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RIFT VALLEY FEVER VACCINE DEVELOPMENT, PROGRESS AND CONSTRAINTS

GF-TADs meeting
January 2011

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2011

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Contents

List of acronyms

ABADRU	Arthropod-Borne Animal Diseases Research Unit
AHRI	Animal Health Research Institute
AHVLA	Animal Health and Veterinary Laboratories Agency
ANSES	Agence nationale de sécurité sanitaire de l'alimentation, de l'environement et du travail
ARBO-ZOONET	International Network for Capacity Building for the Control of Emerging Viral Vector Borne Zoonotic Diseases
ARS	Agricultural Research Service
CDC	United States Centers for Disease Control and Prevention
CFIA	Canadian Food Inspection Agency
chimVLP	chimeric virus-like particles
CIRAD	Centre International de Recherche Agronomique pour le Développement
CISA-INIA	Centro de Investigacíon en Sanidad Animal-Instituto Nacional de Investigacíon y Tecnología Agraria y Alimentaria
CPV	capripox virus
CReSA	Centre de Recerca en Sanitat Animal
CVI-WUR	Central Veterinary Institute of Wageningen University and Research Centre
DIVA	Differentiating Infected from Vaccinated Animals
EC	European Commission
EDEN	Emerging Diseases in a Changing European Environment
EDENext	Biology and Control of Vector-Borne Infections in Europe
EFSA	European Food Safety Authority
ELISA	Enzyme-Linked Immunosorbent Assay
ENCRAD	European Network for the Coordination of Rift Valley Fever Animal Experimentation and Diagnostics
EPIZONE	Network of Excellence for Epizootic Disease Diagnosis and Control
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FLI	Friedrich Löeffler Institut
GALVmed	Global Alliance for Livestock Veterinary Medicines

GF-TADs	Global Framework for the Progressive Control of Transboundary Animal Diseases
GTPV	goat pox virus
IAEA	International Atomic Energy Agency
IFAH	International Federation for Animal Health
IM	intramuscular
IN	internasal
IV	intravenous
KARI	Kenya Agricultural Research Institute
LSD	lumpy skin disease
LSDV	lumpy skin disease virus
MoMLV	Moloney murine leukemia virus
MVA	Modified Vaccinia Ankara
NDV	Newcastle disease virus
NHP	non-human primate
NICD	National Institute for Communicable Diseases
OIE	World Organisation for Animal Health
OP	Onderstepoort
PKR	RNA-dependent protein kinase
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SC	subcutaneous
SPPV	sheep pox virus
USAMRIID	United States Army Medical Research Institute of Infectious Diseases
USDA	United States Department of Agriculture
UTMB	University of Texas Medical Branch
VLP	virus-like particles
VNT	virus-neutralization tests
VPH	Veterinary Public Health

Abstract

In November 2010, the Food and Agriculture Organization of the United Nations (FAO) issued a request to policy-makers, representatives of international organizations and foremost scientists involved in vaccine development for the control of Rift Valley fever (RVF), to attend a workshop entitled "Rift Valley fever vaccine development, progress and constraints". The workshop was organized under the umbrella of the Global Framework for the Progressive Control of Transboundary Animal Diseases (GF-TADs), a joint initiative of FAO and the World Organisation for Animal Health (OIE). It was supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation, and the United States Centers for Disease Control and Prevention (CDC), with the participation of the World Health Organization (WHO), the International Atomic Energy Agency (IAEA) and the Central Veterinary Institute of Wageningen University and Research Centre (CVI-WUR). The meeting was held at FAO headquarters in Rome, Italy from 19 to 21 January 2011. Views on the current and future control of RVF were presented, and the stages of development of candidate vaccines were reported by key stakeholders in vaccine development from international organizations and related industry. The desired characteristics of vaccines for application in different areas of the world were debated, as were the advantages of applying Differentiating Infected from Vaccinated Animals (DIVA) vaccines. The necessity of establishing emergency vaccine banks for livestock was discussed, as was the need for a human vaccine to protect farmers, veterinarians and others at elevated risk for RVF. It was concluded that robust challenge models must become available to facilitate rational selection of novel veterinary vaccines, and that incentives for vaccine manufacturers should be established to ensure that these vaccines come to market in a timely manner. A total of 11 recommendations to policy-makers, industry and the scientific community were formulated to facilitate this process.

Past and present control of RVFV: What is needed

Compilation of presentations provided by Hermann Unger, Pierre Rollin, Stephane de La Rocque, Truuske Gerdes and Samia Ahmed Kamal

Rift Valley fever virus (RVFV) is a phlebovirus of the *Bunyaviridae* family. The virus contains a three-segmented genome, comprising a large (L), medium (M) and small (S) genome segment (Elliott, 1996). The L segment encodes the viral RNA-dependent RNA polymerase, and the M segment encodes at least two non-structural proteins of unknown function, collectively referred to as NSm and the structural glycoproteins Gn and Gc (Gerrard and Nichol, 2007). The L and M genome segments are of negative-sense polarity, whereas the S genome segment is of ambisense polarity. This genome segment contains the non-structural NSs gene in the antigenomic orientation and the gene encoding the nucleocapsid (N) protein in genomic orientation. The NSs protein is responsible for repressing innate host immune responses and is considered the main virulence factor of the virus (Ikegami *et al.*, 2009; Bouloy *et al.*, 2001; Billecocq *et al.*, 2004; Muller *et al.*, 1995; Habjan *et al.*, 2009a).

RVFV was first identified as the causative agent of an epizootic among sheep on a farm near Lake Naivasha in the Great Rift Valley of Kenya in 1930 (Daubney, Hudson and Garnham, 1931). The investigators of this outbreak were able to show that the disease was caused by a virus that infected sheep, goats and cattle, as well as humans, and that the virus was transmitted by mosquitoes (Daubney, Hudson and Garnham, 1931). In the 80 subsequent years, the geographic distribution of the virus expanded to include most countries of the African continent, the Arabian Peninsula (Bakhy and Memish, 2003). and islands in the Indian Ocean, including Madagascar (Gerdes, 2004), Comores and Mayotte (Sissoko *et al.*, 2009). Recent history makes clear that RVFV has a strong capacity to emerge and establish in previously unaffected areas. This capacity is at least partially attributed to the broad host range of the virus, and the ability to be transmitted by at least 30 different species of mosquito belonging to six different genera (for a recent review see [Pepin *et al.*, 2010]). RVFV outbreaks are generally preceded by the mass hatching of RVFV-infected eggs of floodwater *Aedes* mosquitoes, triggered by periods of unusually heavy rainfall. *Aedes* mosquitoes transmit the virus to susceptible mammalian species, and several other mosquito species are subsequently involved in further dissemination of the virus. Mosquitoes potentially capable of transmitting RVFV are not confined to the current habitat of RVFV, which explains the growing concern for future RVFV incursions into Europe, Australasia and the Americas.

Newborn lambs and gestating ewes are the animals most vulnerable to RVFV infection. The disease in newborn lambs generally results in over 90 percent mortality (Coetzer, 1977). The mortality rate in adults is estimated at 20 percent (Coetzer, 1977; Coetzer, 1982), but differs between breeds. Infection of gestating sheep and goats results in a high number

of abortions, which often signals the start of RVF epidemics. The human case-fatality rate is historically estimated to be below 1-2 percent, although several outbreaks have resulted in considerably higher mortality rates (Al-Hamzi *et al*., 2003; Davies, 2010; Adam, Karsany and Adam, 2010; Rakotoarivelo, 2011; Madani *et al*., 2003).

Outbreaks following first incursions of RVFV in a given area can result in explosive epidemics, involving both humans and livestock. This tendency is exemplified by the epidemic that followed the first introduction of RVFV in Egypt in 1977. The epidemic resulted in an estimated 300 000 human clinical cases and 600 deaths. Neonatal mortality and abortions occurred among cattle, sheep, goats and water buffalo. Camels were also affected. Since the first introduction of RVFV in Egypt, the virus has been maintained in an enzootic cycle, and continues to cause occasional outbreaks (Arthur *et al*., 1993).

Between 2000 and 2001, a serious RVFV epidemic occurred in Saudi Arabia and Yemen (Madani *et al*., 2003), and was the first report of RVF outside the African continent. This "virgin-soil" outbreak was very serious, particularly among humans, yielding a mortality rate of 14 percent. Since that time, no major epidemics have occurred in the Arabian Peninsula, although the virus may have persisted in these areas (Elfadil *et al*., 2006).

RVFV is able to persist silently in endemic areas with low levels of circulation, as observed in western and central Africa. The ecology of RVF is still not completely understood, as exemplified by recent outbreaks in South Africa. In 2008, RVF outbreaks occurred in four South African provinces (Limpopo, North West, Gauteng and Mpumalanga). In 2009, small RVF outbreaks were reported in the KwaZulu-Natal Province (KZN). A major epizootic followed in 2010, which was 36 years after the last South African epizootic. Interestingly, no clinical cases of RVFV were reported in the KZN Province in 2010. The National Institute for Communicable Diseases (NICD) identified a new genotype, which was unrelated to isolates collected in 2008 and 2009. This finding suggests either that different strains were recently introduced into the country, or that these viruses silently persisted in this area.

The control of RVF outbreaks requires various actions, from limiting circulation of animals to reducing human risk through health and hygiene awareness campaigns and targeted interventions for populations at risk. FAO and WHO have a common strategy to implement contingency plans during RVF outbreaks, and vaccination is an important tool. Currently, there are two classical RVFV vaccines that are available in South Africa, which have been used to control recent outbreaks. The first, is based on the inactivated whole RVFV. For optimal efficacy, this vaccine requires a booster vaccination and annual re-vaccination. The second vaccine is the so-called live-attenuated Smithburn vaccine (Smithburn, 1949). This vaccine can provide lifelong immunity and is, therefore, a less expensive and more effective alternative to the inactivated vaccine. However, due to residual virulence, the Smithburn virus can cause abortion and foetal malformations when administered to gestating adults. There is need for a vaccine of equal, or greater, efficacy than the live-attenuated Smithburn vaccine, that is as safe as the inactivated vaccine. The recent release of a novel live-attenuated vaccine with improved safety (i.e. the Clone-13 vaccine) is considered a major advance in the battle against RVFV. This vaccine, as well as alternative candidate vaccines, will be discussed below.

The aim of this meeting was to discuss how the most promising RVFV vaccines can be selected and brought to market. Desired characteristics with respect to safety and efficacy

were established, and the advantage of using DIVA vaccines was discussed. This report describes the views from international organizations, policy-makers and industry on the future control of RVFV, and provides an overview of the *status quo* of RVFV vaccine development. The solid conclusions that emanated from the discussions were used to formulate 11 recommendations to the scientific community, policy-makers and industry, which aim to facilitate global preparedness for future RVFV incursions.

View from international organizations and industry

OIE ACTIVITIES AND STANDARDS RELATED TO RVF

François Diaz

OIE is an intergovernmental organization with a mandate from its 178 member countries and territories to improve animal health, veterinary public health (VPH) and animal welfare worldwide.

RVF is a disease listed by the OIE within the current category of multiple species diseases. Arising from its mandate, the OIE has developed different standards, guidelines and recommendations related to RVF. They are laid down in two publications: the *Terrestrial Animal Health Code* (hereafter referred to as the *Terrestrial Code*, and downloadable from http://www.oie.int/en/international-standard-setting/terrestrial-code/access-online/), and the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (hereafter referred to as the *Terrestrial Manual* and downloadable from http://www.oie.int/en/international-standard-setting/terrestrial-manual/access-online/).

The aim of the *Terrestrial Code* is, among other things, to assure the sanitary safety of international trade in terrestrial animals and their products. It is also an essential tool for supporting the mandate of the OIE in the area of improving animal health and welfare worldwide through the application of the standards on animal disease surveillance and recommended control methods. Chapter 8.11 on RVF is based on general provisions for minimum requirements for veterinary services and RVF. Recommendations include provisions for ruminants such as camels and their products. The horizontal chapters, 1.4 on animal health surveillance, 1.5 on surveillance for arthropod vectors of animal diseases and 4.3 on zoning and compartmentalization, also provide useful guidelines for the surveillance and control of RVF.

As a companion volume to the *Terrestrial Code*, the *Terrestrial Manual* provides internationally agreed diagnostic laboratory methods and requirements for the production and control of vaccines and other biological products for all OIE-listed diseases including RVF (Chapter 2.1.14). In particular, it specifies prescribed tests for health screening for international trade or movement of animals. The chapter on RVF provides information on requirements for the production and control of vaccines, based on classical live-attenuated virus, as well as information on vaccines based on inactivated whole virus. The second-generation vaccines, MP-12 and Clone-13 (see below), are mentioned, but requirements of these vaccines are not yet described in the chapter. The *Terrestrial Manual* also contains eleven introductory chapters that deal with a variety of general subjects of interest to veterinary laboratory diagnosticians. Chapter 1.1.8 on principles of veterinary vaccine production is particularly relevant for this workshop. With the objective of ensuring the production and availability of uniform and consistent vaccines of high quality, the chapter describes require-

ments and procedures intended to be general in nature and consistent with published standards that are available for guidance in the production of veterinary vaccines.

In its network of reference laboratories and collaborating centres, the OIE and its members can rely on two OIE Reference Laboratories, located in France and South Africa, for support and expertise.

Finally, different communications on RVF have been done by the OIE and/or in partnership with OIE through publications (e.g. *OIE Scientific and Technical Review*), and regional meetings and workshops (e.g. Workshop on RVF Control and Preventive Strategies in the Middle East and the Great Horn of Africa, Cairo, Egypt, 2007; Laboratory training course on RVF diagnosis, Dar es Salaam, Tanzania, 2008; and Regional Seminar on Re-emergence of Rift Valley fever in Southern Africa: how to better predict and respond, Bloemfontein, South Africa, 2009).

VIEW FROM THE EUROPEAN COMMISSION

Ramunas Freigofas

On 11 October 2005, the European Food Safety Authority (EFSA) published a scientific opinion paper called "*The Risks of Rift Valley Fever Incursion and its Persistence in the Community*" (http://www.efsa.europa.eu/fr/efsajournal/doc/238.pdf). This report assessed three major issues: the risk of introduction of RVFV into the European Union (EU), the risk of exposure to RVFV and the risk of RVFV persistence in the EU. It was concluded that early warning systems should be established. Countries where RVF is endemic should be monitored, and sentinel herds should be established in countries most at risk, predominantly by wind-borne mosquito vectors. The ecology of mosquito vectors must be studied so that the risk of persistence can be better estimated, and effective vector control strategies can be implemented. Veterinarians should be trained to recognize the disease in the field, and contingency plans should be established to be able to respond adequately to a future RVFV incursion. Sufficient laboratory capacity and staff must be available to handle RVF diagnostics, and effective vaccines for both livestock and humans should be developed and made available.

The European Commission (EC) recognizes the threat of a future RVFV incursion into the EU and its potential consequences for the community. This recognition is exemplified by the establishment of the EC-funded Sixth Framework projects: Emerging Diseases in a Changing European Environment (EDEN) [http://www.eden-fp6project.net] and Network of Excellence for Epizootic Disease Diagnosis and Control (EPIZONE) [http://www.epizone-eu.net/default.aspx]; and the Seventh Framework projects: International Network for Capacity Building for the Control of Emerging Viral Vector Borne Zoonotic Diseases (ARBO-ZOONET) [http://www.arbo-zoo.net/about_2/index.html] and Biology and Control of Vector-Borne Infections in Europe (EDENext) [http://www.edenext.eu/]. In all of these programmes, RVF is an important research focus.

In conclusion, the EC recognizes RVF as a real threat to the EU, and acknowledges the need for standardized diagnostics and vaccines that should be established by coordinated international activities. Nevertheless, the European Commission's Directorate General for Health and Consumer Policy document, *Expert opinion on vaccine and/or diagnostic banks for major animal diseases* (SANCO/7070/2010) does not list RVFV as a pathogen for which it is recommended to stockpile vaccines. It is important to note, in this respect, that the

experts who attended the current meeting recommend the establishment of a global RVF vaccine stockpile for emergency vaccination campaigns in all countries at risk.

VIEW FROM THE USDA

William C. Wilson and Cyril G. Gay

The United States Department of Agriculture (USDA) acknowledges the threat of a possible future RVFV incursion into the United States. Currently available commercial vaccines from South Africa and Egypt are considered inadequate for application as emergency vaccines in the United States, and, therefore, the USDA supports the development of alternative vaccines. Supported by the USDA, the Arthropod-Borne Animal Diseases Research Unit (ABADRU, Manhattan, KS, United States of America) has developed enzyme-linked immunosorbent assay (ELISA) tests based on the N, Gn and NSs proteins that, in conjunction with virus-neutralization tests (VNT), can be used to measure immune responses elicited by vaccine candidates. ABADRU has also developed real-time RT-PCR tools to detect and quantify viral RNA. One of the vaccine candidates that is being evaluated with USDA and the United States Department of Homeland Security supported by ABADRU, is the MP-12 vaccine (See section entitled The MP-12 virus). Animal models that are available for studies through the USDA and its partners are a hamster cytokine model at the Colorado State University, young animal models at the Canadian Food Inspection Agency (CFIA) and ABADRU and a larger-scale young animal model at the Kenya Agricultural Research Institute (KARI). The USDA has the objective of evaluating the potential of next-generation RVF vaccines to prevent transmission of the virus in the target animal species, with the ultimate aim of preventing the spread of RVF virus to human populations. The USDA acknowledges the need for a United States emergency stockpile of a selected RVF vaccine for veterinary application, and the added value of a DIVA vaccine for this purpose. Of note, is the need to stockpile vaccines that have been designed for the purpose of controlling disease epizootics. It is important that the development and selection of vaccines suitable for stockpiling be based on a gap analysis of the available scientific information and countermeasures.

VIEW FROM GALVmed

Baptiste Dungu

The objective of the Global Alliance for Livestock Veterinary Medicines (GALVmed) is to make a sustainable difference in access to animal health medicines by impoverished livestock keepers in developing countries. Specifically, the minimum targets are to develop, register and launch four to six vaccines, diagnostic products and pharmaceuticals by 2015. These objectives are to be achieved by collaborating with partner agencies in developing countries to ensure sustainable research, production, delivery and access of these products to poor livestock keepers. The GALVmed activities include the prioritization of diseases that have the highest impact on poor livestock keepers to understand the key barriers to developing new products that will reduce disease impact in developing countries. GALVmed also seeks to identify assets, to fill key expertise gaps and, finally, to plan and manage animal health development projects.

Important points of concern that are recognized by GALVmed are the limited continuous vaccination in African countries due to the cost of vaccination, the safety concerns applicable to the classical live-attenuated Smithburn vaccine, the irregularity of outbreaks

and the lack of a vaccination policy issued by governments. Overcoming these concerns not only requires the availability of improved vaccines, but improved vaccination strategies as well.

The ideal RVF vaccine should be safe to produce, safe to all animals regardless of the physiological stage, should have no residual virulence, should not provide a risk of introduction into the environment (shedding, persistence in animals, etc.) and should not be capable of transmission to humans or other species. The vaccine should provide protection in all susceptible species and provide a quick onset of immunity, in young animals, also. Immunity should be long-lived and the vaccine should stop transmission by preventing virus amplification in the vaccinated animal. The vaccine should be easy to administer, provide protection after a single vaccination, be suitable for stockpiling and cost effective for both producers and users.

Vaccination strategies that are considered by GALVmed can be divided into strategies for endemic regions and strategies for areas with first introductions. For application in endemic areas with irregular occurrence of RVF, the use of multivalent vaccines is considered to be advantageous, encouraging uptake of vaccination and reduction of costs. Development of multivalent vaccines that are supported by GALVmed include the vector vaccines based on capripox viruses (CPV) (See section entitled Capripox viruses as vaccine vectors) and the combination vaccine consisting of the Clone-13 and the lumpy skin Neethling vaccine strain, freeze-dried together. Potentially, these vaccines can be used to control CPV infections (lumpy skin disease [LSD] in cattle, and sheep and goat pox in these two livestock species) and concomitantly to provide immunity to RVF.

To control epidemics in previously free zones, a non-replicating vaccine is preferred that provides rapid onset of immunity, even in young animals. Furthermore, it would be advantageous if such a vaccine would enable DIVA to monitor spread of the virus both within and outside the vaccinated population.

GALVmed supported the launch of the Clone-13 vaccine (See section entitled The Clone-13 virus), which is now used in the field in South Africa, and encourages the establishment of a bank of this vaccine, initially aimed at covering southern and eastern Africa. Field trials with Clone-13 in Kenya and Senegal are planned. In addition, GALVmed is supporting further development of the CPV-vectored vaccines that can be used as multivalent vaccines, together with the registration trials for the combination RVF-LSD vaccine. Finally, GALVmed is aiding the development and establishment of a pen-side diagnostic test from the Agricultural Research Council-Onderstepoort Veterinary Institute (ARC-OVI), by funding the effort and bringing technical expertise from contract research organizations. It is clear that GALVmed not only actively supports the development of novel vaccines and diagnostics, but is also involved in bringing these control tools to poor livestock keepers in the field.

VIEW OF THE ANIMAL HEALTH INDUSTRY

Barbara Freischem

International Federation of Animal Health (IFAH) acknowledges the disadvantages of the classical RVFV vaccines and is following the experiences with the novel Clone-13 vaccine with great interest. IFAH underscores that the safety of any potential RVF vaccine is critical. In view of the known effects of current vaccines, there should be focus on reversion

to virulence, safety in pregnant animals, reassortment potential as well as environmental safety, including the potential uptake by vectors.

There is no shortage of promising experimental vaccines, but there is limited follow-through from research and development to production. A relevant consideration for this lack of follow-through is the limited marketability of a commercial RVFV vaccine. To stimulate the marketing of specific vaccines, it is important to ensure a "level playing field". Characteristics should be defined for new vaccines with respect to safety, efficacy, stability in storage for bulk and manufacturability of the final product, including capacity for "surge production" and DIVA capability. Furthermore, it is important to provide incentives for vaccine manufacturers either through direct public funding for vaccine development, or, more effectively, by creating dependable markets through the establishment of vaccine banks. These vaccine banks could also be created in concerned countries or regions, and be deployed ad hoc to help fight outbreaks elsewhere.

In conclusion, it is important to agree on standards for new vaccines, provide incentives for manufacturers (e.g. research funding, creation of markets) and find new ways of working together. These goals could be achieved by a consortium approach based on a public-private partnership established for the good of all.

Efficacy and safety of novel candidate vaccines

THE MP-12 VIRUS

George E. Bettinger and John C. Morrill

In the 1980s, a human isolate of RVFV was attenuated by serial passage of picked virus plaques in the presence of the mutagen 5-fluorouracil, with the twelfth mutagenesis passage resulting in the MP-12 virus (Caplen, Peters and Bishop, 1985). The virus accumulated mutations on each of the three genome segments, but only those on the M and L segments were later found to account for attenuation. The MP-12 virus was demonstrated to be highly immunogenic, non-pathogenic, and did not cause abortions in ewes that were in the second trimester of pregnancy (Morrill *et al*., 1987). A subsequent study found that the virus induced pyrexia and a low-titre viremia in seven-day-old lambs. No untoward effects were seen, and all of the lambs were protected from a virulent challenge fourteen days post vaccination (Morrill *et al*., 1991). Studies in cattle demonstrated that the vaccine is protective and can safely be applied in this species, as well (Morrill, Mebus and Peters, 1997a; Morrill, Mebus and Peters, 1997b). The safety of MP-12 for young lambs (Hubbard, Baskerville and Stephenson, 1991) and pregnant ewes (Baskerville, Hubbard and Stephenson, 1992) was also reported by other scientists. There is one report of the MP-12 virus causing foetal malformations when administered to sheep in the first trimester of pregnancy; however, these sheep were housed in an uncontrolled outdoor area and received a non-standard vaccine preparation (Hunter, Erasmus and Vorster, 2002).

The MP-12 vaccine is being evaluated at University of Texas Medical Branch (UTMB) and Texas A&M University to establish the safety and efficacy of the MP-12 virus in both humans and livestock. MP-12, as lyophilized vaccine or the virus master seed in culture medium, is stable when maintained cold, and retained full potency following frozen storage at -30^{o} C for over 20 years, suggesting that the vaccine is eminently suitable for stockpiling purposes.

The stability of MP-12 from reversion was tested by serial passage up to 34 times on VeroE6, MRC-5 and foetal rhesus lung cells, at both low (0.001) and high (0.1) multiplicity of infection, and showed no signs of reversion to wild-type by either inoculation into adult mice or by RNA sequence analysis.

Successful clinical testing of the MP-12 vaccine in a total of 63 human volunteers has recently been completed. Following a dose escalation arm, 10^5 plaque-forming units (PFUs) were administered in a single injection via the intramuscular (IM) route with no serious adverse reactions observed. No virus was observed by direct plaque isolation from daily serum or buffy coat samples collected between day 1-14 in 19 subjects in the Phase 2 clinical trial, confirming the hard-to-detect level of viremia post-vaccination with MP-12. The virus was recovered from five subjects by blind passage on Vero cells, and their RNA sequenced and tested in a sensitive 19 day-old mouse model capable of detecting changes

in attenuation. No reversions to the virulent RNA sequence were detected and the virus was found to be attenuated at equal or higher levels than the original MP-12 inoculum. More than 90 percent of the vaccinated individuals developed RVFV-specific antibodies (80 percent plaque-reduction neutralization titre [$PRNT_{80}$] of ≥1/40), and antibodies were maintained at the clinical end point titre of >1/40 at three years post-vaccination.

A successful RVFV animal vaccine should preferably facilitate DIVA. Reverse genetics was used to obtain both a rescued MP-12 (arMP-12) and a virus having a large deletion in the pre-Gn region of the M genome segment (arMP-12ΔNSm) (Won *et al*., 2007).Both arMP-12 and arMP-12ΔNSm induced similar levels of virus-neutralizing antibodies ($PRNT_{80}$) in mice and sheep. Western blots of sera from animals vaccinated with arMP-12 detected antibodies to the NSm protein, while no anti-NSm antibodies were found in animal sera vaccinated with arMP-12ΔNSm. These data support the feasibility of using the arMP-12ΔNSm virus as an animal vaccine with DIVA capability.

Finally, preliminary results from a collaborative study with Gary Adams (Texas A&M University) involving a large group of sheep vaccinated during the first trimester of gestation with either the MP-12 or arMP-12ΔNSm show no evidence of abortigenic or teratogenic effects. The vaccine is now being tested on calves.

In summary, past and recent work suggest that the MP-12 vaccine is highly immunogenic in humans and induces lasting antibodies from just one dose. It is capable of providing protection against a virulent challenge in animals after a single vaccination. Recent experiments with ewes in the first trimester of gestation support the safety of MP-12 virus even in sheep, the most susceptible target species.

THE CLONE-13 VIRUS

Michèle Bouloy

A particularly promising live-attenuated vaccine, which recently came to market in South Africa, is the Clone-13 virus. The Clone-13 virus was isolated from a benign human case in the Central African Republic and was found to contain a large deletion in the NSs gene (Muller *et al*., 1995). This deletion renders the virus avirulent for mice and hamsters, and prompted the studies on the biological function of the NSs protein. The first of these studies demonstrated that the NSs protein functions as an antagonist of interferon (Bouloy *et al*., 2001). Later studies demonstrated that NSs also triggers the specific degradation of double-stranded RNA-dependent protein kinase (PKR) (Ikegami *et al*., 2009; Habjan *et al*., 2009a), thereby interfering with a second major innate antiviral response pathway. Importantly, it was recently demonstrated that the NSs protein interacts with pericentromeric DNA sequences of the host cell, inducing chromosomal cohesion and segregation defects (Mansuroglu *et al*., 2010). This function of NSs could lie at the basis of foetal deformities and abortions characteristic of RVFV infections, highlighting the major role of the NSs protein in disease pathology.

To demonstrate the safety and efficacy of the Clone-13 virus in the major natural target species of RVFV, ewes in different stages of gestation were vaccinated with different doses of the Clone-13 virus and challenged with the virulent RVFV strain 35/74. In a total of three trials involving 38 pregnant ewes, none of the vaccinated animals developed fever or showed any other clinical manifestation of RVF. Furthermore, no abortions occurred and no

teratogenic effects were observed in the offspring. Unvaccinated control ewes developed pyrexia, aborted or succumbed to the infection (Dungu *et al*., 2010). The combined experiments demonstrate that the Clone-13 vaccine is safe and highly efficacious. The vaccine was recently registered and launched in South Africa and will be tested in field trials in RVF-endemic areas.

RVFV LACKING THE NSs AND NSm GENES AND DIVA

Stuart T. Nichol

The availability of RVFV reverse-genetics systems (Bird *et al*., 2007; Ikegami *et al*., 2006; Bouloy and Flick, 2009; Habjan *et al*., 2008) enables the production of RVF viruses with targeted mutations or deletions, and the study of the effect of these modifications on the biology of the virus (for a comprehensive review see [Bouloy and Flick, 2009]). Using reverse-genetics, a recombinant RVFV was created that lacks the genetic information for the NSm proteins (Bird *et al*., 2007; Gerrard *et al*., 2007). These studies demonstrated that the NSm proteins are non-essential for viability, although the biological function of the NSm proteins remains largely unclear (Bird *et al*., 2007). Importantly, whereas inoculation of rats with the parent recombinant RVFV strain was lethal in 100 percent of the rats, the mortality rate was 61 percent in rats inoculated with the NSm deletion mutant (Bird *et al*., 2007). In a subsequent study, a RVFV mutant virus was produced that not only lacks the genetic information of the NSm proteins, but also the NSs gene (Bird *et al*., 2008). This virus (here referred to as ΔNSs/ΔNSm RVFV) was completely innocuous in rats and, therefore, represents a potential vaccine candidate. Indeed, vaccination-challenge experiments demonstrated that the vaccine virus is highly immunogenic and effective in preventing severe disease and mortality in rats (Bird *et al*., 2008). Subsequent studies were performed to establish the efficacy and safety of the ΔNSs/ΔNSm virus in non-pregnant and pregnant ewes, with encouraging preliminary results.

One of the advantages of the ΔNSs/ΔNSm virus is that it originates from a cDNA clone, and is, thus, genetically highly defined. The virus lacks the major virulence determinant NSs, and contains a second attenuating mutation on another genome segment, which could further add to its safety. Finally, it is interesting to note that an experimental NSs ELISA was recently described that potentially can be used as a DIVA test to accompany the ΔNSs/ΔNSm vaccine (McElroy, 2009). Considering that this ELISA can be used to accompany not only the ΔNSs/ΔNSm vaccine virus, but also other candidate vaccines that lack the NSs protein, it will be important to determine if the specificity and sensitivity of this ELISA is sufficient for applications in the field.

CAPRIPOX VIRUSES AS VACCINE VECTORS

David B. Wallace, Reuben K. Soi and Catherine Cêtre-Sossah

A fundamentally different vaccine development approach is based on using live viral vectors. Members of the Poxviridae family are widely used for such applications, with well-known examples being vaccinia virus, Modified Vaccinia Ankara (MVA) and NYVAC, canarypox, fowlpox and the CPVs. Poxviruses are large DNA viruses that can stably maintain large and multiple foreign gene insertions. Poxviruses that are being evaluated as vaccine vectors for the control of RVFV are the attenuated Onderstepoort (OP) and KS-1 vaccine

strains of lumpy skin disease virus (LSDV, CPV genus) (Soi *et al*., 2010; Wallace *et al*., 2006; Wallace and Viljoen, 2005). An important advantage of this approach is that these vaccines can potentially be used for the control of both RVFV and LSDV. Interestingly, due to the antigenic similarity of CPVs, the latter vaccines can also be applied for the prevention of both sheep and goat pox (Kitching, 2003).

The typical manifestation of CPV infections is the formation of skin lesions that may occur all over the body. These skin lesions may erupt, resulting in secondary bacterial infections. The infection can be associated with significantly reduced milk production, a decrease in body mass and permanent damage to hides. Morbidity and mortality rates vary widely among outbreaks and seem to depend on several largely unknown environmental and host factors (Babiuk *et al*., 2008; Woods, 1988). CPV outbreaks cause negative economic impacts not only from direct damage to animal health, but from restrictions of trade with disease-free countries. LSDV seems to be largely confined to the African continent and Madagascar, although sporadic outbreaks of the disease have been reported in several countries of the Middle East (see [Brenner *et al*., 2006] and references therein), explaining the fear that the virus may establish in these areas. Although the geographic distribution of sheep and goat pox virus has historically been restricted to Africa and Asia, recurrent outbreaks have been reported to occur in Greece (Babiuk *et al*., 2008; Mangana, Kottaridi and Nomikou, 2008).

There are two rationales for using CPVs as vaccine vectors for the control of RVFV that are important to note here. First, the CPVs and RVFV share the dominant target species and their habitats overlap. Thus, vaccination with a CPV expressing RVFV antigens can potentially be used to protect cattle, sheep and goats against both RVFV and CPVs. Second, RVFV epidemics tend to occur after periods of exceptionally heavy rainfall (Linthicum *et al*., 1999). The time lapse between such events can be considerable and areas where severe RVFV outbreaks have occurred can then remain disease-free for many years. It is difficult to convince poor farmers of the need for preventative vaccination. Any vaccine that provides protection against other endemic diseases as well as RVFV could improve acceptance of the vaccine, particularly in developing countries.

The South African OP vaccine strain of LSDV was genetically modified to express the RVFV glycoproteins, Gn and Gc, resulting in the vaccine construct, rLSDV-RV (Wallace *et al*., 2006; Wallace and Viljoen, 2005; Wallace *et al*., 2007). In small animal trials, a prime-homologous boost vaccination regime provided full protection in mice (Wallace and Viljoen, 2005). In a subsequent study, one-year-old Merino sheep received a homologous prime-boost with the vaccine and were subsequently challenged with either SPPV or RVFV (Wallace *et al*., 2006). Although the challenge with RVFV was unexpectedly mild, none of the vaccinated animals developed fever after challenge (Wallace *et al*., 2006). Another group of vaccinated sheep were challenged with the Yemen strain of SPPV. All vaccinated animals were found to contain SPPV-specific neutralizing antibodies two weeks after the second vaccination. Unfortunately, no clinical signs were observed in the controls, suggesting that the challenge strain was avirulent in Merino sheep.

More recently, the construct has been redeveloped with removal of the selectable marker genes. To evaluate the protective ability of this construct (rLS-RV.mf), a more virulent strain of RVFV (i.e. strain 35/74) was selected and used for a subsequent single vaccination-

challenge experiment (David Wallace, personal communication). The results of this trial are still being analysed.

There are currently two initiatives that make use of the KS-1 strain [51] to develop a RVFV vaccine. The efficacy of the so-called rKS-1/RVFV vaccine was first evaluated in mice, demonstrating that a single vaccination via different routes provides partial protection (Soi *et al*., 2010). Although protection was only partial, this result was encouraging considering that CPVs replicate poorly in mice.

Groups of sheep (n=5) were then vaccinated with 10^8 50 percent tissue culture infective dose ($TCID_{50}$) of the rKS-1/RVFV vaccine via the subcutaneous route (SC) and boosted 28 days post initial inoculation. All vaccinated animals developed RVFV and SPPV-specific neutralizing antibodies after the first vaccination, and the titres increased after the second vaccination. After 28 days, one group of sheep was challenged with RVFV and another group with SPPV. Three of five control animals challenged with RVFV displayed viremia and all of these animals developed fever. In contrast, only one of the vaccinated sheep displayed fever and none of these animals displayed viremia. Whereas SPPV-challenged non-vaccinated control animals developed fever and skin lesions at the site of inoculation, none of the rKS-1/RVFV-vaccinated sheep displayed these symptoms (Soi *et al*., 2010). The rKS-1/RVFV vaccine construct seems able to provide protection against both RVFV and SPPV. Future experiments will address the duration of immunity and efficacy in cattle and goats.

Another approach makes use of the KS-1 strain for the expression of the NSm-Gn proteins. This vaccine virus, named CPOX-RVF, was administered at a titre of $10^{5.9}$ $TCID_{50}$ to groups of five goats via the SC route on days 0 and 21. Anti-capripox antibodies were detected by immunofluorescence 14 days after the primary immunization, and RVFV-specific neutralizing antibodies were detected at 35 days after the first immunization. Further experiments will focus on the ability of the CPOX-RVF vaccine to protect mice and sheep from virulent RVFV challenge.

AN AVIAN PARAMYXOVIRUS AS A VACCINE VECTOR

Jeroen Kortekaas

Another approach that is based on using a viral vector, makes use of the avian paramyxovirus Newcastle disease virus (NDV). Although NDV is exclusively pathogenic for birds, the virus can replicate efficiently in mammals when artificially inoculated (Huang *et al*., 2003; Bukreyev and Collins, 2008).

The LaSota strain of NDV is used all over the world for the vaccination of poultry and has a high safety record, even in the natural target species. By making use of a genetic modification system of NDV strain LaSota, a recombinant NDV was created that produces the Gn glycoprotein from a newly introduced transcriptional unit (Kortekaas *et al*., 2010a). To study the ability of this vaccine virus to induce RVFV-specific neutralizing antibodies, calves (n=3) were vaccinated twice, with a four-week interval, with 10^7 $TCID_{50}$ via either the intranasal (IN) or intramuscular (IM) route. None of the calves that were vaccinated via the IN route developed detectable antibodies against NDV or Gn. In contrast, the calves that were vaccinated via the IM route, developed detectable antibodies against both NDV and the Gn protein. Importantly, the sera obtained from the calves at three weeks after the second vaccination were shown to be capable of neutralizing virus infectivity.

Although these first results were promising, neutralizing titres were low; therefore, research was aimed at optimization of the efficacy of the vaccine. To this end, a recombinant NDV was created that expresses both the Gn and Gc proteins (here referred to as NDV-GnGc). In a first experiment, mice were either sham-inoculated or were vaccinated via the IM route with $10^{5.3}$ $TCID_{50}$ of NDV-GnGc, or, as a second negative control, with an NDV control virus. Three weeks after the second vaccination, the mice were challenged with a lethal dose of RVFV strain 35/74. Whereas 100 percent of the sham-inoculated group and 90 percent of the NDV control group succumbed to the infection, no clinical signs were observed in the group of mice that were vaccinated with the NDV-GnGc vaccine (Kortekaas *et al*., 2010b).

To study the vaccine efficacy of NDV-GnGc in the main natural target species of RVFV, lambs (n=6) were vaccinated with $10^{7.3}$ $TCID_{50}$ of either NDV-GnGc or an NDV control virus. All NDV-GnGc vaccinated lambs developed neutralizing antibodies after a single vaccination, and these titres were strongly boosted by the second vaccination (Kortekaas *et al*., 2010b). These results suggest that NDV is a highly promising vaccine vector for the control of RVFV in both sheep and cattle. Experiments are planned to study the protective efficacy of the NDV-GnGc vaccine in sheep.

DNA VACCINES AND THEIR COMBINATION WITH MODIFIED VACCINIA ANKARA VECTORS

Alejandro Brun and Sarah Gilbert

Another vaccine development approach makes use of plasmid DNA. It was previously reported that two vaccinations with plasmids encoding the Gn and Gc proteins (pCMV-M4) or the nucleocapsid protein (pCMV-N) protects mice from a lethal challenge dose of RVFV. Whereas the pCMV-M4 vaccine provided complete protection, the pCMV-N plasmid provided partial protection (Lorenzo *et al*., 2010). In the aforementioned studies, a transgenic mouse model was used. The mice that are used in these studies ($IFNAR^{-/-}$) are unresponsive to the actions of type-I interferons and are, therefore, unable to initiate an innate antiviral response upon infection. This factor renders the mouse strain highly susceptible to the otherwise attenuated MP-12 virus. To further evaluate the efficacy of the DNA vaccines, vaccination experiments in BALB/c mice were performed. With the objective of obtaining optimal efficacy, the vaccines were combined with recombinant MVA viruses expressing either the N (rMVA-N) or Gn and Gc proteins (rMVA-M4). The DNA vaccines (100µg/dose, IM) and/or MVA vaccines (10^7 PFU/dose, IP) were administered on day 0 and 15, and mice were challenged with 10^3 PFU of virulent RVFV via the IP route on day 30. Clinical signs were monitored for 21 days.

Neutralizing antibodies were detected after vaccination with rMVA-M4 and pCMV-M4, but not after vaccination with rMVA-N or pCMV-N. Vaccination with pCMV-M4 followed by a boost with rMVA-M4 also induced neutralizing antibodies, although at lower levels. Vaccination with pCMV-N followed by a boost with rMVA-N did not result in the induction of neutralizing antibodies, but enhanced the level of antibody responses against the N antigen. The mice immunized with this approach showed higher survival rates than mice vaccinated with pCMV-N alone upon lethal challenge with a virulent, heterologous strain of RVFV. These mice, however, showed clinical signs long after challenge. In contrast, a

pCMV-M4 + rMVA-M4 regimen did not improve the level of protection achieved by the pCMV-M4 vaccine alone, but none of the surviving mice displayed clinical signs. More interestingly, a single dose of the rMVA-M4 vaccine was sufficient to induce protection in 100 percent of the mice upon viral challenge with no clinical display. These results suggest that the rMVA-M4 is a promising vaccine, which may be applicable for both livestock and, considering its established safety profile (Berthoud *et al*., 2011), humans. Potentially, the efficacy of the DNA vaccines can be improved by including appropriate adjuvants and/or improved delivery routes. Further studies will focus on establishing the efficacy of these vaccine candidates in sheep.

DNA VACCINES AND THEIR COMBINATION WITH ALPHAVIRUS REPLICON VECTORS

Ted Ross

In another study, DNA vaccines were developed that are based on the expression of only the Gn protein, or a fusion protein of Gn and the complement protein C3d. It is well established that the molecular adjuvant C3d can significantly improve antibody responses against antigens delivered by DNA vaccines (Bower, Sanders and Ross, 2005; Green, Montefiori, and Ross, 2003; Ross *et al*., 2001; Mitchell *et al*., 2003; Mkrtichyan *et al*., 2008; Ross *et al*., 2000). Apart from evaluating the efficacy of Gn-based DNA vaccines, a direct comparison with an Alphavirus replicon vector expressing the ectodomain of Gn (Rep-Gn) was performed. Furthermore, the potential added value of a DNA prime/replicon boost was evaluated (Bhardwaj, Heise and Ross, 2010). BALB/c mice (n=5, 6-8 week-old) were vaccinated three times, with a three-week interval. Vaccination with Rep-Gn was performed with a dose of 10^5 infectious units. Vaccination with Gn-DNA or Gn-C3d-DNA was performed with 2µg by gene gun. The prime-boost vaccination consisted of two vaccinations (at week 0 and 3) with Gn-C3d-DNA, followed by a boost with Rep-Gn three weeks later. As a control, mice were vaccinated once with the MP-12 vaccine. Two weeks after the final immunization, all mice were challenged with a lethal dose of RVFV strain ZH501.

Antibody responses specific for RVFV were determined by ELISA. This experiment demonstrated that the MP-12 vaccine was the most immunogenic, followed by the Gn-C3d-DNA/Rep-Gn-vaccinated mice, and, subsequently, Rep-Gn, Gn-C3d-DNA and Gn-DNA. Neutralizing antibodies were detected in all vaccinated groups. The antibody levels were highest in the group of mice vaccinated with the MP-12 vaccine.

To study the cellular immune response elicited, ELISPOT assays were performed using overlapping Gn-derived peptides. Cellular responses were only detected using the splenocytes from mice vaccinated with the replicon vaccine. Two out of four peptides that were identified share the amino acid sequence SYAHHRTLL, which was predicted to be MHC-I restricted (Bhardwaj, Heise and Ross, 2010). This result represents the first identification of an MHC-I restricted epitope in the Gn protein.

In the group of mice that were vaccinated with Gn-DNA, one of five mice did not survive, whereas in the other groups, all mice survived the challenge (Bhardwaj, Heise and Ross, 2010).

In conclusion, these experiments have demonstrated that the candidate vaccines expressing only the Gn protein are capable of protecting mice against a lethal dose of RVFV,

and that C3d is an effective adjuvant to improve the protective immune response. These promising results warrant further studies in large animals and humans.

VIRUS-LIKE PARTICLES AS RVFV VACCINES

Ramon Flick

Expression of the RVFV structural glycoproteins Gn and Gc, with or without the presence of the N protein, results in the assembly and release of virus-like particles (VLPs) (de Boer *et al*., 2010; Mandell *et al*., 2009; Naslund *et al*., 2009; Habjan *et al*., 2009b; Liu, Celma and Roy, 2008; Mandell *et al*., 2010). VLPs are particularly interesting vaccine candidates, since they physically resemble the authentic virus, but are recognized as safe vaccines because they do not contain any viral genetic material (Mandell and Flick, 2010). The major challenge in this approach is to produce a cost-effective vaccine. To this end, the generation of RVF VLPs could be optimized as well as the production methods to maximize yields.

This presentation described the production of RVF VLPs by co-expressing the Gn and Gc glycoproteins with or without the expression of the N protein. Furthermore, RVF VLPs containing the gag protein of Moloney murine leukemia virus (MoMLV) were produced, which are referred to as chimeric VLPs (chimVLPs). The gag protein of MoMLV was added to the VLPs (gag expression plasmid included with RVFV G and N expression plasmids for generation of RVF VLPs) since it was previously shown that incorporation of retroviral gag can increase the uniformity, quantity and stability of VLPs ([66] and references herein). The vaccine efficacy of the different RVF VLP formulations were compared in both mouse and rat models, and efforts were made to optimize the production of the VLPs.

To study RVF VLP immunogenicity to define immune correlates of vaccine efficacy, groups of four mice were vaccinated three times at nine-day intervals, via the SC route, with RVF VLPs containing N (VLP+N), VLPs without N (VLP-N) or chimVLPs. At six months post-vaccination, $PRNT_{80}$ assays demonstrated the presence of neutralizing antibodies in all VLP-vaccinated animals (Mandell, 2009). The cellular immunity induced by three-time RVF VLP vaccination was subsequently studied by measuring antigen-specific cytokine secretion by isolated splenocytes. These experiments demonstrated that splenocytes from VLP-vaccinated mice secreted IL-2, IL-4, IL-5 and IFN-γ upon MP-12 stimulation, consistent with both humoral TH2 and cellular TH1 responses. These results demonstrate that RVF VLP vaccination induces both humoral and cellular immunity, and that immunity is durable.

To study the protective efficacy of RVF VLP vaccination, a pilot study was performed using "suboptimal" conditions to see minor differences in vaccine efficacies of the different vaccine candidates. Groups of 20 mice were vaccinated three times with either chimVLPs, VLP-N or VLP+N, formulated with the Sigma Adjuvant System. Forty-six days after the third vaccination, 16 of the 20 animals were challenged with a lethal dose of RVFV and the remaining 4 mice were used for PRNT80 assays (described above). Whereas all control animals succumbed to the infection between four to six days, 56 percent of the mice vaccinated with VLP+N survived the challenge, but only 19 percent of the mice vaccinated with VLP-N survived. These results suggest that the presence of the N protein improves vaccine efficacy. The best survival rate (68 percent) was obtained in the mice that were vaccinated with chimVLPs, although the difference with VLP+N was not statistically significant. Following this pilot study, mouse challenge studies were performed with RVF VLPs. While 100 percent of vacci-

nated animals survived, all control (unvaccinated) animals died within the first five days post-challenge, demonstrating the efficacy of the RVF VLP-based vaccine candidate against RVFV.

Rats are also frequently used in RVFV animal studies, and are sometimes considered more relevant than mice. To further assess the vaccine efficacy of the chimVLP-based vaccine candidate, groups of six rats were vaccinated with adjuvanted VLPs three times at two-week intervals. Sixty-seven days post vaccination, the rats were challenged with a lethal dose of RVFV. In this experiment, all vaccinated rats survived the challenge without any weight loss, whereas control rats succumbed to the infection within four days. From this experiment, it can be concluded that RVF chimVLPs present a promising subunit vaccine for the control of RVFV. However, RVF VLPs are preferred over chimVLPs simply because they do not contain additional heterologous viral proteins. Therefore, groups of six rats were vaccinated once, twice or three times (days 0, 14 and 28) with adjuvanted RVF VLPs, and subsequently challenged with a lethal dose of wild-type RVFV. Whereas five out of six non-vaccinated rats died within the first five days, all vaccinated rats survived the challenge, including the rats that only received a single vaccination (Mandell *et al.*, 2010). These results demonstrate that RVF VLPs are efficacious even as a single dose.

With the objective of optimizing RVF VLP production, novel mammalian and insect suspension cell-based VLP production systems were developed. A direct comparison between these systems demonstrated that suspension mammalian cells yield at least eight times more RVF VLPs compared with adherent mammalian cells and insect suspension cells on a per cell basis.

In summary, this work demonstrated that adjuvanted RVF VLPs can provide complete protection, even after a single vaccination. Studies on the immune response in sheep, efficacy in livestock, safety in ewes and efficacy in non-human primates will be the subject of further studies. Furthermore, large-scale cGMP production systems will be established.

TRANSCRIPTIONALLY-ACTIVE VLPs AS RVFV VACCINES

Friedemann Weber

The difference between authentic RVFV virus particles and conventional VLPs, is that the latter do not contain viral genome segments. It was recently demonstrated that VLPs of RVFV can transfer genome-like RNAs containing a luciferase reporter protein (Habjan *et al.*, 2009b). These VLPs can be regarded as "infectious VLPs" (iVLPs), since they are capable of infecting cells (indicator cells) and initiate primary transcription of the incorporated minigenome, resulting in low-level reporter gene expression. This primary transcription is mediated by co-packaged L and N proteins. Transfection of the iVLP-infected cells with N and L-expression plasmids provides sufficient amounts of L and N to enable replication of the genome segment and a higher level of reporter gene expression (Habjan *et al.*, 2009b).

In a subsequent study, 30 mice were vaccinated three times with a two-week interval with either 10^5 or 10^6 VLPs/dose. After the third vaccination the sera were analysed for the presence of RVFV-specific antibodies by immunofluorescent analysis. This analysis demonstrated that all mice seroconverted. The virus-neutralizing antibody titres were determined by PRNTs. This experiment demonstrated that all of the mice, with one exception, developed neutralizing antibodies, and that the higher vaccine dose resulted in higher neutralizing antibody titres.

Three weeks after the third and final vaccination, the mice were challenged with a known lethal dose of RVFV. Three out of six mice that were vaccinated with the low dose of VLPs (10^5) survived the infection. Eleven out of 12 mice vaccinated with the high dose (10^6) survived the challenge without displaying clinical signs, whereas only 1 of 12 mice in the control group survived (Naslund *et al*., 2009).

In recent work, so-called N-iVLPs were produced that do not contain a reporter gene in the packaged genome segment, but, instead, contain the gene encoding the N protein (Pichlmair *et al*., 2010). Including the N gene in these particles could improve vaccine efficacy of iVLPs by enhancing viral gene expression or acting as a source of cytotoxic T-cell epitopes. Importantly, this study demonstrated that a single vaccination with N-iVLPs provided complete protection in mice. The combined results demonstrate the high potential of N-iVLPs, and it will be particularly interesting to determine the vaccine efficacy of N-iVLPs in the natural target species of RVFV.

Summary discussion

Rob J. M. Moormann and Jeroen Kortekaas

WHICH VACCINE FOR WHERE?

The general consensus about the need to improve the first generation of RVFV vaccines has resulted in the development of a variety of different candidate vaccines in the past decade. The second generation of live-attenuated vaccines holds great promise for the future. A recent major advance in the battle against RVFV is the launch of the Clone-13 vaccine in South Africa. In contrast to the live Smithburn vaccine that was previously the only available live vaccine, the Clone-13 virus can be safely applied in gestating ewes, which are the most susceptible to disease. The application of this vaccine could greatly reduce the consequences of the current RVFV epidemic in South Africa. Moreover, the use of such second-generation live-attenuated vaccines in endemic areas, provided that they will prove to be safe, could facilitate their future acceptance in countries that are currently free of RVFV.

However, the use of viral vectors for the control of RVFV remains a promising approach, since these vaccines have important advantages. For example, lambs born from seropositive mothers are frequently protected from RVFV infection by maternal antibodies. Due to the presence of these antibodies, these young animals cannot be effectively vaccinated with live-attenuated vaccines to protect them from the virus after maternal antibodies have depleted. Vector vaccines could provide a solution to this problem, since these viral vectors are not neutralized by pre-existing RVFV antibodies. A second potential advantage of vector vaccines is their use as multivalent vaccines. The use of LSDV as a vector of RVFV antigens, could potentially be used to control not only RVFV, but also LSDV, SPPV and GTPV. This feature could be a major advantage to overcome the general reluctance to vaccinate during interepidemic periods, and is one of the main reasons this line of research is supported by GALVmed.

The use of NDV as a vector of RVFV antigens offers distinct advantages. In areas where CPVs are endemic, efficacy of these vaccines may be compromised by pre-existing immunity against the vector. NDV is an exclusive pathogen of birds and pre-existing immunity against this virus in mammals is unlikely to occur.

The live vector vaccine with the highest safety profile is clearly the MVA vector. The use of this viral vector is not only promising for application in livestock, but for humans as well. It will be valuable to study the feasibility of MVA-based RVFV vaccines in the recently established non-human primate model (See section entitled The Need for Robust Animal Models).

The remaining vaccine candidates that were discussed during the meeting are unlikely to face serious safety issues regarding risk for the environment, since these vaccines are incapable of autonomous amplification and spread. Although this quality is a great benefit of these vaccines, it also explains their poorer vaccine efficacy. In addition, production costs of these vaccines are high. However, in the past decade impressive progress has been made

to improve the efficacy of DNA vaccines, and production methods for subunit vaccines have also improved considerably. Therefore, evaluating the efficacy of DNA vaccines, replicon vaccines and (i)VLPs in the natural target species of RVFV should be encouraged in the years to come.

Another potential advantage of the next generation vaccines is their ability to be used as DIVA vaccines. Although the added value of using DIVA vaccines in countries where RVFV is currently endemic is questionable, using such vaccines in previously unaffected areas is, without a doubt, extremely valuable. The first major advantage of using a DIVA vaccine is that it enables close monitoring of virus spread within a vaccinated population. Thus, vaccination strategies can be adapted during outbreaks. Furthermore, the use of DIVA vaccines could reduce economic damage due to trade restrictions with disease-free countries.

Potentially, vector vaccines and subunit or DNA vaccines based on the RVFV glycoproteins can be used as DIVA vaccines with accompanying N-protein-based ELISAs, which are already commercially available. Interestingly, the Clone-13 virus, the MP-12ΔNSm and the ΔNSs/ΔNSm viruses can potentially also be used as DIVA vaccines when diagnostic NSs or NSm ELISAs become available. An experimental NSs ELISA was also recently developed that can potentially be used for this application [40]. It is important to note, however, that the specificity and sensitivity of these ELISAs should be thoroughly evaluated in the field before they can be applied as DIVA tests.

The vaccines that are currently being evaluated are administered using hypodermic needles. Workshop participants were concerned that vaccination of multiple animals using a single needle during an RVFV outbreak would facilitate spread of the virus from viremic to naïve animals. Therefore, efforts should be made to develop needle-free delivery methods that are compatible with existing second-generation vaccines.

THE NEED FOR ROBUST ANIMAL MODELS

Although several highly promising novel candidate vaccines are available, most of these vaccines have not been extensively evaluated in the natural target species of RVFV. To properly evaluate the efficacy of the candidate vaccines, robust challenge models are needed to obtain statistically significant results with the lowest number of animals possible. Such optimized challenge models will facilitate future vaccination-challenge trials and a more scientifically sound comparison of results obtained from experiments performed at different institutes with different vaccine candidates.

Lambs below the age of two weeks are highly susceptible to RVFV infection, resulting in severe clinical signs and high mortality rates. However, using these very young lambs for vaccination-challenge experiments is not practical. Although inoculation of adult sheep with RVFV can result in mortality, infection in most cases manifests as a mild fever or even remains subclinical. Nonetheless, it is plausible to assume that any vaccine that is able to prevent viremia is likely to be effective in preventing disease and dissemination of the virus. Therefore, any challenge model in which challenged control animals consistently develop high viremia and fever should be considered suitable for vaccine efficacy studies. Experience developed in the past years with RVFV challenge models at different research institutes in Europe and South Africa has suggested that induction of high viremia and fever is not

only influenced by the age of the inoculated animal, but also by the challenge strain, the passage history of the challenge virus and the route of inoculation. To establish a robust challenge model in young adult sheep, these parameters must be optimized, preferably in a collaborative action of institutions worldwide.

In the past years, several European institutes have initiated the development of RVFV challenge models. To coordinate these efforts and prevent duplications in research, the European Network for the Coordination of Rift Valley Fever Animal Experimentation and Diagnostics (ENCRAD) was established. Founding of this network was financed by the EPIZONE (EU project number: FOOD-CT-2006-016236). Chaired by the CVI-WUR (J. Kortekaas), this Network brought together key European institutes: CVI-WUR [Netherlands], Centro de Investigación en Sanidad Animal-Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (CISA-INIA) [Spain], Centre de Recerca en Sanitat Animal (CReSA) [Spain], Agence nationale de sécurité sanitaire de l'alimentation, de l'environement et du travail (ANSES) [France], Centre International de Recerche Agronomique pour le Développement (CIRAD) [France], Friedrich Löeffler Institut (FLI) [Germany], Animal Health and Veterinary Laboratories Agency (AHVLA) [England] along with the ARC-OVI [South Africa]. These institutes now share experiences obtained from RVFV animal experiments and diagnostic tools that are used for the analysis of clinical samples. This initiative could be extended geographically and outcomes further shared among the major research institutions.

For application in humans, it is essential to test the efficacy and safety of candidate vaccines in a non-human primate (NHP) model. The current NHP model for RVFV reported in the literature makes use of rhesus macaques, which are challenged with the virus via the intravenous (IV) route. The clinical manifestation of RVFV infection in these animals is not very severe. Therefore, large cohorts of animals are needed to demonstrate statistically significant vaccine efficacy. To overcome this limitation of the macaque model, recent efforts by Darci Smith at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) have focused on the development of a novel NHP model that makes use of the common marmoset (*Callithrix jacchus*). The marmosets were challenged with RVFV not only via the IV route, but also via the SC route to more accurately mimic a natural exposure since RVFV-infected mosquitoes have been found primarily to transmit virus extravascularly (Turell and Spielman, 1992). Preliminary studies demonstrate that marmosets are more susceptible to RVFV infection than rhesus macaques and develop disease that is more reflective of severe RVF in humans (D. Smith, personal communication). Therefore, the common marmoset model may be beneficial for the evaluation of potential vaccines and therapeutics.

There are several highly promising experimental vaccines already available that can potentially be applied in humans. These vaccines should be evaluated extensively for efficacy and safety in the NHP model that is reflective of human RVF disease. These studies would not only lead to the potential development of a licensed human vaccine, but would also provide important safety data for the advancement of veterinary vaccines, such as the removal of live-attenuated vaccines from the select agent list.

A HUMAN RVF VACCINE: ALL IT NEEDS IS A "PULL"

The scale of investments necessary to develop RVFV vaccines for livestock and humans are quite different. The veterinary pharmaceutical industry has clearly recognized the emerging

market for veterinary RVFV vaccines. However, at this time, the market for a human vaccine is not large enough to justify risk-adjusted development costs.

Incentive systems to encourage the development of novel vaccines can be categorized into so-called "push" programmes, which stimulate research efforts, and "pull" programmes, which reward developers for actually delivering the desired product (Kremer and Glennerster, 2004). When there is a need for a vaccine in industrialized countries, the traditional response by policy-makers is to invest in research to develop the desired product. This response is referred to as "push" funding, since a selection is made of several options to push development of a selected product. Generally, this results in research calls to which universities, research institutes and pharmaceutical companies can respond. In these types of programs, researchers not only focus on developing a vaccine that fulfils all requirements for marketability, but also on ancillary goals such as the publication of journal articles. In other words, the product is not the only focus in these programmes. In the alternative "pull" programmes, governments commit themselves to buying, at a reasonable price, whatever vaccine meets predefined requirements. Thus, pharmaceutical companies gain an incentive to invest only in the most promising vaccine candidate. In the opinion of the authors, a "pull" programme for the development of a human RVFV vaccine seems the only feasible way to bring a human vaccine to market within the next decade.

It is important to note that several of the experimental vaccines already developed can be regarded as potential vaccines for humans. The MP-12 virus was tested in a human Phase-II clinical trial, which did not reveal any serious adverse reactions. Based on current knowledge, the other live-attenuated RVF viruses noted above could be even more attenuated in humans, rendering these viruses also potential vaccines for human use. Should a live RVFV not be accepted as a vaccine for application in humans, the vector vaccines presented and discussed in this workshop could be evaluated, as well as vaccines that are not based on live viruses. These vaccines should be evaluated extensively for efficacy and safety in the NHP model, which is now available. Thus, all it takes now is a "pull" for a human RVF vaccine to become available.

It can be concluded that tremendous progress in the development of RVFV control tools has been made in the past decade. The current challenge is to take these control tools to the field. The workshop participants formulated 11 recommendations for policy-makers, industry and the scientific community, which are meant to facilitate the process.

Recommendations

1. In recognition of the role of the OIE and importance of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*), it is recommended that OIE reviews and updates Chapter 2.1.14. of the *Terrestrial Manual* with respect to Rift Valley Fever (RVF) vaccines, taking into account recent scientific advances and the latest available technologies in vaccine development. If appropriate, corresponding changes to Chapter 8.11 of the *Terrestrial Animal Health Code* on RVF should be considered.
2. Points to be considered in the development of RVF veterinary vaccines include the following:
   a. Safety
      i. no reversion to virulence;
      ii. lack of abortion in vaccinated animals; and
      iii. non-teratogenic.
   b. Efficacy
      i. prevention of viremia;
      ii. rapid onset of immunity;
      iii. long-lasting immunity;
      iv. prevention of abortion on challenge;
      v. prevention of clinical disease;
      vi. produce immunity in young animals;
      vii. target key susceptible ruminant species; and
      viii. single-dose regimen.
3. Research recommendations:
   a. Develop robust challenge models in target animal species to evaluate the efficacy of RVF vaccines, as described in 2.b. above. Identification of scientifically critical and desirable characteristics of animal models for RVF research is an important first step in this process.
   b. Identify correlates for protective immunity.
   c. Promote the development of alternative vaccine delivery methods.
   d. Evaluate the benefits of multivalent vaccines to increase uptake of RVF vaccines in specific at-risk populations.
   e. Enhance RVF surveillance and outbreak investigations to improve our understanding of the epidemiology of RVF, including identification of reservoirs of RVFV in inter-epidemic periods, and make results available to the global research community in a timely manner.
   f. Continue to support important RVF research in non-human primates.
4. A scientific review of existing animal models, including state of development and use in current, planned or historical studies, should be undertaken and published.

5. Encourage and facilitate global sharing of state-of-the-art scientific knowledge and technologies.
6. The lack of persistence and prolonged shedding of infectious virus in animals should be considered in the design of RVF control strategies.
7. The relative risks and benefits of RVF vaccination in the face of an outbreak should be evaluated to inform FAO and OIE, and allow them to make the most appropriate recommendations for the integrated control of RVF.
8. Although DIVA is an important property of any future vaccine, a requirement for DIVA should not hinder or block the development or licensing of an effective RVF vaccine.
9. Recognizing that RVF is an important international concern, encourage the development of a strategy for a global RVF vaccine stockpile for use in RVF-endemic areas and emergency vaccination campaigns.
10. In the absence of a registered vaccine for human use, facilitate access to investigational vaccine(s) to protect persons at elevated risk of exposure to RVFV (e.g. veterinarians, livestock officers, abattoir workers and researchers).
11. Recognizing that RVF research benefits greatly from a one-health approach, continue to include the human-vaccine research community in future meetings and discussions on RVF vaccine development and control strategies.

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List of participants

JEAN-CHRISTOPHE AUDONNET
International Federation for Animal Health
Belgium
jcaudonnet@ifahsec.org

GEORGE E. BETTINGER
Western Regional Center of Excellence for Biodefense & Emerging Infectious Diseases
The University of Texas Medical Branch
United States of America
gebettin@utmb.edu

MICHÈLE BOULOY
Institut Pasteur
France
mbouloy@pasteur.fr

ALEJANDRO BRUN
Centro de Investigacíon en Sanidad Animal-Instituto Nacional de Investigacíon y Tecnología Agraria y Alimentaria
Spain
brun@inia.es

CATHERINE CÊTRE-SOSSAH
Centre International de Recherche Agronomique pour le Développement
France
cetre@cirad.fr

STÉPHANE DE LA ROCQUE
Food and Agriculture Organization of the United Nations
Italy
stephane.delarocque@fao.org

PETER DE LEEUW
Food and Agriculture Organization of the United Nations
Italy
peter.deleeuw@fao.org

FRANÇOIS DIAZ
World Organisation for Animal Health
France
f.diaz@oie.int

BAPTISTE DUNGU
GALVmed
United Kingdom
baptiste.dungu@galvmed.org

RAMON FLICK
BioProtection Systems Corporation
United States of America
rflick@bpsys.net

RAMUNAS FREIGOFAS
European Commission
Belgium
ramunas.freigofas@ec.europa.eu

BARBARA FREISCHEM
International Federation for Animal Health
Belgium
bfreischem@ifahsec.org

CYRIL GERARD GAY
United States Department of Agriculture
United States of America
cyril.gay@ars.usda.gov

TRUUSKE GERDES
University of Pretoria
South Africa
gerdes.t001@gmail.com

SARAH GILBERT
University of Oxford
United Kingdom
sarah.gilbert@ndm.ox.ac.uk

DANNY GOOVAERTS
International Federation for Animal Health
Belgium
dgoovaerts@ifahsec.org

MARTIN GROSCHUP
Friedrich Loeffler Institut
Germany
martin.groschup@fli.bund.de

LISA HENSLEY
United States Army Medical Research Institute
of Infectious Diseases
United States of America
lisa.hensley@us.army.mil

SAMIA AHMED KAMAL
Animal Health Research Institute
Egypt
selkabany@yahoo.com

JEROEN KORTEKAAS
Central Veterinary Institute of Wageningen
University
Netherlands
jeroen.kortekaas@wur.nl

TOM KSIAZEK
University of Texas Medical Branch
United States of America
tgksiaze@UTMB.EDU

ROB J. M. MOORMANN
Central Veterinary Institute of Wageningen
University
Netherlands
rob.moormann@wur.nl

STUART T. NICHOL
Centers for Disease Control and Prevention
United States of America
snichol@cdc.gov

PIERRE ROLLIN
Centers for Disease Control and Prevention
United States of America
pierrerollin@comcast.net

TED ROSS
University of Pittsburgh
United States of America
tmr15@pitt.edu

JAN SLINGENBERGH
Food and Agriculture Organization of the United Nations
Italy
jan.slingenbergh@fao.org

DARCY SMITH
United States Army Medical Research Institute of Infectious Diseases
darci.smith1@us.army.mil

REUBEN SOI
Kenya Agricultural Research Institute
Kenya
reubensoi@gmail.com

HERMANN UNGER
Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture
Austria
h.unger@iaea.org

DAVID WALLACE
University of Pretoria
wallaced@arc.agric.za

FRIEDEMANN WEBER
Philipps-University Marburg
Germany
friedemann.weber@Staff.Uni-Marburg.de

HANA M. WEINGARTL
Special Pathogens Unit
National Centre for Foreign Animal Disease
Canadian Food Inspection Agency
hweingartl@inspection.gc.ca

WILLIAM C. WILSON
United States Department of Agriculture-Agricultural Research Service
william.wilson@ars.usda.gov

JAMES ZINGESER
Food and Agriculture Organization of the United Nations
Italy
james.zingeser@fao.org

FAO ANIMAL PRODUCTION AND HEALTH PROCEEDINGS

1. Protein sources for the animal feed industry, 2004 (E)
2. Expert Consultation on Community-based Veterinary Public Health Systems, 2004 (E)
3. Towards sustainable CBPP control programmes for Africa, 2004 (E)
4. The dynamics of sanitary and technical requirements – Assisting the poor to cope, 2005 (E)
5. Lait de chamelle pour l'Afrique, 2005 (F[e])
6. A farm-to-table approach for emerging and developed dairy countries, 2005 (E)
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Rift Valley fever (RVF) is an acute arthropod-borne infection first recognized in Kenya in 1931. Today, the RVF virus has been found in countries across Africa, the Arabian Peninsula and islands in the Indian Ocean, including Madagascar, Comores and Mayotte. This virus has a strong capacity to spread to previously unaffected areas, thanks to its broad host range and ability to be transmitted by at least 30 different mosquito species – some of which are found in Europe, Australasia and the Americas. Outbreaks following first incursions of RVF can result in explosive epidemics involving both humans and livestock.

The control of RVF outbreaks includes vaccination of susceptible animals. Two vaccines are currently available; however, each has significant drawbacks. There is a widely recognized need to develop safer and more efficacious vaccines for animals. *Rift Valley fever vaccine development, progress and constraints* is the report of an international expert workshop that brought together leading experts and policy-makers in RVF virology, epidemiology and vaccine development. The workshop objective was to gain consensus and make recommendations on the desired features of novel veterinary RVF virus vaccines, and to explore how incentives can be established to assure that these vaccines come to market.

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