Development of Canadian diagnostic capability for Rift Valley fever virus (RVFV)

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Standard Clause for Research involving Animal Subjects

In conducting the research described in this report, the investigators adhered to the 'Guide to the Care and Use of Experimental Animals, Vol. I, 2nd Ed.' published by the Canadian Council on Animal Care.

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IMPORTANT INFORMATIVE STATEMENTS

Development of Canadian Diagnostic Capability for Rift Valley Fever Virus (RVFV), CRTI 06-138RD, was supported by the Canadian Safety and Security Program (CSSP) which is led by Defence Research and Development Canada's Centre for Security Science, in partnership with Public Safety Canada.

Led by Canadian Food Inspection Agency National Centre for Foreign Animal Disease partners included Public Health Agency of Canada – National Microbiology Laboratory, University of Calgary, and United States Department of Agriculture.

The CSSP is a federally-funded program to strengthen Canada's ability to anticipate, prevent/mitigate, prepare for, respond to, and recover from natural disasters, serious accidents, crime and terrorism through the convergence of science and technology with policy, operations and intelligence.

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Abstract

CRTI 06-138RD project developed veterinary diagnostic tests for Rift Valley fever virus (RVFV), and identified three species of mosquitoes as potential vectors for RVFV in Canada.

RVFV is a zoonotic virus transmitted to people from mosquitoes or ruminants. It is considered a significant biological warfare agent and a severe natural emerging disease threat to Canada, as it has a high potential for rapid spread, endemic establishment, serious economic and public health consequences. Canada had no veterinary diagnostic capability. Ability to rapidly recognize and control by vaccination a RVF outbreak in ruminants is important for protection of people, livestock industry, economy and wildlife.

The project has developed diagnostic tests for virus and antibody detection transferrable to low containment and field laboratories. The assays were evaluated on positive samples developed in experimentally infected sheep, goats and calves in a high containment facility at NCFAD. ELISA tests for detection of antibodies were evaluated on negative Canadian field samples from all three species. Viremia model in sheep developed during the project was used in efficacy testing of two vaccine candidates.

Canada has now veterinary RVFV diagnostic capability, federal veterinarians trained to recognize RVFV, and veterinary MP12 vaccine (Zoetis) licensed for use in government regulated vaccination program.

Significance for Defence and Security

RVFV is a zoonotic virus on multiple “A lists” (World Organisation for Animal Health, Centers for Disease Control and Prevention, United States Department of Health and Human Services/United States Department of Agriculture Select Agents list), as it is considered a significant biological warfare and bioterrorist agent, and a severe natural emerging disease threat to Canada.

This project built veterinary diagnostic capability transferrable to regional low containment laboratories to facilitate rapid local detection of RVFV incursion. Both virus detection and antibody detection tests are also field deployable using mobile laboratory system. In addition, NCFAD has now capacity for full genomic sequencing important for tracing the source/origin of the virus, especially in case of intentional introduction to Canada.

In summary, the project developed laboratory preparedness for RVFV in Canada, applicable also for long-term consequence management.

The project also assisted in provisional licence of RVFV vaccine by CFIA Veterinary Biologics for government regulated vaccination program, as a countermeasure to limit/control RVFV outbreak should it occur.

Résumé

Le projet CRTI 06-138RD portait sur la mise au point de tests de détection du virus de la fièvre de la vallée du Rift (FVR) et a permis d'identifier trois souches de moustique qui pourraient être vectrices du virus de la FVR au Canada.

La fièvre de la vallée du rift est une zoonose virale qui peut être transmise aux humains par des moustiques ou des ruminants. Elle est considérée comme un agent de guerre biologique important et une grave menace de maladie naturelle émergente au Canada, parce qu'elle peut se propager rapidement et s'établir comme maladie endémique et qu'elle peut avoir des conséquences graves pour l'économie et la santé publique. Le Canada n'avait aucune capacité de diagnostic vétérinaire de cette maladie. Or, il est important de reconnaître la maladie et de l'enrayer avec la vaccination en cas d'éclosion chez des ruminants pour protéger les gens, l'industrie de l'élevage, l'économie et la faune.

Dans le cadre de ce projet, un test diagnostique a été mis au point, qui permet de détecter le virus et les anticorps dirigés contre celui-ci dans des laboratoires de faible niveau de confinement ou des laboratoires aménagés sur le terrain. Les épreuves ont été évaluées sur des échantillons positifs provenant de moutons, de chèvres et de veaux infectés expérimentalement dans des installations à haut niveau de confinement du CNMAE. Les épreuves ELISA de détection des anticorps ont été évaluées sur des échantillons négatifs prélevés sur le terrain, au Canada, chez les trois espèces animales. Le modèle de virémie chez le mouton développé durant ce projet a été utilisé dans la vérification de l'efficacité de deux vaccins potentiels.

Le Canada dispose maintenant d'une capacité de détection du virus de la FVR, de vétérinaires fédéraux formés pour reconnaître le virus et du vaccin vétérinaire MP12 (Zoetis) homologué pour l'utilisation dans le programme gouvernemental de vaccination.

Importance pour la défense et la sécurité

Le virus zoonotique de la FVR figure sur de nombreuses listes d'agents présentant une menace pour la santé publique (Organisation mondiale de la santé animale [OIE], Centers for Disease Control and Prevention [CDC], United States Department of Health and Human Services/United States Department of Agriculture), puisqu'il est considéré comme agent de guerre biologique ou de bioterrorisme important et une grave menace de maladie naturelle émergente au Canada.

Ce projet a permis d'établir une capacité de diagnostic vétérinaire transférable à des laboratoires régionaux de faible niveau de confinement pour faciliter la détection rapide d'une incursion locale du virus de la FVR. Tant le test de détection du virus que le test de détection des anticorps peuvent être déployés sur le terrain, dans des systèmes de laboratoire mobiles. De plus, le CNMAE peut maintenant procéder au séquençage du génome complet du virus, un outil important pour déterminer la source ou l'origine du virus, surtout en cas d'introduction délibérée au Canada.

En résumé, ce projet a permis de préparer des laboratoires à l'éventualité d'une incursion de FVR au Canada et d'assurer la gestion à long terme des conséquences qui pourraient en découler.

Le projet a également contribué à l'homologation provisoire du vaccin contre la FVR par la section des Produits biologiques vétérinaires de l'ACIA pour le programme gouvernemental de vaccination, à titre de contre-mesure pour limiter une éclosion éventuelle de FVR.

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Acknowledgement

We would like to thank Dr. Soren Alexandersen for his support as the champion for the project.

We would like to thank the Animal Care Unit staff at the Canadian Science Centre for Human and Animal Health for the additional effort they had put into this project over several years.

We would like to thank the advisory committee for the project: Dr. Robert McLean, John Pasick, Stuart Nichol, and Mike Turell, for their invaluable advice and also direct input (dr. Turell).

We would like to thank Dr. Myrna Miller and Dr. Barbara Drolet for significant collaborative contribution to the experimental component of the project.

1. Introduction

Rift Valley fever (RVF) is a vector-borne, noncontagious disease of wild and domestic ruminants and a zoonotic disease of humans caused by RVF virus (RVFV). Disease is typically mild or undetected in adult ruminants, however with high viremia permitting transmission back to mosquitoes. The virus causes abortion storms in pregnant ruminants and high fatality rates in infected neonates. In humans, disease may be undetected or exhibited by mild fever and flu-like symptoms; however ocular disease, meningoencephalitis, and hemorrhagic fever syndromes are also possible with case fatality rates ranging from 1% in endemic areas to 25% in naïve populations [1,2]. Humans are amplifying host, and can also transmit RVFV back to mosquitoes. This is different from West Nile virus, where amplifying hosts are only some fo the avian species. Transmission between ruminants is by mosquitoes; human infections can be acquired by mosquito bites or by direct contact with tissues and fluids from infected animals [3]. Aerosol transmission was documented for laboratory exposures, and about 3% of human cases in the 2010 outbreak in South Africa were suspected to be due to drinking of contaminated milk. RVFV it is considered a significant biological warfare and bioterrorist agent, and a severe natural emerging disease threat to Canada.
RVFV has been classified as a Category A High Priority pathogen by the National Institute for Allergy and Infectious Diseases, and is dual listed as a U.S. Department of Agriculture (USDA) select agent due to the high risk to the livestock industry [4], it is also on the OIE (World Animal Health Organization) List A with implication for international trade.

Although to the date, RVFV is endemic to Africa and since 2000 also to Arabian Peninsula [5,6], many mosquito species are competent vectors of the virus, including species present in North America [7,8], and stable and house flies are potential mechanical vectors [9]. The range of competent vectors and susceptible ruminant hosts in Canada would contribute to the rapid spread of the virus if it were introduced, and result in a significant threat to the livestock industry and public health [4,10, 11].

1.1 Issue

In 2006, Canada was essentially unprepared to timely detect incursion of the RVFV, and to control the outbreak in ruminants.

- Neither CFIA nor PHAC had validated diagnostic tests for Rift Valley fever that could be safely distributed to regional diagnostic laboratories
- CFIA did not have the capacity and laboratory tools to handle diagnostic surge should an outbreak occur (the current expectations are that a laboratory is able to handle close to 1000 samples a day including receiving and record keeping).
- Sequence analysis of the circulating virus to be used in epidemiological investigations, tracing the source of RVFV outbreak, both in case of natural occurrence or CBRNE event was not available.
- There were no commercial human and veterinary vaccines were available
- There was no knowledge on competency of Canadian mosquitoes to transmit RVFV.

1.2 Purpose of the work

To prepare for incursion of RVFV into Canada in order to limit the consequences on human and animal health, to mitigate the economic impact on livestock industry and the international trade, and in case of intentional introduction, to be able to trace the origin of the virus.

The development of recombinant technologies based reagents and rapid detection tests, allowing production and use of diagnostic reagents outside biocontainment, will provide first responders and front-line personnel with capability to identify, quickly respond and to contain a CBRNE event, using field deployable and operator safe diagnostic tests. Development of high throughput screening tests for livestock is critical to control an outbreak and minimize the serious economic and public health consequences, and significant impact on the international trade of animals and animal products. The ability to detect the virus rapidly, safely and locally will improve protection of first responders. As part of the high containment animal work, personal decontamination procedures and responder P.E. will be addressed. Establishment of high throughput veterinary tests and creation of new knowledge in the area of pathogenesis and immune response – critical also for vaccine development, will assist with outbreak control and subsequent longer- term consequence management. To start to generate knowledge on RVFV competency and disctribution of mosquitoes, important for risk assessment and control.

Availability of high throughput, rapid diagnostic tests along with sequence analysis of the circulating virus will be used in epidemiological investigations, tracing the source of RVFV outbreak, both in case of natural occurrence or CBRNE event.

1.3 Scope and limits

The scope of the project was ro develop diagnostic preparedness for RVFV in Canada: Specifically, to develop molecular technology based tests and reagents for production and use outside containment applicable also to a field setting, as well as high throughput assays for veterinary diagnostics.

Existing technologies, such as real-time RT-PCR, recombinant protein technology, hybridoma development, ELISAs and immunohistochemistry were to be developed or modified for RVFV.
The scope of the project also included experimental inoculation with RVFV of the three most important livestock ruminant species: cattle, sheep and goat to be conducted in high containment animal facility at NCFAD. The purpose of the animal work was to develop positive control samples and reagents (antiserum), to develop sampling strategy, and a viremia model suitable for vaccine efficacy testing. At the same time basic studies to undertand immune esponse in ruminants were performed. It was originally proposed to consider also wildlife ruminants to determine susceptibility of North merican species to RVFV, but the limits posed by the logistics of the experimental work in high containment with wildlife ruminants, e.g. deer placed this outside the scope of the project.

Selected Canadian mosquito species were evaluated as to their potential to be a RVFV researvoir, and to validate RT-PCR for surveillance.

RVFV has only one serogroup, consequently evaluation fo multiple virus strains for serological tests was not necessary. The virus is also highly conserved acorss all isolates, making it possible to develop generic RT-PCR assays.

1.4. Organization of the work

The high containment live virus work and animal experiments were performed at NCFAD (Dr. Weingartl), with participation of other team members (Dr. Wilosn, Dr. Miller, Dr. Czub), who would travel to NCFAD.

NCFAD, CFIA also evaluated the assays developed in course of the project using samples generated in the animal experiments, including the tri-plex real time RT-PCR and immunohistochemistry developed by ABADRU, USDA (Dr. Wilson).

Recombinant proteins were developed at NCFAD and NML (Dr. Stroeher), two sets of hybridome using recombinant safety tested N and NSs proteins were developed at the University of Calgary (Dr. Czub).

NML, PHAC (Dr. Lindsay) lead the work on vector competency.

The team met once a year together with the advisory committee and the champion for the project to discuss progress and future work.

2. Results/Deliverables

2.1. Approach taken

Rift Valley Fever virus strain ZH501 was kindly transferred to CFIA from NML, PHAC (Dr. Heinz Feldmann and Dr. Ute Stroeher), in addition Dr. Stroeher isolated RVFV from field samples obtained during the 2007 outbreak in Kenya in the NCFAD zoonotic Ag3 laboratory to facilitate the use of this virus in the experimental inoculations of ruminants.

Genomic RNA of the virus was used to develop positive control plasmid for real-time RT-PCR, and the assay based on Bird et al. [12] was modified for veterinary high throughput diagnostic testing by applying one tube protocol, introducing exogeneous internal control to confirm that there are no inhibitors in the samples, and that the RNA was isolated. The assay was later transferred to Dr. Lindsay (NML) to be evaluated for mosquito samples. Initial development and evaluation of the real time RT-PCR was done on *in vitro* generated samples, and later on samples collected during experimental animal infections.

Genomic RNA was also used for development of recombinant proteins representing all RVFV coded proteins (Dr. Stroeher, Dr. Weingartl, Dr. Wilson) except for the large L (polymerase) protein. The nucleoprotein N and the non-structural protein NSs were produced on a larger scale, used for ELISA development, and also transferred to University of Calgary (Dr. Czub) for development of hybridomas producing monoclonal antibodies against the two proteins. The anti-N antibodies were intended for immunohistochemistry, and the NSs antibodies potentially for competitive ELISA. Indirect ELISAs using the N protein, or the NSs protein, the NSm (non-structural) protein, or the glycoprotein Gn were developed, and evaluated on negative serum samples from the NCFAD archives, and on samples generated during experimental animal infections. Microtiter and classical plaque reduction neutralizaiton assays were established as confirmatory tests for detection of antibodies.

ABADRU (Dr. Drolet, Dr. Wilson) developed immunohistochemistry approach for RVFV in fixed animal tissues, and in mosquitoes using polyclonal anti-N antibodies, and later the anti-N monoclonal antibodies developed at ABADRU. The test was transferred to NCFAD, and evaluated on the tissues collected during experimental animal infections.

Concurrently with the *in vitro* work, experimental animal infections in calves, goats and sheep were conducted at NCFAD. Two age groups were used for the inoculations, very young ones (one-to three weeks old) to generate high titers postitive tissue samples, and vaccine age goats and sheep (3 – 4 months old) to develop viremia model (detection fo virus and antibodies in serum) potentially suitable for vaccine efficacy testing, beside the development of positive samples. The *in vivo* studies also developed and tested the

PPE for use with large animals in field should it be required during outbreak control (**Figure 5**).

In the same time frame, Dr. Linday conducted the vector competency studies on wild captured mosquitoes. (Attempts were also made to establish a colony of Culex mosquitoes at NCFAD, but the main studies were done using wild cupture mosquitoes). The mosquitoes were fed on experimentally infected hamsters, and analyzed for RVFV distribution in their organs by real-time RT-PCR.

2.2 Results

2.2.1 Virus detection

SP-NML/SPU-NCFAD isolated a new isolate from human serum collected during the 2007 outbreak of Rift Valley fever in Kenya, and used it for some of the animal inoculations. **Classical virus isolation and virus plaque isolations** were established at SPU/NCFAD.

Two **real-time RT-PCR** approaches were developed and evaluated. The first one targets the L gene of the RVFV, and uses enterovirus RNA as an exogeneous internal control (necessary for samples where host RNA is rare – such as serum, and "house-keeping" genes/mRNA cannot be used). Second approach targets all three segments of the RVFV, and can be used as a confirmatory test, as well as DIVA. Both approaches were published:

> Drolet BS, Weingartl HM, Jiang J, Neufeld J, Marszal P, Lindsay R, Miller MM, Czub M, Wilson WC (2012) Development and evaluation of one-step rRT-PCR and immunohistochemical methods for detection of Rift Valley fever virus in biosafety level 2 diagnostic laboratories. J Virol Methods 179:373-382
>
> Wilson WC, Romito M, Jasperson DC, Weingartl H, Binepal YS, Maluleke MR, Wallace DB, van Vuren PJ, Paweska JT (2013) Development of a Rift Valley fever real-time RT-PCR assay that can detect all three genome segments. J Virol Methods, 193:426-431

In addition, the first real-time RT-PCR was modified for use is mosquitoes.

> Iranpour M, Turell MJ, Lindsay LR (2011) Potential for Canadian mosquitoes to transmit Rift Valley fever virus. Journal of the American Mosquito Control Association, 27: 363-369.

Immunohistochemistry for detection of viral antigen in formalin fixed ruminant tissues was developed employing polyclonal serum against RVFV N protein, and later monoclonal antibody against the N protein as primary detection antibody. This approach is suitable for low containment laboratories, and still widely used in veterinary diagnostics. It can also be potentially used for archived tissue samples, and for mosquito samples. (Drolet et al., 2012) **Figure 1.** and **Figure 2.**

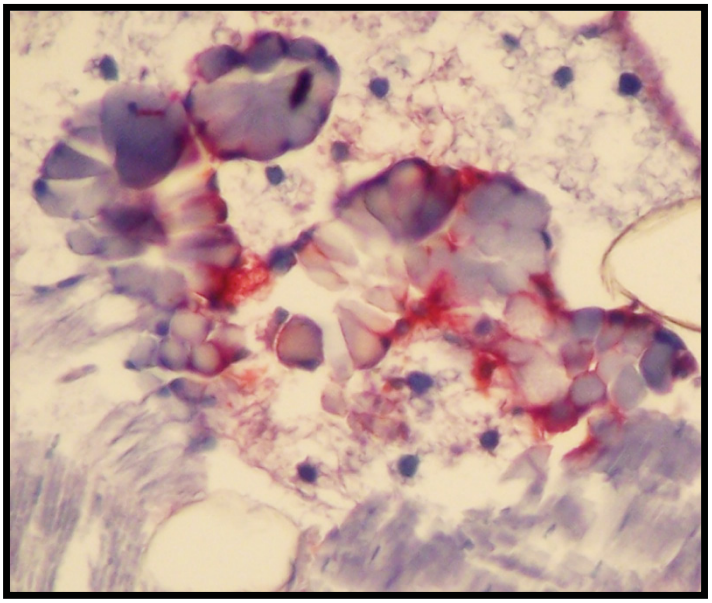

Figure 1. Immunohistochemical detection of wild type Rift Valley fever virus in mosquito salivary glands.

Full genomic sequencing, applicable in tracing outbreak origins was developed in a course of the outbreak and verified using the RVFV ZH501 isolate. **Immune electron miscroscopy** was also developed as an approach in principle, assisting with identification of the virus, if required. **Figure 3.**

Weingartl HM, Zhang S, Marszal P, McGreevy A, Burton L, Wilson WC (2014) Rift Valley fever virus incorporates the 78 kDa glycoprotein into virions matured in mosquito C6/36 cells. Plos One 9:e87385.doi:10.137/journal.pone.0087385

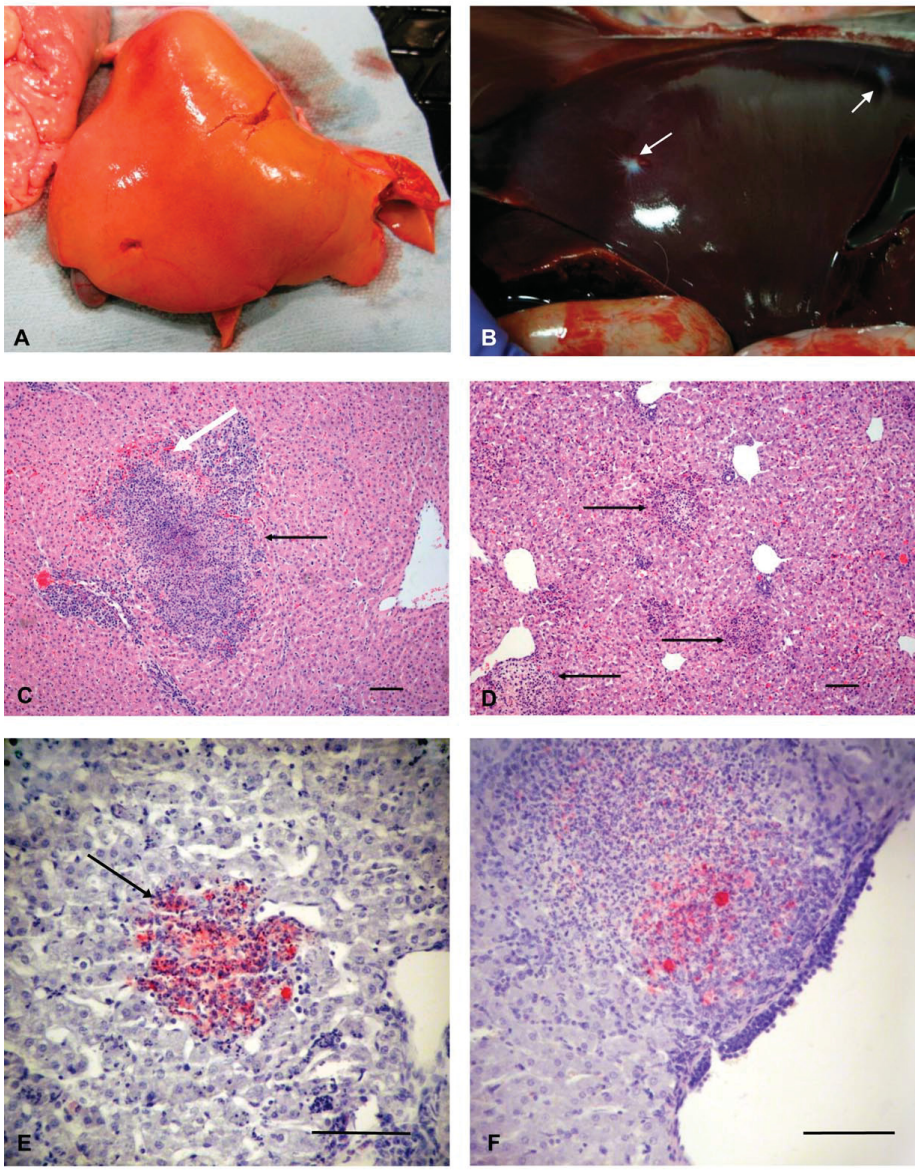

Figure 2. Liver samples from sheep experimentally infected with RVFV. Fig. **A**: Necrotic liver of two-weeks old lamb presenting severe hepatitis, while liver in older animals were essentially free from gross lesions (**B**). Typical microscopic focal lesions in liver of a lamb (**C**) and of a calf (**D**) infected with RVFV. Detection of RVFV N antigen by immunohistochemistry in liver of a lamb (**E**) and a calf (**F**), corresponding with the histological lesions.

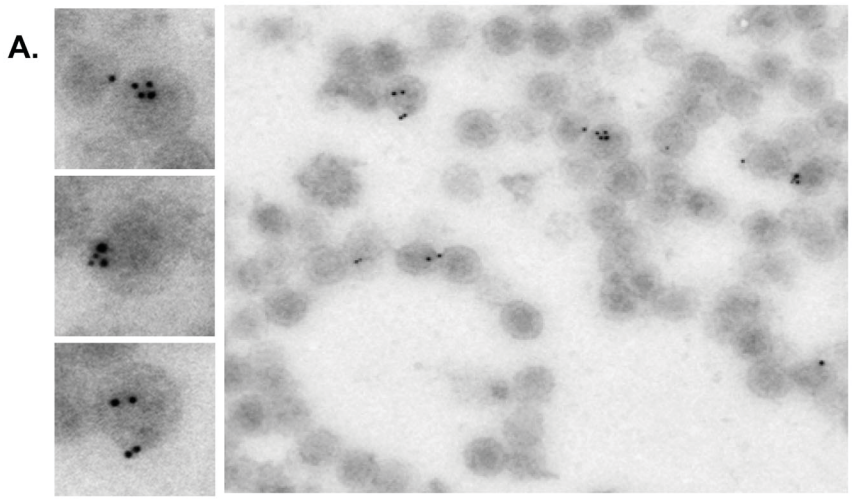

Figure 3. Immune-electron microscopy (IEM) of Rift Valley fever virus virions prepared in mosquito cells C6/36. The immuno-gold particles indicate presence of the large 78kDa glycoprotein in the virions. This protein is unique to RVFV virions from mosquitoes, and the staining could be used for their identification. Staining with monoclonal antibodies against nucleoprotein N, would identify all types of RVFV virions.

All viral protein, except for the large (polymerase coding) L protein encoded by the L genomic segment were expressed in bacterial or baculovirus expression systems. Those were used as a controls to verify which viral proteins can be detected by sera developed in experimentally infected animals, and subsequently used in ELISA developement. Goat serum detected all tested viral proteins, and can be used as a reagent for detection of the virus/viral proteins in cell (and possible tissue) lysates infected with RVFV. Series of **monoclonal antibodies** was developed against individual **recombinant viral proteins**, and can be used in a similar way. (Weingartl et al., 2014; Faburay et al., 2013; **Figure 4**).

Faburay B, Wilson W, McVey DS, Drolet BS, Weingartl H, Madden D, Young A, Ma W, Richt JA (2013) Rift Valley fever virus structural and non-structural proteins: recombinant protein expression and immunoreactivity against antisera from sheep. Vector Borne Zoonotic Dis, 13: 619 - 629

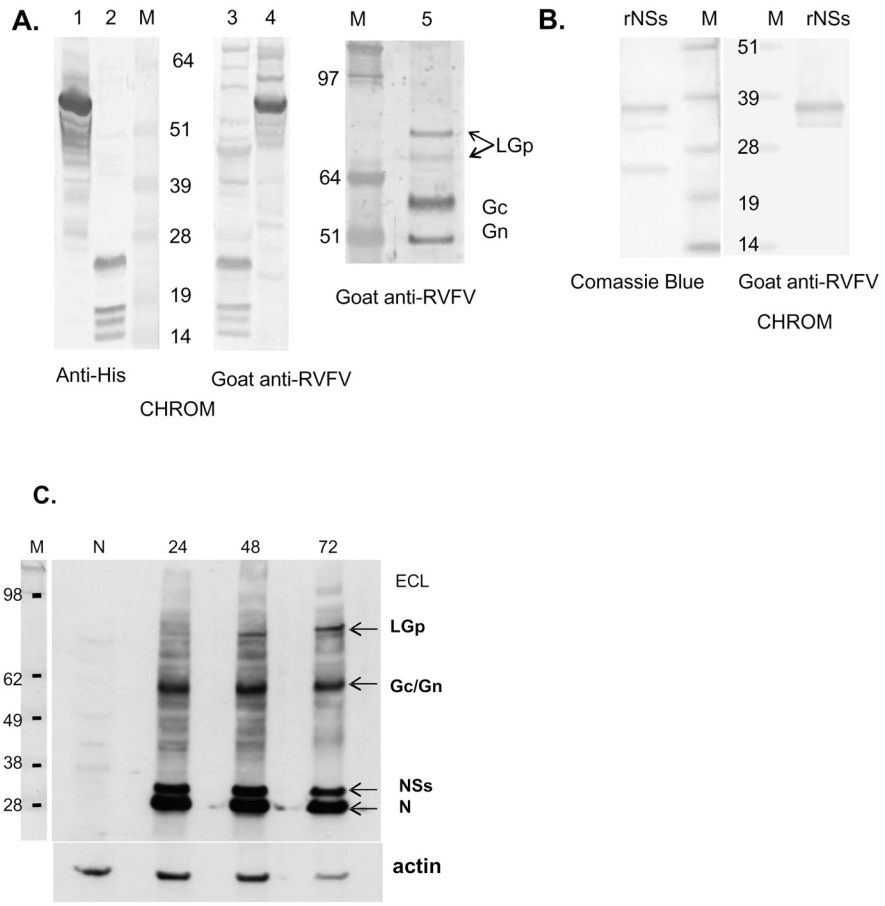

Figure 4. Example of recombinant RVFV proteins and their detection (A, B). Panel **A**: Recombinant Gn protein (strong band between 51 and 64 kDa molecular markers) – lane 1 and 4, and NSm protein (main band between the 19 and 28 kDa molecular markers) – lane 2 and 3 were tagged with six histidines, and detected with anti-His antibodies or with polyclonal goat RVFV antiserum. Confirmation that goat antiserum recognizes both Gn and Gc glycoproteins, as well as large LGp 78 kDa protein, and the NSm protein as a part of the LGp, is illustrated in lane 5.
Panel **B**: Recombinant nonstructural protein NSs as recognized by the goat anti-RVFV serum – lane rNSs far right. Panel **C** illustrates reactivity of goat antiserum with all RVFV proteins important in virus detection or as ELISA antigens. Although the sera was developed as a part of experimental infections of animals, it can be used as a reagent, e.g. in detection of viral proteins.

2.2.2 Antibody detection

Plaque reduction neutralization assay (PRNT) on Vero E6 cells was established as a first step and as a standard for evaluation of ELISAs.

Following recombinant antigens (developed as described above) were evaluated for use in indirect ELISAs using negative field sera and RVFV positive experimental sera:

N nucleoprotein. This protein is a structural protein highly expressed in infected cells. It is also highly immunogenic, however does not induce neutralizing antibodies and can be omitted from recombinant vaccine candidates. It would be suitable for DIVA testing with caution. We have detected higher than desired background in the negative field sheep samples, and were able to deterrmine that antibodies in sheep serum against at least one virus present in Canada can crossreact with RVFV N protein. (Collaboration with Dr. M. Drebot, NML, PHAC detected crossreactivity with Cache Valley virus in abortion cases in sheep, due to PHAC interest in potential exposure of humans.)

NSs protein. This non-structural protein is expressed early post infection of the cells. Antibodies against this are not neutralizing and the protein is omitted from at least one virus vaccine candidate, Detection of antibodies was therefore considered for DIVA development. On our evaluation, it was determined that the onset of development of antibodies in infected animals is much later than for other proteins, and data by others also suggest that the antibodies may be only of a short duration, and high percentage of infected animals would test negative on this test.

Gn protein is ahighly immunogenic structural protein, with neutralizing antibodies developed realatively early post infection. This protein is always a component of vaccine candidates, and on our evaluation serum antibodies against it are well suited to be used for detection of infected animals. Indirect ELISA employing this protein will be the test of choice in an outbreak, as on our evaluation we also did not detect crossreactivity with other viruses or high non-specific background in negative samples.

All the **indirect recombinant ELISA** tests were evaluated on minimum of 300 negative field samples (sheep and cattle; goats on 100 as the sera are not readily available) and on serial bleed sera from 20 calves, 28 goats and 32 sheep, and compared to classical infected cell lysate indirect ELISA (CDC Atlanta protocol, Dr. Nichol) and the PRNT.

Tandem of the N-ELISA and the Gn-ELISA can be used as **DIVA -** with infected sheep being positive on both ELISAs, vaccinated sheep being positive on the Gn ELISA only, and reactors on the N-ELISA only. Those would be considered as false positive, and re-tested by viral neutralization assay (PRNT).

Wilson WC, Weingartl HM, Drolet BS, Dave K, Harpster MH, Johnson PA, Faburay B, Ruder MG, Richt JA, McVey DS (2013) Diagnosatic approaches for Rift Valley fever. Dev Biol (Basel) 135:73 -78

2.2.3. Serum samples development leading to viremia challenge model

In course of development of positive control samples for evaluation fo the above tests, the infection experiments were designed to at the same time develop a viremia model suitable for vaccine efficacy testing, to replace or supplement the only current vaccine efficacy model in ruminants at that time: the abortion model. Model for goats and for sheep were developed, and the later one applied already in vaccine efficacy testing of two vaccine candidates:

modified live virus vaccine MP12, prepared by Zoetis, and now licenced in Canada for government regulated vaccination program by the Veterinary Biologics, CFIA

modified live virus vaccine MP12 with deleted NSm protein expression, prepared by UTMB Texas. This vaccine is now considered for use in Tanzania.

In summary experimental inoculation with RVFV were performed using 20 calves, 32 sheep and 28 goats.

Nfon CK, Marszal P, Zhang S, Weingartl HM (2012) Innate immune reponse to Rift Valley fever virus in goats. PLoS Negl Trop Dis 6:e1623. doi:10.1371/journal.pntd.0001623

Weingartl HM, Miller M, Nfon C, Wilson WC (2014) Development of a Rift Valley fever virus viremia challenge model in sheep and goats. Vaccine 32: 2337 – 2344

Weingartl HM, Nfon CK, Zhang S, Marszal P, Wilson WC, Morill JC, Bettinger GE, Peters CJ (2014) Efficacy of a recombinant Rift Valley fever virus MP-12 with NSm deletion as a vaccine candidate in sheep. Vaccine 32: 2345 – 2349

Efficacy, potential for vector transmission and duration of immunity testing of MP-12, an attenuated Rift Valley fever virus vaccine candidate, in sheep. Myrna M. Miller, Kristine E. Bennet, Barbara S. Drolet, Robbin Lindsay , James O.

Mecham, Will K. Reeves, Hana Weingartl, William C. Wilson. Submitted to Vaccine.

2.2.4. Vector competency studies

Dr. Lindsay lead the work focusing on capability of Canadian species of mosquitoes to replicate and transmit RVFV. In a course of study a Culex mosquito colony was established at NML for future studies if needed, a hamster transmission model, real tiem RT-PCR in mosquitos for surveillance purposes. Dr. Drolet developed an immunohistochemistry for mosquito samples. Currently three species of Canadian mosquitoes were identified and competent vectors for RVFV: *Culex tarsalis, Aedes sticticus and Coquillettidia perturbans*. As only limited number of species were tested, this indicates that in Canada the range of mosquito species capable to replicate and transmit RVFV is likely quite large.

Iranpour M, Turell MJ, Lindsay LR (2011) Potential for Canadian mosquitoes to transmit Rift Valley fever virus. J Amer mosquito Contr Assoc 27: 363-369

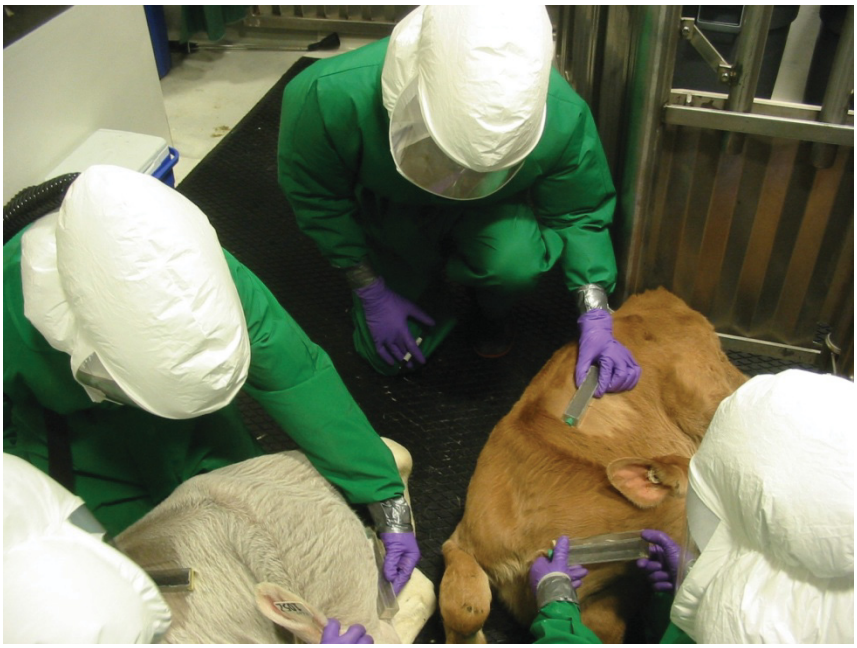

Figure 5. The illustration of vector competency studies also illustrates the personal protective equipment which can be used in the field: Rubber boots, fisherman coveralls, double gloves taped to the coverall over a full Tyvek suit with full Tyvek hood and HEPA filtered battery operated respirator.

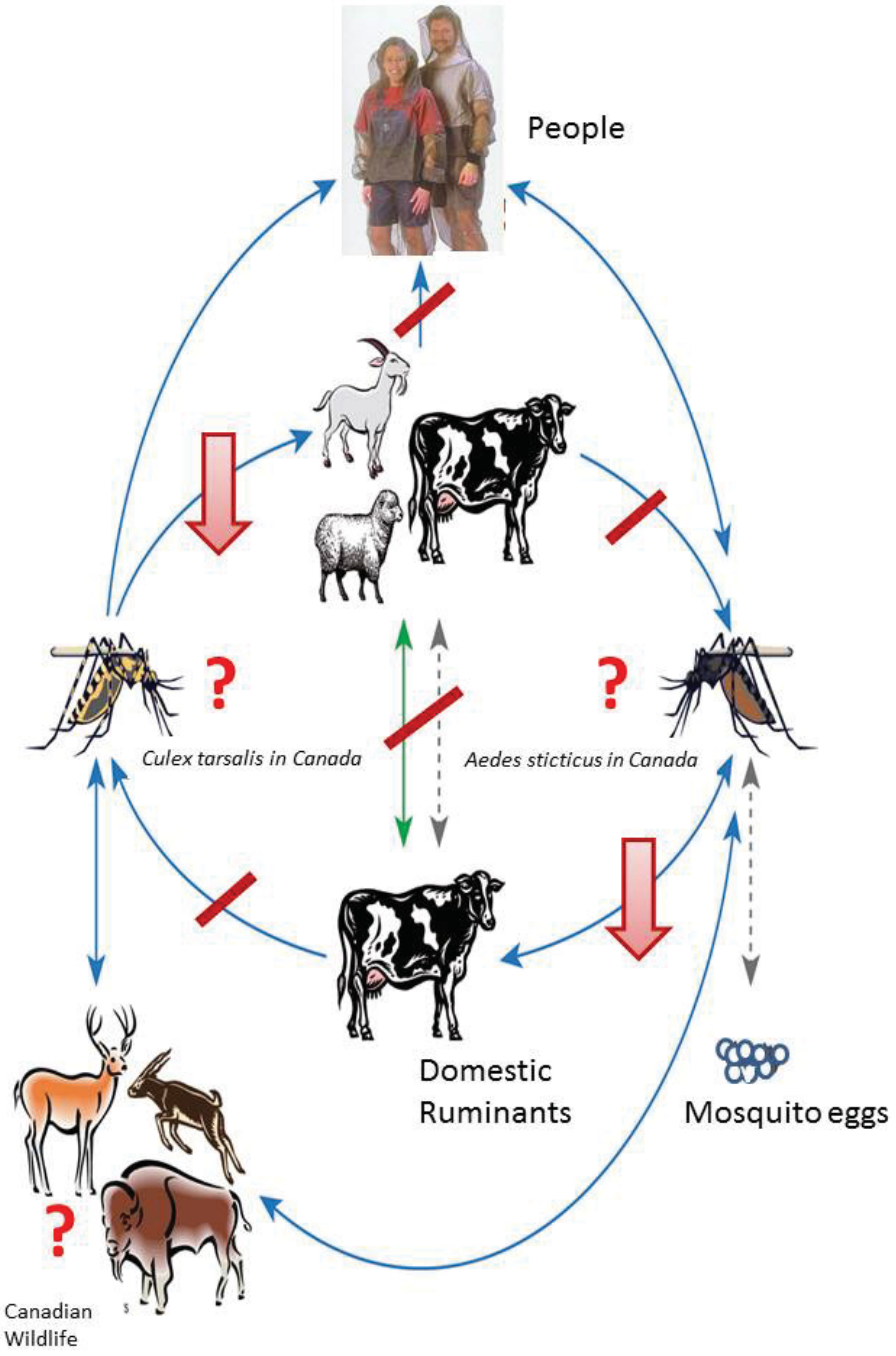

Figure 6. Overview or the transmission cycle of Rift Valley fever virus (adapted from Institut Pasteur for Canadian situation) with paths in the cycle interrupted or reduced by vaccination of livestock (red lines). The question marks indicate unknown susceptibility of Canadian wild life and mosquito species to RVFV.

3. Outcome and conclusions

The project developed veterinary diagnostic capability for Rift Valley fever virus for use in regional, low containment laboratories, transferrable also to field. This would assist rapid detection of the introduction of RVFV to Canada, as well as outbreak control. In addition in case of an intentional introduction of RVFV, Canada would be able to trace the origin and likely laso source of the virus.

Obtained knowledge on competency of Canadian species of mosquitoes will assist in risk assessment and application of countermeasures, such as mosquito control where possible.

Additional significant outcome of the project is also a provisional licence for RVFV vaccine in livestock in government regulated program (such as outbreak control) in Canada and USA (the USDA Center for Biologics has issued a 2-year conditional licence for this vaccine based on the reasonable expectation of safety and efficacy.

Figure 6 provides an overview of the RVFV transmission cycle with paths which would be now either interrupted or reduced. It has to be understood, that in case of introduction of RVFV into North American continent, the virus will very likely become endemic. Current tools developed under this project will however limit and slow down the spread of the virus, providing time for protection of human population and livestock. In combination with use of personal protective equipment, such as mosquito-net clothing and repellents, the impact on human health would be significantly decreased.

4. Publications:[1]

1. Iranpour M, Turell MJ, Lindsay LR (2011) Potential for Canadian mosquitoes to transmit Rift Valley fever virus. Journal of the American Mosquito Control Association, 27: 363-369.

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4. Wilson WC, Romito M, Jasperson DC, Weingartl H, Binepal YS, Maluleke MR, Wallace DB, van Vuren PJ, Paweska JT (2013) Development of a Rift Valley fever real-time RT-PCR assay that can detect all three genome segments. J Virol Methods, 193:426-431

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6. Weingartl HM, Zhang S, Marszal P, McGreevy A, Burton L, Wilson WC (2014) Rift Valley fever virus incorporates the 78 kDa glycoprotein into virions matured in mosquito C6/36 cells. Plos One 9:e87385.doi:10.137/journal.pone.0087385

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10. Efficacy, potential for vector transmission and duration of immunity testing of MP-12, an attenuated Rift Valley fever virus vaccine candidate, in sheep. Miller MM , Bennet KE, Drolet BS, Lindsay R , Mecham JO, Reeves WK, Weingartl H, Wilson WC. Submitted to Vaccine.

[1] Many of the publications are open source and can be found on the web- for those requiring subscription access please consult your librarian or CSS at: css-info@drdc-rddc.gc.ca

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