

arbo-zoonet

ARBOZONET FP7 SYMPOSIUM:
Interventions against WNV, RVFV and CCHFV:

Where are we?

19-20 NOVEMBER 2009
GRANDOZTANIK HOTEL

ISTANBUL - TURKEY

ABSTRACT BOOK

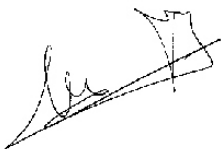


Preface

Dear colleagues,

This symposium will cover intervention studies against Crimean Congo Hemorrhagic Fever, Rift Valley Fever, and West Nile Fever. These are multisectoral diseases including medicine, veterinary medicine, ecology, basic sciences, and public health. There are many questions in each of these fields to be answered. By this meeting we bring leading international experts together and will update the information on these infections .

We are looking forward to meet you all and enjoy together the strong and attractive scientific programme, as well as the exciting and historical city of Istanbul.



Rob Moormann



Onder Ergonul

ARBOZONET FP7 SYMPOSIUM:

Interventions against WNV, RVFV and CCHFV: Where are we?

Program: 19 November 2009

Morning:

8.50-9.00: Opening

Workshop: Vaccines against RVFV, CCHFV and WNV

a) New vaccine development

Moderators: Michele Bouloy/Rob Moormann

9.00-9.30

Exploiting reverse-genetics to create novel bunyavirus vaccines. Richard M. Elliott.

9.30-10.00

Rift Valley fever outbreak dynamics and reverse-genetics generated vaccine. Brian Bird, César Albariño, Amy Hartman, Bobbie Erickson, Jane Githinji, Joseph Macharia, Jacqueline Kasiiti, Stephen Gacheru, Joseph Musaa, Jonathan Towner, Serena Carroll, Jennifer Oliver, Thomas Stevens, Laura Morgan, Marina Khristova, James Comer, Anita McElroy, Pierre Rollin, Thomas Ksiazek, and Stuart Nichol.

10.00-10.30

Reverse-genetics of Rift Valley fever virus: Applications and Implications. Matthias Habjan and Friedemann Weber.

10.30-10.45

Less is more: The size of the inoculum determines the outcome of Rift Valley fever virus infections. A. F. Antonis, J. Kortekaas, J. Kant-Eenbergen, R. J. M. Moormann.

10.45-11.15: Coffee break

11.15-11.45

DNA and Alphavirus replicon based vaccines are immunogenic and protect mice against virulent Rift Valley fever virus infection. Nitin Bhardwaj, and Ted M. Ross.

11.45-12.00

Protection against lethal RVFV infection in transgenic IFNAR^{-/-} mice induced by different DNA vaccination regimens. Gema Lorenzo, Raquel Martin, Esther Hevia, Hani Boshra, and Alejandro Brun.

12.00-12.15

Inoculation of calves with an experimental Newcastle disease virus-based vector vaccine elicits neutralizing antibodies against Rift Valley fever virus. J. Kortekaas, A. Dekker, K. Weerdmeester, R. P. M. Vloet, A. J. de Wit, J. van der Laan, B. P. H. Peeters, R. J. M. Moormann

12.15-12.30

The Use of Self-Adjuvants in RVFV DNA vaccines. Hani Boshra, Gema Lorenzo, Alejandro Brun.

12.30-12.45

Rift Valley fever virus subunit vaccines confer complete protection against a lethal virus challenge. Boer de SM, Kortekaas J, Antonis AF, Kant J, Oploo van JL, Rottier PJM, Moormann RJM, Bosch BJ.

12.45-14.00: Lunch break

Afternoon:

b) Experiences with existing vaccines

Moderator: Stuart Nichol

14.00-14.30

Evaluation of the efficacy and safety of the RVF Clone 13 vaccine in sheep. B

Dungu, I Louw, B von Teichmann, A Lubisi, P Hunter¹ and M Bouloy

14.30-14.50

Bulgarian vaccine against CCHFV. I. Christova.

14.50-15.10

Applications of the ALVAC vector for immunization of horses against West Nile virus and African Horse Sickness virus. Jean-Christophe Audonnet.

15.10-15.40: Coffee break

Workshop: Anti-vector therapies

Anti-vector vaccines

Moderators: Yabbar Ahmed/Önder Ergonül

15.40-16.10

The contribution of anti-tick vaccines to the control of tick borne disease. Peter Willadsen, Shelley Hope, Tony Vuocolo and Varda Shkap.

16.10-16.40

Anti-Mosquito vaccines: Effects on survival, fertility and transmission of *Plamodium berghei*, of immunization with extracts from *Anopheles stephensi* and *Anopheles gambiae* Midguts. A.P.G. Almeida.

16.40-18.00

Wrap-up first day and general discussion: Identification of research gaps.

18.00-19.00

Informal get together with drinks

19.30-22.00

Dinner

Program: 20 November

Morning:

Workshop: Antivirals against CCHFV, RVFV and WNV

a) The efficacy of existing drugs

Moderator: Stuart Nichol

9.00-9.30

The use of ribavirin for therapeutic treatment of patients infected with CCHFV.

Önder Ergonül.

9.30-9.45

Comparison of antiviral activity of commercially available recombinant antiviral and multiferon. Helen Karlberg, Gunnel Lindegren, and Ali Mirazimi.

9.45-10.00

The antiviral effect of nitric oxide on two viruses belonging to two different genera within the family Bunyaviridae. Sara Åkerström, Melinda Simon, and Ali Mirazimi.

b) New and potential molecules

Moderator: Ali Mirazimi.

10.00-10.30

Novel minigenome assay for screening Crimean-Congo hemorrhagic fever antivirals and identification of the SKI-1/S1P protease as a promising antiviral target. Éric Bergeron, Martin Vincent, César Albariño, Marina Khristova, and Stuart Nichol.

10.30-11.00: Coffee break

11.00-11.30

Establishment of a stat-1 knockout mouse model for Crimean-Congo hemorrhagic fever. Dennis Bente, Judie Alimonti, Gaëlle Camus, Ute Ströher, Shieh Wung-Ju, Sherif Zaki, Steven Jones.

11.30-11.45

Assistance with the development of interventions against Crimean-Congo Hemorrhagic fever (CCHF) virus. S. Dowall, J.Chamberlain, R. Hewson.

11.45-12.00

The virus-host cell interaction: Aim of therapeutic interventions?
M. Keller, K. Schmidt, U. Ziegler, M.H. Groschup.

12.00-12.45

Wrap-up morning session and general discussion: Identification of research gaps.

12.45-14.00: Lunch break

Afternoon:

Satellite workshop: Epidemiology and diagnosis

Moderator: Beate Kummerer

14.00-14.30

The second seasonal appearance of human West Nile neurological invasive disease in Northern Italy: Evidence for environmental viral persistence and necessity for improvement of control measures. Vittorio Sambri, Francesca Cavrini, Paolo Gaibani, Anna Maria Pierro, Giada Rossini and Maria Paola Landini.

14.30-14.45

Evidence of recent Rift Valley Fever virus circulation in Mayotte, a French island of the Indian ocean. Catherine Cêtre-Sossah, Agnès Billecocq, Cédric Defernez, Jacques Favre, Michèle Bouloy, Dominique Martinez and Emmanuel Albina.

14.45-15.00

Recombinant indirect immunofluorescence test for the serological diagnosis of Crimean-Congo hemorrhagic fever. Nadine Litzba, Ramon Flick, Yavuz Uyar, Gülay Korukluoğlu, Ahmet Carhan, Ana Saksida, Tatjana Avšič-Županc, Sadegh Chinikar, Janusz Paweska, Önder Ergönül, Sabine Lederer, and Matthias Niedrig.

15.00: Closure

EXPLOITING REVERSE GENETICS TO CREATE NOVEL BUNYAVIRUS VACCINES

Richard M. Elliott

Centre for Biomolecular Sciences, School of Biology,
University of St Andrews, St Andrews, Fife KY16 9ST, Scotland, UK

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Bunyaviruses are characterised by a tripartite, negative sense RNA genome, and impinge on human health and well-being, either directly in causing diseases ranging from encephalitis (e.g. La Crosse virus) to haemorrhagic fever (e.g. Crimean-Congo haemorrhagic fever virus, Rift Valley fever virus), or indirectly by causing disease in animals (e.g. Rift Valley fever, Cache Valley and Akabane viruses) or crop plants (e.g. tomato spotted wilt virus). Furthermore, this family contains prime examples of emerging and re-emerging viruses. We developed a highly efficient reverse genetics system to recover the prototype bunyavirus, Bunyamwera (BUNV), from cloned cDNA (Lowen et al., *Virology* **330**, 493-500; 2004), and this system was subsequently applied to recover La Crosse and Rift Valley fever viruses. This technology can be exploited to make attenuating mutations in the genome, either by mutating or deleting virulence genes, or by mutating non-coding cis-acting control sequences. In addition, specific marker sequences can be incorporated into the viral RNAs and/or proteins. Examples using BUNV will be presented that demonstrate the power of this technology and the plasticity of the bunyavirus genome. Furthermore, suggestions on how to create genetically inert or non-reassortable bunyaviruses will be described, and their application as potential live attenuated vaccines will be discussed.

RIFT VALLEY FEVER OUTBREAK DYNAMICS AND REVERSE-GENETICS GENERATED VACCINE

Brian Bird¹, César Albariño¹, Amy Hartman¹, Bobbie Erickson¹, Jane Githinji², Joseph Macharia², Jacqueline Kasiiti², Stephen Gacheru², Joseph Musaa², Jonathan Towner¹, Serena Carroll¹, Jennifer Oliver¹, Thomas Stevens¹, Laura Morgan¹, Marina Khristova¹, James Comer¹, Anita McElroy¹, Pierre Rollin¹, Thomas Ksiazek¹ and Stuart Nichol¹

¹Special Pathogens Branch, Centers for Disease Control and Prevention, Atlanta GA, USA; ²Ministry of Livestock and Fisheries Development, Division of Veterinary Services and Disease Control, Kabete, Kenya

Rift Valley fever (RVF) virus is a mosquito-borne human and veterinary pathogen associated with large disease outbreaks throughout Africa, Madagascar and the Arabian peninsula. Infection of livestock can result in sweeping “abortion storms” and high mortality among young animals. Human infection results in self-limiting febrile disease that in ~1-2% of patients progresses to more serious complications including hepatitis, encephalitis, retinitis or a hemorrhagic syndrome with high fatality. The most recent large RVF outbreak occurred in eastern Africa in 2006-2007, particularly Kenya, southern Somalia and Tanzania. Detailed analysis of over 3000 livestock and wildlife specimens in Kenya, showed evidence of RVF infection in almost 10% of animals tested, and across 23 Districts. The complete S, M and/or L genome segment sequence was obtained from 31 representative RVF virus specimens. Analyses revealed the concurrent circulation of multiple virus lineages, gene segment reassortment and common ancestry of the 2006-2007 outbreak viruses with those from the 1997-1998 eastern Africa RVF outbreak. Evidence of recent increases in genomic diversity and effective population size 2 to 4 years prior to the 2006-2007 outbreak was also found, indicating ongoing RVF virus activity and evolution during the inter-epizootic/epidemic period. Such findings highlight the need for safe and effective vaccines.

The virus S segment-encoded NSs protein and the M segment-encoded NSm proteins are important virulence factors. Recombinant RVF viruses have been successfully engineered by reverse genetics to contain either full length complete virus genome (wt) or precise deletions of the NSs alone or NSs/NSm genes in combination, thus creating attenuating deletions on multiple virus genome segments. These viruses were highly attenuated with no detectable viremia or clinical illness observed at high challenge dosages (10^4 PFU) in a rat lethal disease model. A single immunization induced robust anti-RVF virus IgG antibodies (titer ~1:6400) by day 26 post-vaccination. All vaccinated animals subsequently challenged with a high dose of virulent RVF virus survived infection and could be serologically differentiated from naïve experimentally infected animals by the lack of NSs antibodies. These rationally designed marker RVF virus vaccine viruses show great promise for use in endemic regions or following natural or intentional introduction into previously unaffected areas.

REVERSE GENETICS OF RIFT VALLEY FEVER VIRUS: APPLICATIONS AND IMPLICATIONS

MATTHIAS HABJAN AND FRIEDEMANN WEBER

Department of Virology, University of Freiburg, Germany

Rift Valley fever virus (RVFV), a member of the family *Bunyaviridae*, is a mosquito-transmitted BSL-3 pathogen which causes large epidemics among humans and domestic animals in Africa and the Arabian peninsula. RVFV is capable to infect a variety of hosts and vectors, and global warming and international livestock trade have led to an increase in number and severity of outbreaks. RVFV is hence considered a serious threat to public health and economy in industrialized countries.

We have developed a set of molecular tools to genetically manipulate RVFV (« reverse genetics »). A minireplicon system was established which consists of the viral polymerase L, the nucleocapsid protein N, and a reporter gene flanked by viral promoter sequences (minireplicon), all expressed in mammalian cells. The RNA minireplicon is specifically packaged by N and transcribed by L, resulting in the formation of recombinant RVFV nucleocapsids. This system allows to easily measure and quantify viral polymerase activity. The nucleocapsids can be packaged into virus-like particles (VLPs) by additional expression of the viral glycoproteins. VLPs are useful for studies on RVFV particle formation, attachment and infection of cells, viral transcription, and the influence of antiviral host factors (1). Moreover, VLPs expressing a reporter gene facilitate the measurement of neutralizing antibodies and were shown to be an efficient and safe vaccine protecting from a lethal RVFV challenge (2). By replacing the minireplicon with constructs for full-length viral genes, infectious recombinant RVFV particles could be rescued. This allows the targeted introduction of mutations into the viral genome (3). Using the rescue system, we demonstrated that the nonstructural protein NSs of RVFV has two different mechanisms to fight the innate immune system of the host. One function, which is conserved among bunyaviral NSs proteins, is the inhibition of interferon synthesis. The second function is the specific degradation of the antiviral host kinase PKR (4). This second activity appears to be unique for RVFV and may contribute to its extraordinary virulence.

Together, our set of reverse genetics tools facilitates research on RVFV and enables to efficiently test diagnostic, therapeutic and preventive measures under non-BSL3 conditions.

REFERENCES :

- 1 : HABJAN et al. (2009): *Virology* 385:400-408
- 2 : NÄSLUND et al. (2009): *Virology* 385:409-415
- 3 : HABJAN, et al. (2008), *J. Gen. Virol.* 89: 2157 – 2166
- 4 : HABJAN et al. (2009): *J. Virol.* 83:4365-4375

LESS IS MORE: THE SIZE OF THE INOCULUM DETERMINES THE OUTCOME OF RIFT VALLEY FEVER VIRUS INFECTIONS.

A. F. Antonis, J. Kortekaas, J. Kant-Eenbergen, R. J. M. Moormann*

Department of Virology, Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands

Rift Valley fever virus (RVFV) is a mosquito-transmitted RNA virus that can cause severe disease in humans and livestock. Laboratory mice are frequently used in animal models for RVFV. As in other mammals, three major outcomes of RVFV infection can be distinguished in mice. The infection can run a subclinical course, result in fulminant hepatitis, or result in delayed onset neurological disease. The underlying mechanisms of these different manifestations remain largely elusive. In our experiments, the minimal dose required for productive infection resulted in the highest case fatality. This finding has important implications for animal models of RVFV infection and could provide new insights into RVFV pathology.

DNA AND ALPHAVIRUS REPLICON BASED VACCINES ARE IMMUNOGENIC AND PROTECT MICE AGAINST VIRULENT RIFT VALLEY FEVER VIRUS INFECTION

Nitin Bhardwaj^{1,2}, DVM, MS and Ted M. Ross, PhD²

¹Department of Infectious Diseases and Microbiology, Graduate School of Public Health;²Center for Vaccine Research, University of Pittsburgh, Pittsburgh, PA, USA 15261.

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Background: Rift Valley Fever virus (RVFV) is an arthropod-borne phlebovirus (family *Bunyaviridae*) associated with abortion storms, neonatal mortality in livestock and hemorrhagic fever or fatal encephalitis in a proportion of infected humans. Although, the inactivated RVFV vaccines have been used in livestock, there is no licensed vaccine available to protect the human population. Therefore, there is an urgent need for developing safe and effective vaccine that rapidly elicits protective immunity against RVFV infection.

Methodology/Principal Findings: To address this, DNA plasmid and alphavirus replicon vectors expressing RVFV Gn glycoprotein were constructed and evaluated for their ability to induce protective immune responses in mice against RVFV. Briefly, DNA plasmids expressing ectodomain of RVFV Gn glycoprotein in conjunction with three copies of molecular adjuvant C3d were constructed and analyzed along with Gn expressing alphavirus replicon for their ability to act as potent vaccine candidates against RVFV in mice. An experimental live-attenuated vaccine (MP-12) was used as a benchmark for comparison. These vaccines produced the RVFV glycoprotein to high levels *in vitro* and elicited anti-RVFV antibody responses in immunized mice, as determined by RVFV specific ELISA, IgG isotype ELISA, and demonstration of a neutralizing antibody response. Interestingly, these candidate vaccine strategies were able to elicit cellular immune responses as determined by Gn specific ELISPOT assay. More importantly the vaccines were able to protect immunized mice from virulent Rift Valley Fever virus challenge.

Conclusion/Significance: An ideal vaccine produces broader immune response by stimulating both humoral and cell mediated arms of immunity. In our approach with DNA and replicon derived vaccines, we were able to elicit neutralizing antibodies along with an adaptive cell mediated immune response which conferred protection against lethal Rift Valley Fever virus infection. These experimental studies not only directly assess the potential of our vaccine candidates, but also significantly enhance our general understanding of anti-RVFV immunity.

PROTECTION AGAINST LETHAL RVFV INFECTION IN TRANSGENIC IFNAR^{-/-} MICE INDUCED BY DIFFERENT DNA VACCINATION REGIMES

Gema Lorenzo, Raquel Martín, Esther Hevia, Hani Boshra, Alejandro Brun

Centro de Investigación en Sanidad Animal (CISA-INIA), Valdeolmos. 28130 Madrid (Spain)

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Rift Valley Fever is a viral zoonosis transmitted by mosquitoes causing a severe, fatal disease in neonatal livestock, as well as in humans in the form of haemorrhagic fever with frequent fatal consequences. The disease is endemic in Sub-Saharan Africa, although it has been shown to propagate to northern latitudes. There are no specific treatments against this disease, but symptomatic treatments in severe human cases exist. Vaccines against RVF have been developed using many different approaches, but none of them have yet to be licensed for veterinary use in non-endemic countries. In this work, we have developed plasmid constructs encoding two different M segment ORFs, as well as the nucleoprotein N, and have used them in different vaccination regimes to test protection against a lethal RVFV MP12 strain in a transgenic mouse model with impaired interferon type I response (IFNAR^{-/-}). We obtained dose dependent protection in animals immunized with a construct encoding both mature glycoproteins, whereas only partial protection was obtained in animals vaccinated with either N construct or a combination of both plasmids. No protection was achieved with a plasmid encoding the full polyprotein sequence encoded by the viral RNA M segment. The protection elicited by the expression of the mature glycoproteins, but not that of the nucleoprotein, could be directly related to the induction of neutralizing antibodies against them. The combination of both vaccine constructs did not enhance their individual protective effects but induced nucleoprotein specific lymphoblast proliferation after a single vaccine dose.

INOCULATION OF CALVES WITH AN EXPERIMENTAL NEWCASTLE DISEASE VIRUS-BASED VECTOR VACCINE ELICITS NEUTRALIZING ANTIBODIES AGAINST RIFT VALLEY FEVER VIRUS.

J. Kortekaas*, A. Dekker, K. Weerdmeester, R. P. M. Vloet, , A. J. de Wit, J. van der Laan, B. P. H. Peeters, R. J. M. Moormann.

Department of Virology, Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands

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In the past decade, the use of Newcastle disease virus (NDV) as a vaccine vector for the prevention of economically important livestock diseases as well as for human diseases has been extensively explored. In this study, we have constructed a recombinant NDV vaccine virus, named NDFL-Gn, that produces the Rift Valley fever virus (RVFV) Gn glycoprotein. Inoculation of calves with NDFL-Gn elicited antibodies against both NDV and the Gn protein. The RVFV-neutralizing activity of the antisera was demonstrated, suggesting that NDV is a promising vaccine vector for the prevention of RVF in calves.

THE USE OF SELF-ADJUVANTS IN RVFV DNA VACCINES

Hani Boshra, Gema Lorenzo, Alejandro Brun

CISA-INIA (Valdeolmos, Spain)

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Over the past few years, DNA vaccines have been shown to be a potentially useful tool for combating Rift Valley Fever outbreaks. Specifically, previous studies using DNA constructs encoding for the glycoproteins against RVFV were shown to confer complete protection against viral challenge in mice. Furthermore, it has been previously shown that DNA constructs encoding for the RVFV nucleoprotein (RVFV N) provided partial protection in mice challenged with the virus. Based on these observations, we are currently attempting to determine whether the immunogenicity of RVFV N can be increased using plasmid constructs encoding for self-adjuvants. Our strategy involves the generation of chimeric proteins, in which RVFV N is fused to a variety of self-adjuvants, known to increase the immune response when expressed in the form of a DNA vaccine. Preliminary experiments using RVFV N, fused with LIMPII were performed in IFNAR $-/-$ mice, which were then subsequently challenged with the MP12 attenuated strain of RVFV. Initial results indicate that survival of N-LIMPII inoculated mice increased significantly, with higher antibodies titers also being observed. These results have initiated further studies using other self-adjuvants, which will also be discussed.

RIFT VALLEY FEVER VIRUS SUBUNIT VACCINES CONFER COMPLETE PROTECTION AGAINST A LETHAL VIRUS CHALLENGE.

Boer de SM^{a,b}, Kortekaas J^a, Antonis AF^a, Kant J^a, Oploo van JL^b, Rottier PJM^b, Moormann RJM^{a,b,*}, Bosch BJ^b.

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^b Division of Virology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

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Rift Valley fever virus (RVFV) is an emerging mosquito-borne virus causing significant morbidity and mortality in livestock and humans. Rift Valley fever is endemic in Africa, but also outside this continent outbreaks have been reported. Here we report the evaluation of two vaccine candidates based on the viral Gn and Gc envelope glycoproteins, both produced in a *Drosophila* insect cell expression system. Virus-like particles (VLPs) were generated by merely expressing the Gn and Gc glycoproteins. In addition, a soluble form of the Gn ectodomain was expressed and affinity-purified from the insect cell culture supernatant. Both vaccine candidates fully protected mice from a lethal challenge with RVFV. Importantly, absence of the nucleocapsid protein in either vaccine candidate potentially allow differentiation between infected and vaccinated animals using a commercial recombinant nucleocapsid protein-based indirect ELISA.

EVALUATION OF THE EFFICACY AND SAFETY OF THE RVF CLONE 13 VACCINE IN SHEEP

B Dungu¹, I Louw¹, B von Teichmann¹, A Lubisi¹, P Hunter¹ and M Bouloy²

¹Onderstepoort Biological Products Ltd., Onderstepoort, Republic of South Africa and ²Institut Pasteur, Unité de Génétique Moléculaire des Bunyavirus, Paris, Cedex 15, France

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A Rift Valley Fever virus isolated from a non-fatal case of RVF in the Central African Republic was passaged in mice and Vero cells, and plaque purified in order to study the virus subpopulations. A clone designated 13 which did not react with specific monoclonal antibodies against NSs was further investigated and found to be avirulent in mice, yet immunogenic. By reassortment between Clone 13 and a virulent strain, it was demonstrated that the large internal deletion in the NSs gene was responsible for attenuation. Further work showing that wild type NSs is an antagonist of interferon beta gene activation as well as a general inhibitor of cellular transcription was indeed defective in Clone 13.

RVF Clone 13 was evaluated for its safety and efficacy as a possible veterinary vaccine in South Africa. Vaccination with Clone 13 and challenge experiments were conducted in sheep in order to (1) evaluate the safety of the RVF Clone 13, through its ability to generate immunity in pregnant ewes without triggering clinical manifestations such as elevated body temperature, teratogenicity or abortion and (2) evaluate the efficacy of the RVF Clone 13 vaccine, through the evaluation of the ability to trigger a protective immunity in early and late pregnancy in vaccinated ewes challenged with a virulent dose of wild-type RVF virus. In conclusion, the present study confirms the absence of virulence of RVF Clone 13 in sheep, the most sensitive ruminants and indicates the suitability of RVF Clone 13 to be used as a safe, cost-effective and efficacious vaccine for the control of RVF.

BULGARIAN VACCINE AGAINST CCHFV

I. CHRISTOVA

National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria

Bulgaria is a country endemic for Crimean Congo hemorrhagic fever (CCHF). Various investigations of different tick and animal species for infection with CCHF virus (CCHFV) have proven wide distribution of the virus. In humans, CCHFV causes severe and often lethal infection. Officially reported in Bulgaria are between 20 and 30 cases per year with a maximum in 2002 – 52 CCHF cases. A CCHF vaccine was developed and introduced in 1974. As a result, the diseases incidence dropped over 4 times. The vaccine consists of inactivated CCHFV antigen, Bulgarian strain V 42/81, adsorbed in $Al(OH)_3$. The vaccine is designed for protection against infection with CCHFV for medical workers, border army units, agriculture workers and other people living in CCHF endemic regions. The vaccine builds specific active protective immunity against CCHFV 14 days after injection. Significant increase in antibody levels after vaccination was established. No significant side effects were noticed.

APPLICATIONS OF THE ALVAC VECTOR FOR IMMUNIZATION OF HORSES AGAINST WEST NILE VIRUS AND AFRICAN HORSE SICKNESS VIRUS.

Jean-Christophe AUDONNET and Jules Minke

Biologicals R&D, Laboratoire Lyon Gerland, Merial SAS.

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One of the most significant changes in the field of veterinary medicine has been the introduction of several recombinant vaccines based on the canarypox (ALVAC) vector platform. Its high safety profile and ability to induce broad immune responses against the transgene without the need for adjuvants have been the driving forces for the generation of a number of commercial vaccines. The ALVAC technology platform facilitates the rapid generation of new constructs and as soon as the sequences of the protective genes of a micro organism are known, synthetic genes can be made and inserted into the ALVAC genome. This has proven to be an advantage in the case of emerging diseases such as WNV and Nipah virus and could be a major asset for the development of safe and efficacious vaccines for African Horse Sickness (AHS). Studies on the ALVAC-WNV vaccine have shown that the vaccine induced a rapid onset of immunity which is long lasting. The vaccine stimulated both humoral and cell-mediated immunity and these responses were not inhibited by subsequent immunizations with the same vaccine. Notwithstanding the evident success of the polyvalent modified live vaccines against AHS in endemic areas, there are concerns about their use in epidemic situations because of their inherent biological safety risks. Therefore, their deployment in case of an outbreak in Europe would be viewed with concern by some veterinary authorities. The recent successful demonstration of efficacy of a canarypox vaccine expressing the VP2 and VP5 proteins of the related BTV confirms the viability of an ALVAC vaccination strategy for AHS. An additional advantage of the use of the ALVAC platform is that tests based upon the non-structural proteins will enable differentiation between naturally infected and vaccinated animals

THE CONTRIBUTION OF ANTI-TICK VACCINES TO THE CONTROL OF TICK-BORNE DISEASE

Peter Willadsen, Shelly Hope, Tony Vuocolo and Varda Shkap

CSIRO Livestock Industries, Brisbane, Australia and the Kimron Veterinary Institute

Historically the control of vector-borne disease has relied heavily on the control of the vector. Of the vector control technologies potentially available, successful vaccination would offer a number of advantages. Nevertheless, despite encouraging though fragmentary results, almost nothing has been done to examine the applicability of anti-tick vaccines to a program for the control of diseases like CCHFV. Such a vaccine may need to control a number of tick species, with a focus on *Hyalomma* species, be effective in several host species and have efficacy sufficient to interrupt or at least decrease disease transmission. Current evidence, although limited, suggests that vaccines based on Bm86 or its homologues would have good efficacy against *Hyalomma* sp. We now know that the tick host species itself may be an important contributor to vaccine efficacy, although very little data is available, while the effect of vaccination on disease transmission has rarely been examined. More efficacious anti-tick vaccines would undoubtedly help but again there is need for more research. The number of tick antigens continues to increase, though slowly, and few give strong protection. Multi-antigen vaccines may be a solution, but the design of effective antigen combinations has received virtually no experimental examination. In short, current evidence on the contribution that could be made by anti-tick vaccines to controlling tick-borne disease is encouraging, but much research remains to be done.

ANTI-MOSQUITO VACCINES: EFFECTS ON SURVIVAL, FERTILITY AND TRANSMISSION OF *PLASMODIUM BERGHEI*, OF IMMUNIZATION WITH EXTRACTS FROM *ANOPHELES STEPHENSI* AND *ANOPHELES GAMBIAE* MIDGUTS.

Almeida, A.P.G.

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Mosquito borne diseases, such as malaria and many arbovirosis like dengue and West Nile fever, are amongst the most serious public health problems, causing high morbidity and mortality (WHO, 2004, 2008). Mosquito control has faced varied problems such as resistance to insecticides, and their effects of the on the environment and public health have limited even further their use in residual spraying as the main vector control weapon.

Antivectorial vaccines constitute a potential alternative for vector control by decreasing mosquito longevity or fertility, and integrate a malaria control programme or Integrated Vector Management (WHO, 2004). The bloodmeal would be the vehicle for antibodies that could either i) attack the mosquito causing its death before malaria parasites complete their cycle, ii) reduce fertility decreasing mosquito population, or iii) interfere with the mosquito/parasite interaction rendering the former incapable as a vector. All these possible interferences would have strong repercussions on the epidemiology and malaria transmission according to Macdonalds vectorial capacity model .

Considering that a better knowledge of mosquito antigens relevant for such an immunological approach was needed, studies focusing on midgut epithelium as potential targets, have been carried out.

Immunizations of laboratory mice have been performed using several extracts from *An. stephensi* mosquitoes: head and thoraxes, midguts, ovaries and fat-bodies, with varying degrees of reduction of mosquito's longevity and/or fertility. Further work involved the separation of subcellular fractions, namely microvilli (MV) and basolateral membranes (BLM) from the midguts of sugarfed mosquitoes, involving homogenization in glass/glass grinder, differential centrifugation, followed by transmission electron microscopy confirmation of MV presence by the visualization of uniform membrane vesicles, and enrichment of aminopeptidase activity. Microvilli extracts induced a strong antibody reaction against the midgut, which was manifest as decreased survival and fecundity, and reduced the mean intensity of *P. berghei* oocysts when fed *in vitro*.

Several protein bands were detected when MV extracts from either *An. stephensi* or *An. gambiae* were separated eletrophoretically by PAGE. Horizontal gel sections, were used to immunize BALB/c mice. After 5 sequential immunizations, antibody titres reached $1:10^5$ with different and cross reactivities in immunoblotting. Mosquitoes that took three bloodmeals on these mice were monitored for survival and fecundity. In some groups, mortality was significantly higher, striking 75% of mosquitoes by day 10 as opposed to 20-25% in the control groups. The effect of these antibodies in the transmission of rodent malarial parasites, *P. berghei* was variable, as some groups increased the prevalence and/or intensity, while others decreased the intensity, of the infection, in the positive midguts.

Further research into effective targets in the mosquitoes, using new antibody technology, is needed.

THE USE OF RIBAVIRIN FOR THERAPEUTIC TREATMENT OF PATIENTS INFECTED WITH CCHFV.

ONDER ERGONUL

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Ribavirin was early shown to be effective against CCHFV in vitro. In suckling mice, ribavirin treatment reduced CCHF virus growth in the liver; significantly decreased, but did not prevent, viremia; and significantly reduced mortality and extended the geometric mean time to death. No randomized clinical trials of the efficacy of ribavirin against CCHF have been performed, but the effectiveness of its use was described by several observational studies. The first of these reports was published in 1995, and the observation was limited to 3 health care workers infected with CCHFV. In observational studies, ribavirin was reported to be beneficial in CCHF infections. The ribavirin could be useful at the early stages of the infection.

The mechanism by which ribavirin might be acting in cases of CCHF has not been determined. It is possible that the drug has an immunomodulatory effect, but this has not yet been studied. In this regard, the cytokines IL-6 and TNF- α were found to be higher among fatal CCHF patients than in nonfatal cases.

Most all the authors claimed that they were not able to perform a randomized clinical trial of ribavirin therapy because of ethical constraints. Since the lack of randomization is the main criticism for these observational studies, the qualities of the observational studies could be improved. The observational studies could provide qualified information. Three leading confounders make make observational studies difficult to interpret. These are, Severity of the infection, Number of days from onset of illness, and severity of the gastrointestinal symptoms. If these confounders are not controlled, then misclassification bias is inevitable. In order to minimize the confounders, the course of the infection should be reviewed, and the place of ribavirin in the treatment of CCHF should be detailed on the course of the infection.

Prophylaxis is suggested after a high-risk contamination, such as a needle stick injury of a health care worker from a CCHFV infected source patient. Daily follow-up by checking complete blood count and biochemical tests for the exposed individuals is highly recommended.

COMPARISON OF ANTIVIRAL ACTIVITY OF COMMERCIALY AVAILABLE RECOMBINANT ANTIVIRAL AND MULTIFERON

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As a first line of defense against a virus infection, mammalian cells elicit an innate immune response, characterized by secretion of type I Interferons and the up-regulation of interferon stimulated genes (ISGs). Crimean-Congo Hemorrhagic Fever Virus (CCHFV) and Rift valley fever virus (RVFV), member of family *Bunyaviridae*, is the causative agent of severe hemorrhagic fevers and has previously shown to be sensitive to antiviral activity of Interferon. Interferons are an important component of the immune system and are produced naturally in the body. In this study we compared the antiviral activity of the commercially available recombinant antiviral and Multiferon, a natural interferon-alpha (IFN- α). Human leucocyte Interferon- alpha Multiferon is obtained from the leukocyte fraction of human blood and therefore contains several naturally occurring IFN- α subtypes compared to recombinant inteferons which only contain one subtype. Recombinant inteferons, are used today as treatment of infectious diseases such as hepatitis. Methods: Recombinant Interferons and Multiferon treated A549 cells were infected with CCHFV (Ibar strain) and RVFV (Nigerian strain) at different moi. Supernatant and cell lysate were analysed by Western Blot and viral RNA was prepared for quantitative RT-PCR.

THE ANTIVIRAL EFFECT OF NITRIC OXIDE ON TWO VIRUSES BELONGING TO TWO DIFFERENT GENERA WITHIN THE FAMILY BUNYAVIRIDAE.

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Nitric Oxide (NO) is an important signalling molecule between cells, which has been shown to have an inhibitory effect on some virus infections. The family Bunyaviridae consists of five genera, we have examined whether NO has an inhibitory effect on two viruses from two different genera. The viruses we have examined are Crimean-Congo hemorrhagic fever virus (Nairovirus) and Rift Valley Fever virus (Phlebovirus). Vero E6 cells were infected with either virus and then stimulated with different concentration of the organic NO donor S-nitroso-N-acetylpenicillamine (SNAP) or, as negative control, N-acetylpenicillamine (NAP) which lacks the NO-donating S-nitroso group. Supernatant and cell lysates were harvested at different time points post infection and analysed for the yield of progeny virus, viral RNA and protein expression.

NOVEL MINIGENOME ASSAY FOR SCREENING CRIMEAN-CONGO HEMORRAGIC FEVER ANTIVIRALS AND IDENTIFICATION OF THE SKI-1/S1P PROTEASE AS A PROMISING ANTIVIRAL TARGET

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-- Crimean Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus (genus *Nairovirus*, family *Bunyaviridae*) associated with high case fatality disease outbreaks in regions of Africa, Europe and Asia. The CCHFV genome consists of three negative strand RNA segments, S, M and L. The unusually large virus L polymerase protein, the complexity of the M polyprotein and the need for BSL4 containment has hampered CCHFV research. The L protein has an ovarian tumor (OTU) protease domain located in the N-terminus which has led to speculation that the protein may be autoproteolytically cleaved to generate the active virus L polymerase and additional functions. We report the successful development of efficient CCHFV helper virus-independent minigenome system for analysis of virus RNA and protein features involved in replication. The OTU domain located in the N-terminus of the expressed virus L protein was shown to be a functional protease. However, no evidence of L protein autoproteolytic processing was found, and the OTU protease activity was dispensable for virus RNA replication. This newly developed assay was used to test efficacy of antivirals against CCHFV minigenome replication. Ribavirin, a broad antiviral compound inhibited minigenome replication with efficacy comparable to *in vitro* studies with live CCHFV. These results demonstrated the utility of the minigenome system for antiviral drug screening in BSL2 laboratory settings.

In addition, the processing of CCHFV M segment polyprotein was investigated to define if this step was essential for CCHFV replication. The M polyprotein was shown to be rapidly processed into two glycoproteins precursors (PreGn and PreGc). Further cleavages by cellular endoproteases produce the mature structural glycoproteins, Gn and Gc. Our previous published findings identified subtilisin kexin isozyme-1/site-1 protease (SKI-1/S1P) as the cellular PreGn convertase. Furthermore, we recently identified that SKI-1/S1P also cleaves PreGc at a SKI-1/S1P motif (RKPL₁₀₄₀). When cells deficient in SKI-1/S1P were infected with CCHFV, we found that cleavage of both precursors was impaired and no infectious virus was released. Complementation of these cells with a SKI-1/S1P expression vector was sufficient to restore processing of the glycoprotein precursors and release of infectious virus to high titers ($>10^6$ pfu/ml). CCHFV infections of cells lacking SKI-1/S1P activity resulted in the accumulation of PreGn and PreGc at the virus site of assembly (i.e. Golgi). Despite the correct trafficking of the glycoprotein precursors, only nucleoprotein containing particles which lacked detectable levels of either structural glycoproteins or glycoprotein precursor forms were secreted in the absence of SKI-1/S1P. We conclude that the processing of CCHFV glycoprotein precursors by SKI-1/S1P is required for the incorporation of viral glycoproteins into virions. Therefore, SKI-1/S1P represents an attractive CCHF antiviral target.

ESTABLISHMENT OF A STAT-1 KNOCKOUT MOUSE MODEL FOR CRIMEAN-CONGO HEMORRHAGIC FEVER

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Crimean-Congo hemorrhagic fever virus (CCHFV) has the most extensive geographic range of medically significant tick-borne viruses. Human infection with CCHFV often results in severe hemorrhagic disease with high mortality rates of up to 70%. In the contrary, CCHFV does not cause disease in a broad range of domestic and wild animals or laboratory animals other than newborn mice. Unlike most hemorrhagic fever viruses, CCHFV has not been found to cause disease in commonly used species of nonhuman primates. Therefore, no suitable animal model is available to study pathogenesis or test antivirals and vaccines. Recent *in vitro* studies have shown that the interferon (IFN) response plays a crucial role in controlling CCHFV replication. These studies also demonstrate that replicating CCHFV delays substantially the IFN response and cells infected with CCHFV are insensitive to IFN- α treatment. Therefore, we hypothesize that the IFN type 1 response is critical in controlling initial and early virus replication. To define CCHFV pathogenesis in a small animal model, we used mice containing a homozygous disruption of the STAT-1 gene and complete lack functional STAT-1 proteins which eliminates the intracellular interferon response. STAT-1 knockout mice were infected with different doses of the two CCHFV strains IbAr10200 and C698031 to determine mean lethal dose. Subsequently, animals were challenged with 100 PFU of each strain and clinical presentation, viral replication and tropism, immune response, clinical pathology and histopathology were studied through the course of infection. The model is uniformly lethal for both CCHFV strains even with a low virus dose. This indicates how important the IFN response is in controlling the virus. However, a significant phenotypical difference in terms of pathogenesis between the two strains was noticed. We were also able to show similarities between the animal model and human cases in regards to cytokine profile, histopathology, and clinical pathology. Ribavirin, a nucleoside analogue with antiviral activity, has been used for post-exposure prophylaxis for patients with Crimean-Congo hemorrhagic fever, however, evidence for its efficacy is very limited. Thus, Ribavirin treatment was tested in STAT-1 knockout mice at different time points post infection to determine if it protects the animals. Ribavirin was able to protect the animals when a low dose of challenge virus was used. We find that the STAT-1 knock-out mouse strain is an excellent model to study CCHFV pathogenesis, antivirals, and virus attenuation.

ASSISTANCE WITH THE DEVELOPMENT OF INTERVENTIONS AGAINST CRIMEAN- CONGO HAEMORRHAGIC FEVER (CCHF) VIRUS

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The Health Protection Agency (HPA) has a long history of working with arboviruses and haemorrhagic fever (VHF) viruses, including CCHF. This experience has led to the CEPR laboratory being designated a World Health Organisation (WHO) collaborating centre for virus reference and research (arboviruses and VHF's) with links to many international groups.

A hindrance to laboratory work with CCHF however, is the mandatory requirement of using high containment facilities (Containment Level/Bioafety Level 4) to handle infectious virus. While such facilities are available at Porton Down a lot of the research work on CCHF has lagged behind other infectious disease. Importantly there is an absence of a suitable animal model of disease which has precluded vaccine and therapeutic studies. HPA is aiming to address this deficiency by undertaking research work to adapt CCHF virus to mice, and more recently in determining the susceptibility of rhesus macaques.

A range of techniques can be used to assess clinical responses in animals including telemetry, clinical chemistry, haematology, and recording of behaviour. With projects using many different high containment pathogens, the HPA has expertise in studying the immune response after vaccination and during infection with many different bacterial and viral agents. These tools can be transferred to studying responses to CCHF infection as a prelude to vaccination studies to tackle the spread of the virus. These immunological tools include the measurement of multiple analytes by luminex analysis, the use of flow cytometry, determination of antibody levels and assessment of immune cell function.

In addition to the work of the research group, the Special Pathogens Reference Unit at the HPA undertakes the diagnosis of emerging and re-emerging tropical infectious diseases, including CCHF. To improve the diagnosis of CCHF, an ELISA assay has recently been developed to produce a simplified system in which to measure levels of CCHF-specific IgG and IgM. Results from this assay will be presented, showing the potential of this assay.

THE VIRUS–HOST CELL INTERACTION: AIM OF THERAPEUTIC INTERVENTIONS?

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Species which are naturally infected by West Nile Virus strains show differences in the susceptibility to the virus and in the severeness of the disease. Most of infected birds show normally no symptoms of a disease but some species, e.g. crows, are heavily affected meaning the death of the animal in a short time after infection in the most cases. Infection of the “dead-end-host” horse results for 40% of infected animals in a fatal outcome. Infections in humans are in most of the times subclinical. However, about 20% of the infected humans develop the so called West Nile Fever and less than 1% show clinical signs of encephalitis. Differences in the transmission efficiency and outcome of infection may originate in the differences in the molecular pathogenesis of the viral infections. First of all the immunological status of the infected animal plays an important role. The second step is the entry of the virus into cells including the binding of the virus to a cellular receptor. Eventually virus needs to be replicate in the infected cell and be released to infect other cells. During the viraemic stage differences in all the mentioned steps (immunological status, virus entry, replication and release) can be responsible for differences in the course of the disease.

The aim of our studies is to reveal the role of the cellular receptor for the West Nile Virus for the susceptibility to a viral infection of the cell. The cellular receptor was described by Chu and Ng (journal of Biological Chemistry 279(52):54533-41, 2004) to be the integrin alpha v beta 3. we have established cell culture models comprising wildtype cells and embryonic mouse fibroblasts which are deficient for the Integrin subunits alpha v, beta 3 and beta 1, respectively. These cells were infected with three different WNV strains, namely NY99 (Lineage 1), Uganga (Lineage 2) and the less pathogenic strain Sarafend (Also Lineage 2). Results from infection experiments with the cell culture models mentioned above are presented. The elucidation of the role of integrins during the attachment of the virus to the host cell and other membrane proteins involved in this binding may lead to mechanisms for therapeutic interventions through inhibition of the virus attachment to its host cell.

THE SECOND SEASONAL APPEARANCE OF HUMAN WEST NILE NEUROLOGICAL INVASIVE DISEASE IN NORTHERN ITALY: EVIDENCE FOR ENVIRONMENTAL VIRAL PERSISTENCE AND NECESSITY FOR IMPROVEMENT OF CONTROL MEASURES.

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During late summer of 2008, the first cases of human neuro-invasive disease caused by West Nile virus (WNV) have been described in the Po river valley, in the North-East of Italy. The small epidemic consisted of five laboratory confirmed cases and all of these patients were living in or nearby areas with marsh and flooded lands. The animal infection background that was under this first human epidemic outbreak consisted of several wild birds and horses identified as infected by WNV. In addition, the entomological survey detected the viral genome in 3 pools of mosquitoes collected nearby the areas where patients were living. After this first epidemic outbreak an active surveillance system was set up in order to monitor the possible re-appearance of WNV infection for the 2009 summer mosquitoes activity season. The surveillance involved an enlarged geographical area in respect with that where WNV activity was identified in 2008, since a simple model of diffusion suggested that the virus could spread westbound along the Po river valley. This hypothesis was made after a serological survey performed on wild birds captured in the area and demonstrating a substantial absence of any specific antibody response to WNV. The monitoring activity was started in May and involved both veterinarian and entomological branches in addition to human suspected patients. A weekly report containing all the information collected by the surveillance system was issued and made available to all the branches in order to have a timely updated situation. During the last week of August the Laboratory of CRREM identified the first four human patients suffering from WNV neuro-invasive disease. Two of these patients lived in the area near Ferrara where WNV activity was detected in the late summer of 2008, and two were from the province of Modena, located west of the 2008 affected sites. The total number of WNV neuro-invasive disease identified up to September 18th was 14 in an area that included the Provinces of Padova, Rovigo, Modena, Reggio Emilia, Bologna, Ferrara and Mantova. The total surface was three times that of the 2008 affected sites and the diffusion of the virus was confirmed westbound. A particular aspect of the WNV surveillance was dedicated to blood donation and organ transplant. In order to ensure the biological safety of blood and organs (including stem cell from cord and bone marrow) all donations were controlled by using a NAAT method (which are currently capable to detect WNV genome in a range of 10-40 copies/sample). This screening activity identified up to September 18th two blood and one organ donors during the viremic asymptomatic stage. The veterinary surveillance systems identified (as of September 9th) 22 PCR positive birds over a total of 955 controlled and 25 horses suffering from WNV neurological syndrome. A total of about 150.000 mosquitoes (about 140.000 belonging to the *Culex pipiens* species) were tested in pool (total pools evaluated: 1211) by PCR. More than 20 of these pools showed the presence of the WNV genome (sequence identity > 99.0 % with the genome of the 2008 WNV isolates). These data confirmed the persistence of WNV in the Po river valley and the geographical expansion (west bound) of the affected area, strongly suggesting the over winter survival of WNV in Italy. The results obtained confirmed the necessity of an active WNV surveillance and control system.

AFFILIATION EVIDENCE OF RECENT RIFT VALLEY FEVER VIRUS CIRCULATION IN MAYOTTE, A FRENCH ISLAND OF THE INDIAN OCEAN

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Rift Valley Fever (RVF) is a serious emerging arthropod-borne viral anthroponosis. It is reported primarily to infect sheep, cattle and goats, producing high mortality in newborn animals and abortion in pregnant animals. RVF causing epidemics of flu-like syndrome with some cases of haemorrhagic or neurologic complications. Outbreaks of the disease occur when particularly heavy rains favour the breeding of the mosquito vectors. Many sub-Saharan tropical and sub-tropical countries in Africa have reported outbreaks of RVF. Periodic large-scale RVF epidemics occurred in African countries, the latest was in Madagascar which has already reported 17 human deaths and 418 suspected infections in April 2008. In this context, the veterinary services of Mayotte Island decided to set up three different serological studies. The analysis was performed with an IgG competitive ELISA recently validated in different species such as humans, domestic and wild ruminants. A first serological survey was focused on samples collected in 2007 in one area selected for a risk of illegal introduction of animals from Comoros. 79 cattle and 23 illegally imported goats were sampled. 13 samples were found positive for IgG and 3 for IgM. Only one goat scored positive for IgG. A follow-up of the negative bovines in April 2008 and of additional goats from illegal import led to the conclusion of a recent circulation of the virus since 2 goats over 29 were found IgM positive and one bovine seroconverted. Therefore, it was decided to test a total number of 304 bovine sera collected between June 2007 and May 2008 distributed all over the Island. An overall percentage of 11% (95%IC:7-14) was detected. Finally, in order to set up a serosurveillance of this disease, 5 herds with a range of 4 to 35 animals in each herd are followed up every 6 to 8 weeks. So far, in December 2008, 5 of the animals seroconverted in 2 different herds. These studies illustrate the recent circulation of RVF in Mayotte. Studies are ongoing to detect a possible reactivation of the virus circulation and also to trace back the possible period of initial introduction of the virus in the island.

RECOMBINANT INDIRECT IMMUNOFLOUORESCENCE TEST FOR THE SEROLOGICAL DIAGNOSIS OF CRIMEAN-CONGO HEMORRHAGIC FEVER

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In collaboration with EUROIMMUN Medizinische Labordiagnostika AG we developed and evaluated a non-infectious recombinant indirect immunofluorescence test (IIFT), using cells expressing the GPC and N protein of CCHFV. Until now, there was no commercial assay available for the serological diagnosis of Crimean-Congo hemorrhagic fever (CCHF) and diagnostics were relying on in-house assays.

The assay presented here was evaluated with 3 different serum panels from CCHFV-infected patients originating from different geographical areas. The first panel from Turkey consisted of 184 IgM and 148 IgG positive samples. The second panel was from Kosovo (70 IgM and 58 IgG positive sera) and the third panel from Iran (18 IgM and 52 IgG positive samples).

A panel of 45 healthy German blood donors served as negative control. The sera were tested with the IgM and IgG IIFT to determine the sensitivity and specificity of the IIFT compared to the corresponding in-house ELISA assay results. For the IgM analysis of the Turkish panel additionally 50 CCHF-negative Turkish people and 110 ELISA IgM-negative CCHF patients served as negative control.

The IgG IIFT revealed with the panel from Turkey a sensitivity of 92.1% and a specificity of 100 %, with the panel from Kosovo it showed a sensitivity of 83.3 % and a specificity of 100 %; and with the Iranian panel, a sensitivity and specificity of 100 %.

The results of the IgM IIFT sensitivity varied strongly between the three different panels. In the Turkish panel the sensitivity for IgM was 97.2 % and the specificity 97.5 %. Whereas the assay revealed a sensitivity of 54.3 % and a specificity of 98 % with the panel from Kosovo; with the Iranian panel the sensitivity was 44.4 % and the specificity 97.8 %.

This study showed a good specificity of the anti-CCHFV IIFT both for IgM and IgG and a high sensitivity for IgG, but the sensitivity for IgM was varying strongly among the panels. The reason for this might be due to quality of the in-house assays, which are not standardized and the difference in the pre-treatment of the samples in the different panels. This study was retrospective and some of the panels were already tested in the in-house assays and stored until tested in the IIFT. As IgM is very sensitive to freezing and thawing cycles the IgM reactivity might have been diminished in some panels before IIFT testing. Taken together the anti-CCHFV IIFT represents a good non-infectious alternative to time-consuming in-house assays.

