmission rate is influenced by contact structure between pigs and slower spread between pens should be taken into account when the course of an FMDV infection in pig herds is analysed or modelled. Also, transmission between different units and, more importantly, herds, probably develops more slowly than as estimated in within-herd transmission experiments. Although the reduction of transmission caused by limited contact structure by itself might be insufficient to halt an epidemic, the combination with e.g. vaccination might reduce transmission sufficiently.

Furthermore, data such as presented here can help when quantified parameters are needed e.g. to estimate the period of virus introduction in a herd, to develop surveillance programmes or to analyse or model the effectiveness of (implemented) intervention measures.

5. Acknowledgements

This work was supported by the European Union (FAIR5-PL97-3665) and the Ministry of Agriculture, Nature and Food Quality, The Netherlands.

SWINE VESICULAR DISEASE: ANALYSIS OF THE RISK FACTORS HIGHLIGHTED IN THE OUTBREAKS DETECTED IN LOMBARDIA REGION IN ORDER TO CARRY OUT BIOSECURITY GUIDELINE

S. Bellini, G. Zanardi, D. Avisani, M. Gradassi, C. Nassuato, L. Alborali

Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy

Key words: Swine Vesicular Disease, Risk Factors, Bio security Measures

Swine Vesicular Disease (SVD) was first observed in Italy in 1966, when it was clinically recognised as foot-and-mouth disease (FMD). SVD was included in the List A of the OIE for the similarity of its lesions to those produced by FMD, however SVD is often mild in nature and may infect pigs without manifesting itself by clinical symptoms. SVDV is extremely resistant to the environment and to normal disinfectants, a feature of major importance in the epidemiology and control of the disease. Direct contact of susceptible animals by infected pigs or contaminated materials, premises or means of transport is usually necessary for disease transmission.

In Europe in the last decade, Swine Vesicular Disease (SVD) has been persistently reported in Italy and for this reason surveillance and eradication activities are in place. The central-northern parts of Italy have been qualified SVD-free and have maintained this health status since 1997, while others Southern regions never gained the disease free-status. However, from time to time SVD outbreaks have occurred and spread also in northern regions (1998-1999 and 2002) and have always been eradicated following the adoption of rigorous control measures.

In 2006 a recrudescence of SVD was reported in Italy and outbreaks were identified in Northern Regions where the presence of the disease caused heavy economic losses. The 2006 SVD epidemic compare to the previous ones was characterized by a rapid spread of the condition. Lombardia, which is a densely populated pig area, resulted the region mainly affected: 36 outbreaks were detected and 92.219 pigs were stamped out.

In Lombardia some 4.5 million pigs are bred, which makes up 35 % of the national pig population. The region has an extension of 23.834 km² and a pig density of 120,70/animals km², distributed in 5108 pig holdings.

On the basis of the risk factors highlighted in the outbreaks (movement of pigs, introduction of contaminated hauliers' vehicles, proximity to other outbreaks, use of manure on agricultural land as fertilizer) bio security guideline were realized to minimize the risk of SVD introduction and spread. The different typology of pig farms, according to the production cycle and hence management and frequency of animals and vehicles movements, played a different role in disease spreading and different levels of risk were recognized.

Taking into account the risk factors highlighted during the 2006 epidemic, SVD surveillance and eradication activities had to be reviewed and bio-security guideline for the disease established.

References

1. Bellini S., Abrami S., Cominardi F., Nigrelli A., Zanardi G., Brocchi E., Alborali L. *Malattia Vesicolare del Suino: analisi dei fattori di rischio evidenziati nei focolai lombardi al fine della predisposizione di misure di biosicurezza. Atti della Società Italiana di Patologia ed Allevamento dei Suini*, pp 213-224, Modena 29-30 Marzo 2007;
2. Nardelli L., Lodetti E., Gualandi G.L., Burrow R., Goodridge D., Brown F. & Cartwright B. *A foot and mouth disease syndrome in pigs caused by an Enterovirus. Nature* 1968, 219, 1275-6;
3. Lin F. & Kitching R. P. *Swine Vesicular Disease: An Overview. The Veterinary Journal* 2000, 160, 192-201.

FULL LENGTH SEQUENCE ANALYSES AND PHYLOGENETIC COMPARISON OF GENOME SEGMENT 2 OF BLUETONGUE VIRUS SEROTYPE 2 AND 9

S. Maan, N. Maan, C. Batten, A. Samuel, P Mertens

Institute for Animal Health, Pirbright Laboratory, Ash Road, Pirbright, Surrey, United Kingdom

Key Words: Sequence, Bluetongue, Serotype

1. Introduction

Bluetongue virus is the prototype species, genus *Orbivirus*, family *Reoviridae*. Bluetongue viruses (BTVs) can infect most ruminants, causing a haemorrhagic disease 'bluetongue' in sheep. The BTV particle contains 10 dsRNA genome segments and 7 structural. The virus also generates 3 non-structural proteins in infected cells. The outer capsid proteins VP2 and VP5 are the most variable viral proteins and determine the identity of 24 distinct BTV serotypes. Sequencing studies of genome segment 2 and 6 (encoding VP2 and VP5 respectively) of BTV isolates from documented origins (see: www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/ReoID/BTV-isolates.htm) have been used to create a sequence database to support molecular epidemiology studies (see: www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/btv_sequences.htm).

2. Materials and Methods

Seventeen virus isolates of BTV-2 and twelve of BTV-9 (including the South African reference and vaccine strains, as well as isolates from recent outbreaks in the Mediterranean region, and isolates from other geographical regions) were used for full length sequence analyses and phylogenetic studies.

3. Results

All of the isolates of both serotypes have typical bluetongue virus RNA termini (+ve RNA strands - 5' GTTAAA ACTTAC 3' - see www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/CPV-RNA-Termin.htm). In each case Seg-2 of BTV-2 and BTV-9 was 2943 and 2921 base pairs (bp) in length respectively, with a single open reading frame of 2985, or 2864 bp encoding a 962, or 955 amino acid (aa) protein (VP2). Multiple alignments of full-length Seg-2 sequences from 21 isolates of BTV-2 (including the four sequences previously described) revealed a maximum divergence of 28% (BTV-2 SA vaccine and BTV-2 India - 1982). These data also divide the isolates into eastern and western groups. Within the western group (European and S. African isolates) <5% nucleotide (nt) sequence divergence was detected in Seg-2. The American strain (BTV-2/M21946/US) is similar to some of the European and African strains (with only 2.4% and 1.9% divergence in aa and nt sequences respectively). All of the recent European field isolates have very similar VP2 aa sequences with only minor variation apparent (< 0.4%) between them. The European isolates also showed a high level of nt similarity (~98%) with some of the African isolates suggesting a recent common ancestry.

Similar studies of Seg-2 from BTV-9 isolates also revealed two divergent groups (eastern and western) with up to 31.2% nt and 27.4% aa variation between them. The western group viruses (which include the S. African vaccine and reference strains) are closely related to each other with only 0.4% nt and 1.3% and aa differences overall. The recent European isolates also form a tightly related cluster (with <0.6% and <1.5%, nt and aa differences respectively) which has an eastern origin and is related to the Australian and Indonesian strains (up to 13.1% nt and 10.6% aa differences).

Phylogenetic analysis of Seg-2 from BTV-2 and BTV-9 can already be used to 'topotype' individual virus isolates, and by establishing the genetic relationships between different strains, can help to trace the movement and likely source of an outbreak. Addition of more Seg-2 / VP2 sequences to the database, for strains from distinct and precisely identified sources, will help to improve the precision of these phylogenetic studies and the identification of novel isolates. The same also appears to be true for other BTV types.

HIGH THROUGHPUT DIAGNOSIS OF AVIAN INFLUENZA: APPLICATION TO SURVEILLANCE OF WILD BIRDS

S. Hammoumi¹, P. Gil¹, N. Gaidet¹, T. Dodman², D. Martinez¹, E. Albina¹

¹CIRAD, Département BIOS, UPR Contrôle des Maladies, Montpellier, France

²Wetlands International, Wageningen, the Netherlands

Key words: influenza surveillance, wild birds

1. Introduction and Objectives

In the context of highly pathogenic avian influenza (HPAI) emergence in Africa in early 2006, it has become necessary to increase the surveillance level in this region, including surveillance of wild birds suspected to play a role in transmission. A first surveillance campaign was launched in Africa in early 2006, within the framework of a regional Technical Cooperation Programme (TCP) of FAO. It showed a prevalence of 4.2% of LPAI in wild birds out of 5288 samples analysed by hand [1]. Here, we present our strategy for the development of a high throughput diagnostic line of AIV applied to wild bird surveillance in Africa and Eastern Europe during winter 2006/2007, within the framework of a second regional TCP of FAO.

2. Material and methods

Field collection of samples was carried out by Wetlands International and CIRAD. Cloacal and tracheal swabs collected from wild birds were immediately placed in conservative medium and sent to the laboratory where an automation workstation (Biomek FxP, Beckman) was used for RNA extraction. Specific real-time quantitative RT-PCR were then run on a Stratagene Mx3000P to identify influenza A virus positive samples and H5 or H7 subtypes. These subtypes could be further characterized by sequencing of the hemagglutinin cleavage site and by virus isolation.

3. Results

At the time of writing the summary, the analyses were still in process. Around 2,500 samples from 5 countries out of 16,000 expected from 16 countries have been tested so far. No high pathogen viruses have been detected.

4. Discussion and Conclusions

The high throughput diagnosis of AIV from tracheal and cloacal swabs has been developed in the laboratory. It is based on the use of an automation workstation for nucleic acid preparation on which a commercial kit (Macherey Nagel) gave us the best output compared to 4 others. Samples were distributed in a 96-well plate format and their RNA was extracted in 50 minutes without problems of contamination. The real-time RT-PCR adopted were those recommended by the FAO/OIE reference laboratory IZSve (Padova) and the Community reference laboratory VLA (Weybridge). The whole process including sample preparation, RNA extraction and real-time PCR for the M gene allowed to test up to 400 samples a day.

5. Acknowledgements

We acknowledge the participation of and permissions granted by numerous national and local agencies in the participating countries. We are also grateful to the numerous ornithologists and veterinarians who collaborated in this surveillance program by collecting bird samples, as well as the IZSve (G Cattoli) and VLA (M Slomka) for their help in setting up the diagnostic methods. Thanks also to the laboratory persons for their help in transport medium preparation. This extensive survey has been coordinated by FAO through its Technical Cooperation Program

6. References

1. Gaidet N, Dodman T, Caron A, Balança G, Desvaux S, Goutard F, et al. Avian influenza viruses in water birds, Africa. *Emerg Infect Dis* [serial on the Internet]. 2007 Apr [date cited]. Available from <http://www.cdc.gov/EID/content/13/4/626.htm>

RISK FACTORS SIGNIFICANTLY INFLUENCING FUTURE AUJESZKY'S DISEASE ERADICATION PROGRAMME IN POLAND

A. Lipowski, Z. Pejsak

National Veterinary Research Institute, Pulawy, Poland

Key words: Aujeszky's disease, eradication, risk factors

Summary

Aujeszky's disease (AD) is still very important disease from both epidemiological and economical point of views. The latter is especially important due to requirements of Commission Decision 2001/618 on additional guarantees in intra-Community trade of pigs relating to Aujeszky's disease. Because most of the European Union countries are actually free of AD, Poland, being on the EU market with the yearly pig production of over 18 million animals, has to implement an AD eradication programme. However, there are several risk factors that could have a great impact on successful realization of the programme. One of them is a large number of pig holdings in our country. According to available data, there is over 700 thousand pig farms from which more than 70% are those with 1-25 pigs. Moreover, there is a very intensive trade of animals in this group of pig producers. Additionally, the animal movement on the whole territory of our country is practically without any control. Until now, certificates of health as well as of place of origin are not obligatory. Its implementation is foreseen in the AD eradication programme, but in small villages with intensive pig trading between family members as well as close neighbours could be difficult to supervise, resulting in AD virus spreading. With medium and large pig farms there is no problem as they regularly monitor their health situation to certificate an AD free status. Furthermore, the owners of those small pig holdings actually are not interested in exporting live animals to other EU Member states and therefore it will be very difficult to change their understanding how and why AD eradication is so important for our country. The next risk factor that has to be taken into account is an enormous number of samples to be collected, especially in the first year of the programme. The first sampling has to detect 20% of infected animals with 95% confidence. It means that there will be almost 4.9 million blood samples to take. In the second sampling (to detect 5% of infected animals with 95% confidence), in pig farms potentially free of AD, as detected after the first sampling, it will be almost 14.7 million pig sera to sample. However, the number of blood samples to be taken will vary from 0.18 in regions with rather small pig production to 3.4 million in regions with very intensive pig production. The overall number of 19.6 million serum samples to be taken create a significant challenge for veterinary service. The mentioned extremely high number of samples to be taken is connected with large percentage of small pig holdings in which all or the majority of pigs have to be tested. The next step after collecting the serum samples is to test them for AD virus antibodies. Although ELISA is the fastest, cheapest, and easiest test to perform there will be necessary to provide a man power to complete the results in reasonable time span. So, taking into account the above-mentioned problems with sampling and testing almost 19.6 million serum samples it seems that these will be the most critical points in the whole AD eradication programme. And last but not least a cost of the programme is of great importance. In Polish law at present, all eradication programmes of infectious diseases of animals are covered, as a whole by government budget. The cost of the first year of the programme will be very high due to not only sampling and testing the animals but also due to the necessity of employment of many veterinarians who will bleed pigs, technicians who will test the samples, and co-ordinators who will supervise realization of the programme, from both administrative and epidemiological points of view. Summarizing, there are several well known and defined risk factors that could have a great negative impact on successful AD eradication programme in Poland and its success depends not only on understanding the importance of AD from epidemiological and economical points of view but also on the involvement of all parties in all respects in this programme.

Abstracts Theme 5 meeting

USE OF BACTERIOPHAGE LAMBDA PARTICLES AS POTENTIAL NOVEL DELIVERY SYSTEM OF MUCOSAL VACCINES

T. Vahlenkamp, W. Fuchs, G. Keil, T. Mettenleiter

Friedrich-Loeffler-Institut, FLI, Greifswald-Insel Riems, Germany;

Key words: Mucosal Immunity, Vaccination, Host-Pathogen Interactions

1.Introduction and Objectives

Bacteriophage lambda containing reporter genes under control of a CMV promoter have been used as vehicles for nucleic acid immunisation (Clark and March, 2004). Following i.m. injection immune responses were elicited in mice and rabbits (March et al., 2004). Macrophages incubated with lambda particles containing the green fluorescent protein (GFP) gene under the control of a CMV promoter also showed protein expression. Results suggest that targeting of antigen-presenting cells by phage 'vaccines' may be possible. The objective of this work is to explore the possibility of mucosal delivery of recombinant lambda particles.

2.Material and methods

Recombinant bacteriophage lambda particles containing genes encoding immunogenic structural proteins derived from rota- and caliciviruses under the control of the CMV promoter were constructed. Rota- and Caliciviruses were chosen as model viruses for this system because infected hosts are known to rely in particular on the induction of a mucosal immune response for protection. The rotavirus genes encoding the inner (VP6) and outer (VP4, VP7) shells were expressed under control of an eucaryotic promoter. The expression cassettes were subsequently inserted into bacteriophage lambda genomic DNA using the Lambda ZAP II vector kit (Stratagene). Similar experiments were performed using the rabbit haemorrhagic disease virus (RHDV) VP60 encoding gene.

3.Results

Expression of the model antigens in this study, bovine rotavirus VP4, VP6 and VP7 after transient transfection into RK13 cells was successful as analyzed by indirect immunofluorescence. The expression cassettes were inserted into bacteriophage lambda genomic DNA. Transfection experiments resulted in the expression of VP6. VP4 and VP7 could not be detected by indirect immunofluorescence. RHDV VP60 inserted similarly into the bacteriophage lambda genomic DNA resulted in the strongest fluorescence signal after transfection into RK13 cells. Currently we use the bovine macrophage cell line 'BoMAC' as antigen presenting cells to further analyse the expression of the generated recombinant lambda particles in vitro.

4.Discussion and Conclusions

The results from the study will verify, whether bacteriophages can be used as potential new vaccine delivery system by incorporating a eukaryotic promoter-driven gene within their genome. In eukaryotic hosts, bacteriophages are unable to replicate and in the absence of a suitable prokaryotic host, behave as inert particulate antigens. Recent work showed that whole phage particles can be used to deliver vaccines in the form of immunogenic peptides attached to modified phage coat proteins or as delivery vehicles for DNA vaccines. While both approaches are promising by themselves, in future there is also the possibility of creating a hybrid phage combining both components to create phages that are cheap, easy and rapid to produce and that deliver both protein and DNA vaccines in the same construct (Jepson and March, 2004).

5.Acknowledgements

We would like to thank Kerstin Wink for her technical support.

6.References

- (1) Clark, J.R. and J.B. March, 2004. Bacteriophage-mediated nucleic acid immunisation FEMS Immunol. Med. Microbiol. 40: 21-26
- (2) Jepson, C.D. and J.B. March, 2004. Bacteriophage lambda is a highly stable DNA vaccine delivery vehicle. Vaccine 22: 2413-2419.
- (3) March, J.B., Clark, J., Jepson, C., 2004. Genetic immunisation against hepatitis B using whole bacteriophage lambda particles. Vaccine 22: 1666-1671.

NEW DNA VACCINE STRATEGIES AGAINST AFRICAN SWINE FEVER VIRUS

J. Argilaguet^{1*}, E. Pérez-Martín^{1*}, C. Gallardo², J. Salguero², M. Pérez-Filgueira³,
J. Pujols¹, M. Nofrarías¹, N. Fernández-Borges¹, E. Blanco², J. Escribano³ & F. Rodríguez²

¹CReSA, UAB, Bellaterra, Barcelona; ²CISA-INIA, Valdeolmos, Madrid

³Dpto de Biotecnología INIA Madrid; * both authors contributed equally to this job.

Key words: African swine fever virus (ASFV), DNA vaccines, antigen presentation, cellular responses

1. Introduction

African Swine Fever (ASF) is a highly infectious disease of obligatory declaration in the OIE. Despite in many countries it was eradicated in the mid 90's, it remains endemic in many others of the Sub-Saharan Africa, so possibilities of disease reintroduction in our countries clearly exist. ASF is caused by a dsDNA virus (ASFV), which is able to express more than 100 different proteins and against which there is not a vaccine available. Thus, the control of the disease is based on a rapid diagnostic and the sacrifice of the animals. Work done in the mid 90s clearly demonstrated that immunization with the viral proteins p54 and p30 or with the viral Hemmagglutinin (HA) with homology to CD2, expressed in baculovirus, was able to induce partial protection by inducing neutralizing antibodies and by unknown mechanisms, respectively (1 and 3). The aim of the present paper is to develop new vaccine strategies against ASFV. To do so, we planned to use the above described ASFV antigens and diverse vaccine strategies based on plasmid DNA immunization.

2. Materials & Methods

The ORFs encoding the ASFV p30 and p54 were cloned in a modified pCMV vector (Clontech) to obtain the pCMV-PQ. Aiming to enhance the immune response induced, we constructed new plasmid versions based in previous results obtained working with mice (2): i) pCMV-UbPQ encoding PQ as a fusion with ubiquitin in order to enhance CTL induction; ii) pCMV-PQLII encoding PQ as a fusion with the lysosomal targeting signal from LIMP2 in order to enhance T-helper induction. Finally, a new plasmid was generated: iii) pCMV-sHA/PQ, a plasmid expressing a soluble form of ASFV HA (sHA) fused to PQ. Thus, sHA should drive antigens encoded in our vaccine to the antigen presenting cells (APCs) expressing the CD2 receptors. Upon immunization, the kinetic of induction of both antibodies and cellular responses were followed and finally all animals were challenged with a lethal dose (100DI₅₀) of the homologous virulent strain of ASFV (E75L7).

3. Results & Discussion

pCMV-PQ, containing the two immunorelevant antigens P30 and p54 alone, did not develop any detectable ASFV specific immune responses. In clear contrast, pigs vaccinated with pCMV-sHA/PQ showed strong humoral and cellular responses measured by ELISA and an IFN γ ELISPOT, respectively. In spite of the strong responses induced, no protection was afforded and after viral challenge, if any, an exacerbation of the viremia was observed. On the other hand, and in spite no antibody responses were detected upon vaccination with pCMV-UbPQ and/or pCMV-PQLII, partial protection was afforded.

4. Conclusions

i) Immune responses induced by DNA immunization in pigs can be exponentially improved by fusing them to a secretory form of the ASFV Hemmagglutinin. ii) Protection can be afforded in the absence of antibodies. And iii) There is no correlation between the level of specific antibodies and IFN γ expressing cells induced by a given vaccine and the level of protection.

5. References

- (1) Gomez-Puertas et al. *Virology* 1998, 1243(2): 461-71.
- (2) Ruiz Gonzalvo, F. et al. *Am J Vet Res.* 1986, 47(6): 1249-52.
- (3) Rodriguez, F. et al. *Virology* 2000, 268: 233-2384

DEVELOPMENT OF A REVERSE GENETICS SYSTEM FOR PESTE DES PETITS RUMINANTS VIRUS (PPRV)

D. Bailey, L. Chard, A. Banyard, P. Dash and T. Barrett

Institute for Animal Health, Pirbright, Woking.

1. Introduction and Objectives

Peste-des-petits-ruminants virus (PPRV), a member of the *Morbillivirus* genus, is the cause of a serious disease of small ruminants, mostly sheep and goats, endemic in many developing countries in Africa and southern Asia³. We have determined the sequences of two virulent isolates of PPRV, one isolated from goats in the Ivory Coast (ICV 1989) and from sheep during a more recent outbreak in Turkey (Tu 2000)¹, and are developing a full reverse genetics system for these viruses.

2. Methods

Functional minigenomes containing CAT reporter genes have been made for both virus isolates and we are using these to analyse the functional sequence motifs in the viral promoter elements. We have also constructed chimeric minigenomes using the Tu 2000 minigenome and a previously constructed rinderpest virus (RPV) minigenome.

3. Results

Using the PPRV Tu 2000 minigenome system in comparative studies with the RPV minigenome, we observed differences in the ability of combinations of the PPRV and RPV helper proteins, N (nucleocapsid), P (phosphoprotein) and L (large polymerase) to rescue the minigenomes. Chimeric RPV/PPRV minigenomes were produced and were functional, although much less efficient at CAT production than their wild-type homologous counterparts.

4. Discussion

Experiments using homologous Tu 2000 or RPV minigenomes and combinations of helper proteins highlighted important differences between the same proteins of different viral origins. For example, it appears that PPRV L has a much broader binding specificity than RPV L. Use of the chimeric minigenomes suggested that RPV helper proteins are more sensitive to a foreign AGP than genome promoter, whereas the opposite is true for PPRV helper proteins.² We have now constructed a full length cDNA representing the Tu 2000 strain of PPRV and are attempting to rescue the virus in mammalian cells. This will be the first such system for PPRV and will allow investigation into the molecular determinants of virulence and pathogenesis. This system should also allow the development of marker vaccines for PPRV to aid the eradication of the disease.

5. References

- 1) Bailey, D., Banyard, A., Dash, P., Ozkul, A and Barrett, T. (2005) Full genome sequence of peste des petits ruminants virus, a member of the Morbillivirus genus. *Virus Research* 110: 119-124
- 2) Bailey, D., Chard, L.S., Dash, P., Barrett, T and Banyard, A.C. (2007) Reverse Genetics for Peste-des-Petits-Ruminants Virus (PPRV): Promoter and Protein Specificities. *Virus Research* (in press).
- 3) Dhar, P., Barrett, T., Corteyn, M. and Bandyopadhyay, S.K. (2002) Recent Epidemiology of Peste des Petits Ruminants Virus (PPRV). *Veterinary Microbiology* **88**: 153-159.

IMMUNIZATION OF RABBITS WITH RECOMBINANT CSFV E2 GLYCOPROTEIN LOADED PLGA MICROSPHERES

N. Brandhonneur ¹, C. Loizel ², F. Chevanne ¹, E. Hutet ³, P. Wakeley ⁴, A. Jestin ², P. Le Corre ¹, M.F. Le Potier ³

¹ Laboratoire de Pharmacie Galénique, Biopharmacie et Pharmacie Clinique, Université de Rennes I - 2, Rennes Cedex, France.

Agence Française de Sécurité Sanitaire des Aliments (AFSSA),

² Unité de Génétique Virale et Biosécurité ³ Unité de Virologie et Immunologie Porcines Les Croix, Ploufragan, France

⁴ Veterinary Laboratories Agency (VLA), Woodham Lane, New Haw, Addlestone, Surrey, UK

Key words: Immunization, E2 glycoprotein, Microspheres

1. Introduction and Objectives

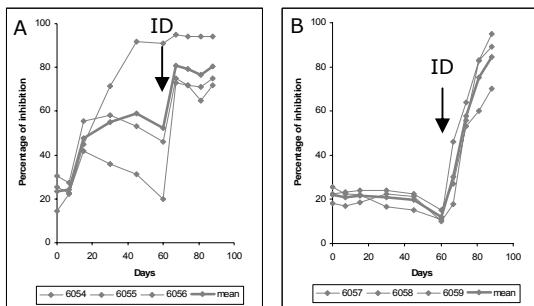
E2 is the most immunogenic envelope protein of classical swine fever virus (CSFV) inducing neutralizing antibodies in pigs. For wildlife or farm animals, oral administration of antigen-encapsulated microparticles via bait or food is of interest to induce a mucosal immunisation directly at the site of infection [1]. E2 glycoprotein loaded poly (D,L-lactide-co-glycolide) microspheres PLGA-MS could be a potential oral vaccine, offering the possibility to discriminate CSFV infected from vaccinated pigs. The purpose of this study was to evaluate the specific immune response against purified E2 glycoprotein encapsulated in PLGA-MS in rabbits after intramuscular (IM) or intranasal (IN) administration.

2. Material and methods

Recombinant E2 (rE2) glycoprotein loaded microspheres were prepared using a double emulsion solvent extraction evaporation process. The *in vivo* study was performed in 11 rabbits. Each rabbit received 10 µg of rE2 glycoprotein encapsulated in PLGA-MS (E2-MS) by the IM or IN route. As a negative control, rabbits received empty PLGA-MS. As a positive control, rabbits received 10 µg of rE2 glycoprotein by IM dosing. After 60 days, each rabbit was challenge with 5 µg of rE2 glycoprotein by intradermal (ID) administration. Blood samples were collected weekly for 90 days. Antibodies specific for E2 protein were measured using the Bommeli CHEKIT CSF ELISA kit.

3. Results

The average diameter of the rE2 protein loaded microspheres was 4µm. The encapsulation efficiency of rE2 glycoprotein was 80% ± 5%. 14 days after IM administration of rE2-MS, specific antibodies for E2 were detected, the level of which increased 1.5 fold after ID administration of rE2-MS (Fig. A). By nasal route, a dose of 10 µg E2-MS did not in itself induce a specific Ig response but a priming immunization was established as demonstrated by the immediate and large increase of antibody levels following intradermal administration of the immunogen (Fig. B).



Induction of total anti-E₂ immunoglobulin in rabbits immunized with E2-MS by IM route (A) or by IN route (B)

4. Discussion and Conclusion

Administration of rE2-MS either through the intra-muscular or intra-nasal route primes the immune response to further boosts. Oral immunization with rE2-MS is currently under evaluation in rabbits.

DEVELOPEMENT OF A SUBUNIT VACCINE AGAINST TUBERCULOSIS: PLANT-PRODUCED ESAT-6/AG85 FUSION ANTIGEN PROTEIN AND CATIONIC LIPOSOMES DELIVERY SYSTEM.

M. Mockey¹, U. Conrad², P. Holzmuller¹, L. Dedieu¹.

¹CIRAD, Département Elevage et Médecine Vétérinaire, Montpellier, France ;

²Institut für Pflanzengenetik und Kulturpflanzenforschung, GATESLEBEN, Leibniz Institute, Germany.

Key words: Tuberculosis, ESAT-6-Ag85, Hsp70, Vaccine, Liposomes.

1. Introduction and Objectives

Tuberculosis (TB) is a major infectious disease problem with 8 million people developing the active disease and 2 million dying of TB each year. The attenuated *Mycobacterium bovis* Bacillus Calmette Guerin (BCG) is the only available vaccine against TB. However, different trials have shown that this vaccine do not provide consistent protection against TB. Furthermore, BCG is a live vaccine which is potentially pathogenic and not recommended in immunodepressed people. An improved second-generation vaccine is therefore urgently needed. Based on the identification and characterization of several major antigens of *M. tuberculosis*, we have selected the ESAT-6/Ag85 fusion antigen protein to develop a new subunit vaccine against TB. Included in the European consortium Pharma-Planta projects, we evaluate *in vitro* and *in vivo* immunogenicity of plant-produced ESAT-6/Ag85B antigen and design liposome-based delivery systems. Previous results using ovalbumin antigen have shown *in vitro* efficacy of cationic liposomes to improve the Ag immunogenicity. Moreover, we have demonstrated strong immunostimulatory properties of Hsp70 from *T. congolense* on murine and bovine antigen-presenting cells (APCs) Therefore, we now develop optimized liposome formulations for TB antigen delivery and assess Hsp70 protein or peptide-derived as adjuvant in TB vaccine development.

2. Material and methods

Under patent procedure

PHARMACOKINETIC AND TOXICITY STUDY IN PIGS OF AN IMIDAZOPYRIDINE, AN IN VITRO INHIBITOR OF THE REPLICATION OF CLASSICAL SWINE FEVER VIRUS

R. Vrancken¹, J. Paeshuyse², G. Puerstinger³, J. Rozenski², M. Wright⁴, J. Neyts², F. Koenen¹

¹VAR, Dep. of Virology, Ukkel, Belgium; ²Rega Institute for Medical Research, KU Leuven, Leuven, Belgium; ³Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria; ⁴Gilead Sciences, Drug Metabolism and Pharmacokinetics, Foster City, CA, USA

Key words: classical swine fever, antiviral, BPIP, pestivirus

1. Introduction and objectives

The classical swine fever virus is the etiological agent of classical swine fever (CSF) and represents a major threat for the pig industry. Since general vaccination was banned within the EU, CSF-outbreaks are controlled by stamping-out of the infection and pre-emptive culling. An alternative strategy could be the use of antiviral agents for curbing the viral spread in case of an outbreak. It was the purpose of this study to investigate the pharmacokinetic properties and possible adverse effects of BPIP, an imidazo[4, 5-c]pyridine derivative that selectively inhibits the *in vitro* replication of pestiviruses (1,3). These results will be used to refine the doses and schedule for BPIP-treatment in further infection studies.

2. Material and methods

Compound and administration

BPIP was synthesised as described previously (2) and was administered orally as pellets of a homogeneous mixture of pig feed and BPIP.

Animals

Eight-week old conventional weaner pigs (Belgian Landrace x Piétrain) of ca. 20 kg.

Pharmacokinetic and acute toxicity study

Three animals received a single oral dosage of BPIP at a concentration of 200 mg/kg. Acute toxicity was monitored for 3 days and blood samples were taken at 1, 2, 3, 4, 6 and 24 h after administration.

Chronic toxicity

Two groups of five animals were treated with BPIP for 15 days. A first group was administered BPIP daily at 50 mg/kg. A second group was treated twice a day at 25 mg/kg. Toxic effects were monitored until two weeks after BPIP-treatment and blood samples were taken at a daily basis.

Sample analysis

Plasma-levels of BPIP were determined using a HPLC-MS technique (1).

3. Results

Pharmacokinetic study and acute toxicity

No acute toxic effects could be observed. Analysis of the BPIP-concentration in plasma revealed a biphasic uptake and pharmacokinetic parameters were determined ($T_{max} = 3.3 \pm 2.5$ h; $C_{max} = 10.2 \pm 4.32$ µM; $AUC_{inf} = 337.12 \pm 286$ µMh; $t_{1/2} = 20.2 \pm 13.6$ h; $Cl/F = 2.42 \pm 1.4$ L/h/kg).

Chronic toxicity

Neither group, single nor double administration of BPIP, exhibited toxic effects. A decreased food consumption was observed when BPIP was administered once a day resulting in a lower average weight gain (2.6 kg) than the control group (4.45 kg). A normal weight gain was observed when administered twice a day (4.2 kg).

4. Discussion and Conclusions

After a single oral administration, BPIP was readily absorbed, indicating a good bioavailability and a prolonged absorption, maintaining a plasma concentration exceeding the EC₉₀ of 0.88 µM for at least 24 h. The mechanism underlying the appearance of a double plasmapeak of BPIP is not yet clear. No significant toxic effects, acute nor chronic, were observed at the used dosage and period of treatment. Further, the reduced food consumption of the group treated once a day could not be unequivocally linked to BPIP. This study resulted in an indicative working dose. Studies to further refine the BPIP-dose for infection studies are ongoing.

5. Acknowledgements

This work was supported by a grant from the EU in the framework of a FP6 project (Grant Nr.: NOE project: 16236).

6. References

1. Paeshuyse, J. et al. (2006) *J. Virol.*, 80: 149-160.
2. Puerstinger G. et al. (2006) *Bioorg Med Chem Lett.* 16: 5345-9.
3. Vrancken, R. et al., *Antivir. Res.*, submitted.

FOOT-AND-MOUTH DISEASE IMMUNOPROPHYLAXIS USING SINGLE-DOMAIN ANTIBODY FRAGMENTS

M. Harmsen¹, H. Fijten¹, A. Dekker², P. Eblé²

¹*Animal Science Group, ID-Lelystad, Lelystad, The Netherlands*

²*Central Institute for Animal Disease Control Lelystad, CIDC, Lelystad, The Netherlands;*

Key words: VHH, nanobody, foot-and-mouth disease, passive immunisation

1. Introduction and Objectives

Foot-and-mouth disease (FMD) is a contagious viral disease. Outbreaks of FMD with severe economic consequences occasionally occur in the European Union due to the non-prophylactic-vaccination policy. Our objective was to produce FMDV-neutralizing recombinant llama single-domain antibody fragments (VHHs) suitable for the passive immunization of livestock to confer rapid protection in FMD outbreak situations.

We previously isolated and characterized 87 FMDV serotype O binding VHHs for this purpose (1). VHH immunotherapy is complicated by their rapid renal clearance from the body due to their small size. The serum half-life can be increased a 100-fold by making genetic fusions of FMDV binding and porcine IgG binding VHHs, resulting in bispecific VHH2s (2). To increase the FMDV neutralizing potency we now produced genetic fusions of two FMDV binding VHHs. These VHH2s were then fused to an immunoglobulin binding VHH, resulting in VHH3s. We then analysed the immunotherapeutic efficacy of these VHH3s in pigs.

2. Material and methods

The FMDV binding VHHs M180 and M200 (1) were genetically fused using the llama IgG short hinge sequence as a linker. Two M200 VHHs were similarly fused. These VHH2s were genetically fused to the porcine IgG binding VHH VI-8, resulting in the VHH3s M180shM200ggsVI-8 and M200shM200ggsVI-8.

VHH3s were produced in bakers yeast using 100-L fermentation, purified by IMAC and subsequent IEC and concentrated to 200 mg/ml in PBS. Ten conventional 6-week-old pigs received 50 mg/kg of a mixture of both VHH3s intravenously. Six control pigs received PBS. Pigs were housed in isolation rooms in groups of two. In each room one pig was challenge infected with FMDV O1 manisa 24 h after VHH3 administration. The development of clinical signs of FMD was followed the following days for both the seeder and sentinel pigs.

3. Results

Both VHH3s were secreted at the high level of 3 g/L by yeast. The VHH3s neutralized FMDV O1 manisa at about 0.1 mg/ml in vitro. As compared to their monovalent counterparts the VHH3s cross reacted more efficiently to FMDV serotype Asia1 in ELISA. In the transmission experiment the three control pigs transmitted FMDV within 1 day whereas the five pigs that received the VHH3s transmitted FMDV within 3 days (3 pigs) or did not transmit FMDV (2 pigs). Furthermore, the pigs that received VHH3s always had strongly reduced clinical signs, lower viraemia and lower viral shedding than the control pigs.

4. Discussion and Conclusions

We have produced new VHH3s with increased in vitro FMDV neutralizing capacity, a high production level in yeast and broader reactivity with other FMDV serotypes. Administration of a high dose (50 mg/kg) inhibited both FMD clinical signs and transmission. The production costs of such a VHH3 dose are estimated at 38€ per 100-kg pig. The efficacy of this immunotherapy at lower doses, using the single VHH3s and the interference of passive immunisation with active immunisation remain to be determined.

5. References

- (1) Harmsen MM, Van Solt CB, Fijten HPD, Van Keulen L, Rosalia RA, Weerdmeester K, Cornelissen AHM, De Bruin MGM, Eblé PL, Dekker A (2007) *Vet Microbiol* 120: 193-206
- (2) Harmsen MM, Van Solt CB, Fijten HPD, Van Setten MC (2005) *Vaccine* 23: 4926-4934

Participants Annual Meeting EPIZONE 2007

Please find the e-mail addresses in the contact list at:

www.epizone-eu.net/cps

Family name	First name	Institute				
Blanchard	Yannick	AFSSA	WPL 4.2			
Castric	Jeannette	AFSSA				
Dory	Daniel	AFSSA				
Jestin	Veronique	AFSSA				
Kuntz-Simon	Gaëlle	AFSSA				
Le Gall-Recule	Ghislaine	AFSSA				
Le Potier	Marie-Frédérique	AFSSA		TL 5		
Renson	Patricia	AFSSA				
Vannier	Philippe	AFSSA			CF	
Zanella	Gina	AFSSA				
Zientara	Stéphan	AFSSA				
Backx	Anoek	CIDC				
Bongers	Johan	CIDC	WPL 2.3; 2.4			
de Vos	Clazien	CIDC				
Dekker	Aldo	CIDC				
Eblé	Phaedra	CIDC	WPL 6.3			
Elzenaar	Jeroen	CIDC	Financial officer			
Engelsma	Marc	CIDC				
Oosterga-Land	Jitty	CIDC	Project manager			
van Rijn	Piet A.	CIDC	WPL 1.1	TL 1	CF	
Vedder-Rootjes	Margriet	WUR	Communication officer			
Albina	Emmanuel	CIRAD	WPL 3.2	TL 3	CF	
Camus	Emmanuel	CIRAD				GB
Hammoumi	Saliha	CIRAD				
Keita	Djénéba	CIRAD				
Kwiakek	Olivier	CIRAD				
Dedieu	Laurence	CIRAD				
Libeau	Geneviève	CIRAD				
Manso-Silvan	Lucia	CIRAD				
Minet	Cécile	CIRAD				
Mockey	Michael	CIRAD				
Perrin	Aurelie	CIRAD				
Thiaucourt	Francois	CIRAD	WPL 6.4			
Bárcena	Juan	CISAINIA				
Borrego	Belen	CISAINIA				
Brun	Alejandro	CISAINIA	WPL 5.1		depCF	
Fernández	Jovita	CISAINIA				
Gallardo	Carmina	CISAINIA				
Martínez	Marta	CISAINIA				
Ortego	Javier	CISAINIA				

Real Sevilla Tafalla Rodriguez	Gustavo Noemí Carolina Fernando	CISAINIA CISAINIA CISAINIA CRESA		
Oude Ophuis van Hassel	Peter Victor	DiVa DiVa		CF
Minguez-Tudela	Isabel	EU		
Beer Bergmann Conraths Gall Hoffmann Keil Mettenleiter Vahlenkamp	Martin Sven Franz Astrid Bernd Günther Thomas Thomas	FLI FLI FLI FLI FLI FLI FLI FLI	WPL 7.2 WPL 4.1 WPL 5.2	TL 4 CF TL 7
Chen Fu Qiu Tong Wang Wang Yu Zhang	Hualan Chaoyan Hua-Ji Guang-Zhi Yunfeng Xiaomei Li Yun	HVRI HVRI HVRI HVRI HVRI HVRI HVRI HVRI		CF
Meindl-Böhmer Moennig	Alexandra Volker	HVS HVS	WPL 2.2	CF
Bankowska Bankowski Bashiruddin Batten Chapman Chard Dixon Dukes Herbert King Knowles Mellor Parida Reid Shirley Stirling Waters Zhang zhang	Kasia Bartek John Carrie David Louisa Linda Juliet Rebecca Donald Nick Philip Satya Scott Martin Julie Ryan Fuquan zhidong	IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH IAH		TL 5 CF GB

Harmsen	Michiel	ID-L			
Hulst	Marcel	ID-L			
Kortekaas	Jeroen	ID-L			
Moormann	Rob	ID-L			
van Boven	Michiel	ID-L			
Van der Poel	Wim	ID-L	WPL 2.1	TL 2	CF
de Jong	Mart	WUR			
Bellini	Silvia	IZSLER			CF
Massirio	Ivano	IZSLER			
Moreno Martin	Ana	IZSLER			
Bovo	Giuseppe	IZS-Ve			
Cattoli	Giovanni	IZS-Ve			
Capelli	Gioia	IZS-Ve			
Ceglie	Letizia	IZS-Ve			
Ceolin	Chiara	IZS-Ve			
Dalla Pozza	Manuela	IZS-Ve			
Dundon	William	IZS-Ve			depCF
Ferrè	Nicola	IZS-Ve			
Monne	Isabella	IZS-Ve			
Mori	Antonio	IZS-Ve			
Toffan	Anna	IZS-Ve			
Ging	Qiu Chang	LVRI			
Jing	Zhi Zhong	LVRI			
Qiang	Zhang	LVRI			
QIU	CQ	LVRI			
Shang	Youjun	LVRI			
Sun	Shiqi	LVRI			
Yin	Hong	LVRI			CF
Antychowicz	Jerzy	NVRI			
Bigoraj	Ewelina	NVRI	Organisation		
Domanska-Blicharz	Katarzyna	NVRI			
Dutkiewicz	Wacław	NVRI	Organisation		
Grzebalska	Agnieszka	NVRI	Organisation		
Jasik	Agnieszka	NVRI	Organisation		
Kowalczyk	Andrzej	NVRI			
Kozyra	Iwona	NVRI	Organisation		
Kuzmak	Jacek	NVRI			
Lipowski	Andrzej	NVRI			
Lizak	Radosław	NVRI			
Markowska-Daniel	Iwona	NVRI	Organisation		
Materniak	Magdalena	NVRI			
Matras	Marek	NVRI			
Minta	Zenon	NVRI			
Niemczuk	Krzysztof	NVRI	Organizing committee		
Pejsak	Zygmunt	NVRI			CF
Przeniosło-Siwczyńska	Monika	NVRI	Organisation		
Rachubik	Jarosław	NVRI	Organisation		

Rola	Marzena	NVRI		
Smietanka	Krzysztof	NVRI		
Stadejek	Tomasz	NVRI		
Szczotka	Anna	NVRI	Organisation	
Szóstak	Paweł	NVRI	Organisation	
Szymanek	Katarzyna	NVRI	Organizing committee	
Szymańska-Czerwińska	Monika	NVRI	Organisation	
Walczak	Małgorzata	NVRI	Organisation	
Wieczorek	Kinga	NVRI	Organisation	
Wijasza	Tadeusz	NVRI		
Żmudziński	Jan	NVRI		
Żmudziński	Piotr	NVRI	Organisation	
Firat SARAÇ	Müge	SAP		
Fuat	Özyörük	SAP		CF
sareyyupoglu	Beyhan	SAP		
Berg	Mikael	SVA		
Blomström	Anne-Lie	SVA		
Carlsson	Ulla	SVA	CF	
Engvall	Anders	SVA		GB
Gyarmati	Peter	SVA		
Jansson	Eva	SVA		
Lindberg	Ann	SVA		
Ståhl	Karl	SVA	depCF	
Tamm	Staffan	SVA		
De Clercq	Kris	VAR		
Goris	Nesya	VAR		
Koenen	Frank	VAR	WPL 5.4	CF
Marché	Sylvie	VAR		
Van Borm	Steven	VAR		
Vrancken	Robert	VAR		
Heegaard	Peter	VET-DTU	WPL 5.3	
Klein	Jörn	VET-DTU		
Madsen	Mogens	VET-DTU		TL 3
Madsen	Sanne	VET-DTU		
Olesen	Niels Jørgen	VET-DTU	WPL 6.1	
Paisley	Larry	VET-DTU	WPL 7.1	
Uttenthal	Åse	VET-DTU	WPL 4.3	
Vigre	Håkan	VET-DTU		
Alexandersen	Søren	VETINDK		TL 6 CF
Finnegan	Christopher	VLA		
Healy	Derek	VLA		
Kelly	Louise	VLA	TL 7	depCF
Kerr	Lauren	VLA		
Newell	Diane	VLA		GB
Phipps	Paul	VLA		
Slomka	Marek	VLA		

Snary
Wakeley

Emma
Philip

VLA WPL 7.4
VLA WPL 4.4

EAP members

Czub
Griot
Donaldson
Erlacher-Vindel

Markus
Christian
Alex
Elisabeth

University of Calgary
IVI
Bio-Vet Solutions Ltd
OIE

Invited speakers

Fitzpatrick
Hussain
Stärk

Julie
Manzoor
Katharina

Moredun Research Institute, Scotland
FAO
Royal Veterinary Public Health, Londen