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Meeting Review: 6th International Conference on Emerging Zoonoses

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Impacts

- Summaries provide reviews of specific zoonoses and threats to human and animal health.
- Genomic analysis and systems and computational biology are increasingly important research tools whatever emerging disease is being investigated.
- The continuum of basic research leading to understanding a disease and then to managing that disease and finally to preventing it offers a pattern of scientific discovery that is relevant to many emerging zoonotic diseases.

Key Words:
Zoonoses; bacteria; prions; influenza viruses; pathogens; wildlife

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Summary

The 6th International Conference on Emerging Zoonoses, held at Cancun, Mexico, February 24-27, 2011, offered 84 participants from 18 countries a snapshot of current research in numerous zoonoses caused by viruses, bacteria or prions. Co-chaired by Professors Heinz Feldmann and Jürgen Richt, the conference explored 10 topics: (1) The ecology of emerging zoonotic diseases; (2) The role of wildlife in emerging zoonoses; (3) Cross-species transmission of zoonotic pathogens;(4) Emerging and neglected influenza viruses; (5) Hemorrhagic fever viruses; (6) Emerging bacterial diseases; (7) Outbreak responses to zoonotic diseases; (8) Food-borne zoonotic diseases; (9) Prion diseases; and (10) Modeling and prediction of emergence of zoonoses. Human medicine, veterinary medicine and environmental challenges are viewed as a unity, which must be considered under the umbrella of 'One Health'. Several presentations attempted to integrate the insights gained from field data with mathematical models in the search for effective control measures of specific zoonoses. The overriding objective of the research presentations was to create, improve and use the tools essential to address the risk of contagions in a globalized society. In seeking to fulfil this objective, a three-step approach has often been applied: (1) use cultured cells, model and natural animal hosts and human clinical models to study infection; (2) combine traditional histopathological and biochemical approaches with functional

genomics, proteomics and computational biology; and (3) obtain signatures of virulence and insights into mechanisms of host defense response, immune evasion and pathogenesis. This meeting review summarizes 39 of the conference presentations and mentions briefly the 16 articles in this Special Supplement, most of which were presented at the conference in earlier versions. The full affiliations of all presenters and many colleagues have been included to facilitate further inquiries from readers.

1. The Ecology of Emerging Zoonotic Diseases

As new zoonotic diseases continually emerge, one learns to expect the unexpected. The emerging zoonotic disease situations considered during the opening day of the Cancun Conference were the West Nile Virus, Lyme disease, interactions among multiple tick-borne pathogens, Hantaviruses in the context of rodent dynamics, the Usutu virus (a mosquito-borne flavivirus) and a novel astrovirus enterotoxin. Furthermore, the importance of systems and computational biology was stressed, whatever emerging disease was being investigated (Poland et al., 2011). In addition to the summaries below of six presentations on this topic, this Special Supplement includes an article, **Monitoring of West Nile Virus Infections in Germany** by Dr. U. Ziegler et al. which identified West Nile Virus (WNV) antibodies in migratory birds, but not in resident birds, domestic poultry or in local horse populations throughout Germany. The WNV antibody-positive species were found in birds that migrate to tropical Africa or southern Europe; however, WNV-specific RNA could not be found in any of the samples.

The Conference opened with a presentation from Professor M. A. Diuk-Wasser and her colleagues J. Simpson and C. M. Fosom-O'Keefe (all Yale School of Public Health, New Haven, CT, USA) and G. Molei, P. M. Armstrong, and T. G. Andreadis (Center for Vector Biology and Zoonotic Species at the Connecticut Agricultural Experiment Station, New Haven, CT, USA), **Ecology of West Nile Virus in the Northeastern United States.** Professor Diuk-Wasser began by noting that West Nile Virus (WNV) was introduced into New York City in 1999 by unknown means and was now considered endemic throughout the USA, with 29,700 human cases and 1,180 deaths in the USA since 1999. It had been hypothesized that increased biodiversity leads to a decreased risk of exposure to zoonotic pathogens (Keesing, Holt and Ostfeld, 2006). At issue is whether this 'dilution effect' or 'Zooprophylaxis' for vector-borne pathogens applies only when vectors are generalist feeders, because the link between host diversity and pathogen transmission might break down when vectors exhibit host preferences.

In the northeastern United States, WNV perpetuates in an enzootic transmission cycle involving *Culex spp.* mosquitoes and virus-competent avian hosts. Previous studies had detected that a large proportion of *C. pipiens* and *C. restuans* bloodmeals were derived from American robins (*Turdus migratorius*), suggesting a key role for this bird species in the WNV transmission cycle (Molaei et al, 2006; Kilpatrick et al, 2006). The New Haven-based research team tested for preferential feeding by conducting equal choice experiments (robins vs. other bird species) (Simpson et al., 2009) and by comparing the proportion of *Culex spp.* bloodmeals acquired from robins to the proportion of robins in the local bird community. Both methods indicated preferential feeding for robins. They were also able to identify robin communal roosts as amplification foci in greater New Haven (Diuk-Wasser et al., 2010). Then, through field-informed mathematical modeling, they determined that host preferences were indeed key drivers of WNV transmission, and that landscape attributes (such as urbanization), in combination with mosquito abundance and a measure of host community competence were the strongest predictors of pathogen prevalence (Simpson et al., 2011). Thus it was clear that pathogen prevalence and human risk of infection

were best predicted by assessing the relative pathogen competence and attractiveness to vectors of all species in the host community, rather than using simple measures of biodiversity.

In the next presentation, **Interactions among Multiple Tick-borne Pathogens in a Natural Reservoir Host**, Professor Fish and his colleagues J. Brown, M. Fitzpatrick, S. Usmani-Brown, P. Cislo and P. Krause (Yale School of Public Health, New Haven, CT, USA) stressed that species interactions within a parasite community drive infection risk in a wildlife population (Telfer et al., 2010). At least five tick-borne pathogens are known to be transmitted by *Ixodes scapularis*, the principal vector of Lyme disease in the United States: (1) *Borrelia burgdorferi*, an agent of Lyme disease; (2) *Anaplasma phagocytophilum*, an agent of human anaplasmosis; (3) *Babesia microti*, an agent of human babesiosis; (4) *Borrelia miyamotoi*, an agent of relapsing fever; and (5) the Powassan encephalitis virus. Two or more of these pathogens can be transmitted either simultaneously by a single tick or sequentially by successive tick-bites, resulting in 240 different permutations of mixed-infection studies. In the context of pathogen prevalence of *Ixodes scapularis* nymphs, *Borrelia burgdorferi* has been found in 19.8 percent of samples from the Northeast and Midwestern United States, while *Babesia miroti* has been found in 14.7 percent of samples from Block Island, Rhode Island.

Professor Fish explained that several types of co-infections have been explored in an experimental system employing laboratory colonies of *I. scapularis* ticks and *Peromyscus leucopus* white-footed mice, a natural reservoir host for these pathogens. Outcomes of mixed infections in mice have been measured by R_o, the fitness parameter and basic reproductive rate which indicates the number of secondary tick infections resulting from a primary infection (Levin and Fish, 2004). The observed outcomes of dual mixed infections have been variable with both positive and negative effects on R_o, while interactions have been mutual, unidirectional or null. These diverse pathogen interactions play an important role in determining the infection prevalence of host-seeking nymphs in nature, and consequently, in the risk of infection for humans.

Professor H. Henttonen (Finnish Forest Research Institute, Vantaa, Finland) and his team H. Leirs, E. R. Kallio, K. Tersago and L. Voutilainen in collaboration with University of Antwerp, Belgium; University of Liverpool, United Kingdom; and the Universities of Helsinki and Jyväskylä, Finland, studied **Biome Specific Rodent Dynamics and Hanta Epidemiologies in Europe**. Their research sought to understand the main biomes and forest coverage in Europe, the European hanta viruses and their carriers, and the biome specific dynamics of hanta virus carriers and the biome specific transmission dynamics and epidemiologies.

Within the *Bunyaviridae* family of viruses, hantaviruses infect rodents (and insectivores) and cause haemorrhagic fever with renal syndrome (HFRS) in humans in the Old World and hantavirus cardiopulmonary syndrome (HCPS) in the New World. In a large European Union project, EDEN (Emerging Diseases in a Changing European Environment, 2011), rodent-borne (robo) viral infections have been studied, along with tick-borne pathogens, leishmaniasis, West Nile Virus, malaria and Rift Valley Fever. The most important aim of Professor Henttonen and his colleagues was to clarify the differences in boreal (Northern) and temperate Europe in the human epidemiology of *nephropathia epidemica*, by far the most common hantaviral disease in Europe, caused by Puumala hantavirus (PUUV). The population dynamics of the host species, the bank vole, differ greatly in various parts of Europe, driven by predation in the north and masting events in the temperate zone. Consequently, the causes of rodent fluctuations are different. In addition, the role of landscape patterns (homogenous Taiga vs.

fragmented temperate forests) in rodent/virus dispersal is significant, as well as local environmental conditions (e.g. temperature and moisture), which affect virus survival outside the host. For example, in room temperature PUUV remains infectious for at least two weeks outside the host, and possibly for much longer in cold temperatures and in moist conditions. These research findings are essential for human risk evaluation with regard to both long-term and seasonal occurrence of PUUV in the environment. In spite of chronic infection of bank voles and the excretion of PUUV in their faeces, urine and saliva, the shedding period is limited, which has significant implications for seasonal transmission dynamics in rodents. Thus within the same host/virus system, biome specific PUUV epidemiologies occur (Kallio et al., 2009. Tersago et al., 2009), thereby highlighting the need for geographically comparative studies in Europe (METLA, 2012).

Professor V. Sambri and his team, P. Gaibani, F. Cavrini, A. M. Pierro, M. P. Landini and G. Rossini (all Regional Centre for Microbiological Emergencies [CRREM], Unit of Clinical Microbiology, St Orsola-Malpighi University Hospital, Bologna, Italy) investigated **Usutu: A Novel Human Pathogenic Mosquito-borne Flavivirus**. This virus belongs to the Japanese encephalitis serogroup within the mosquito-borne cluster of the genus *Flavivirus* in the family *Flaviviridae*. First isolated from mosquitoes of the genus *Culex* in South Africa in 1959, the Usutu virus (USUV) has since been isolated from mosquitoes, rodents and birds throughout Sub-Saharan Africa and Europe. The virus is thought to be maintained in nature in a mosquito-bird transmission cycle in areas with a minimum of at least ten hot days >30^{o}C, but no mammalian reservoir has yet been identified.

Professor Sambri pointed out that it was not until September 2009 that USUV was found in the liver of a patient who underwent an orthotropic liver transplant (Gaibani et al., 2010). Further study of the plasma and genome sequencing analysis confirmed the presence of USUV viremia. Then USUV was detected in the livers of an additional four patients from the same area suffering from acute meningo-encephalitis during 2008/2009. Both serological assay and molecular assay have been used as new tools for the diagnosis of USUV infection. Thus it is now clear that USUV is a new emerging flavivirus pathogenic for humans.

Further studies are required to discover both the geographic distribution of this virus and the mechanisms by which humans acquire the virus. Since this conference presentation there has been increased awareness of the seriousness of USUV (Vázquez et al, 2011).

According to the World Health Organization (WHO) and UNICEF (2009), 1.5 million children under the age of five die from diarrhea annually. Professor S. Schultz-Cherry and her colleagues, A. Burnham and P. Freiden (all Department of Infectious Diseases, St Jude Children's Hospital, Memphis, TN, USA), L. A. Moser (Department of Medical Microbiology and Immunology, University of Wisconsin, Madison, WI, USA) and M. D. Koci (Department of Poultry Science, North Carolina State University, Raleigh, NC, USA) presented the evidence they had gathered on the **Identification of a Novel Astrovirus Enterotoxin: Potential Zoonotic Risk?**

Astroviruses cause infections within the small intestine and are associated with at least 10 percent of all sporadic cases and greater than 25 percent of all hospitalised cases. These rapidly evolving, nonenveloped, single-stranded RNA viruses can be transmitted directly from infected individuals and animals, and indirectly through contaminated food and water. Professor Schultz-Cherry's laboratory was the first to demonstrate that astroviruses induce diarrhea by a novel mechanism: they possess an

enterotoxin that disrupts intestinal epithelial barrier function independent of cellular damage or an inflammatory response (Koci et al., 2003). This occurs within 24 hours post-infection due to reorganization of the tight junction protein occludin and the actin cytoskeleton (Moser et al, 2007).

In essence, within a complex pathogenic process, astroviruses cause diarrhea by increasing intestinal barrier permeability. This is the first evidence showing that a viral coat protein is an enterotoxin. Of great interest, the toxin can act independently of species barriers. Given the increasing isolation of astroviruses from diverse species, there is increasing evidence that toxicogenic astroviruses could be associated with zoonotic disease.

Professor M. G. Katze (Department of Microbiology and Washington National Primate Research Center, University of Washington, Seattle, WA, USA) set out a unifying approach to molecular biology in his presentation, **Systems and Computational biology: Emerging Tools for Exploring Emerging Viruses**. He emphasized that modern day virologists and immunologists *must* do better in their search to understand how a virus kills and how effective vaccines can be developed, especially because traditional virology has yielded surprisingly little information about why some virus strains cause severe diseases while others remain innocuous. He pointed out that the case fatality rate for the 1918 influenza pandemic was about 2.5%, and that particular H1N1 virus may have infected as much as one-third of the world's population. Issues arise not only in understanding a virus, but also in understanding how hosts respond. For example, the 1918 virus infection resulted in very high expression of inflammatory, antiviral and immune cell genes very early in host infection (Kash et al., 2006).

Significant progress in overcoming existing and emerging viruses depends on biologists, mathematicians and computer specialists working together within a systems biology paradigm. Such research begins with either *in vitro* studies of virus replication on cell lines or primary cell cultures, moving to nonhuman primate models of virus infection. Then samples from the experiments are investigated at multiple time points and conditions; and high throughput data is then examined by data processing to prepare systematic evaluations of different host responses. Data integration involving data analysis and modeling of key genes and pathways is then possible, followed by iterative processing of host perturbations and the use of viral mutants to discover specific applications to translational research. Such a systems biology approach requires not only continuing experiments with virus-infected experimental systems but also significant efforts to maintain the hardware and software of an extensive lab computational infrastructure. It is this computing infrastructure, which permits the lab to go quickly from samples to pathway visualization, as the data analysis workflow moves from microarray images to gene expression data to pathway models.

The mission of this ViroLab is to develop steadily over the years to come a virtual laboratory to confront the viruses involved in 14 infectious diseases—influenza, Ebola, Marburg, Hepatitis C, SARS-CoV, vaccinia, Herpes simplex, West Nile, HIV-1, SIV, measles, Lassa, Chikungunya and Dengue Fever. The three key characteristics of this integrated approach to so many infectious diseases are: (1) to use cell culture, primary cells, nonhuman primate and human clinical models to study viral infection; (2) to combine traditional histopathological, virological and biochemical approaches with functional genomics, proteomics and computational biology (Haagmans et al., 2009); and (3) to obtain signatures of virulence and insights into mechanisms of host defense response, viral evasion and pathogenesis (Casadevaill et al., 2011). For example, with the study of all respiratory viral diseases a unifying hypothesis is that highly pathogenic respiratory viruses use both unique and common strategies to remodel the host cell to

enhance virus replication, regulate disease severity and promote virus transmission (Chang et al, 2011).

A highly significant new tool for studying these emerging viruses is Next Generation Sequencing (NGS) which has already 'changed the way we think about scientific approaches in basic, applied and clinical research' to such an extent that 'the potential of NGS is akin to the early days of Polymerase chain reaction (PCR), with one's imagination being the primary limitation to its use' (Peng et al, 2011). Already, a good understanding of the 'timing' and extent of immune (innate) mediated injury after virus infection has been achieved. Furthermore, molecular 'disease' signatures associated with different pathogens in multiple animal species have been described at microRNA, mRNA, protein level, metabolite and lipid levels. Such successful modelling of molecular events has made possible verifiable prediction about key nodes and bottlenecks, enabling the identification of novel host cell drug targets (Diamond et al, 2010). The translational impact of this research, in Professor Katze's view, will be immense, revealing a completely new and expanded host defense repertoire consisting of non-annotated non-coding RNAs.

Despite all of these achievements, four crucial questions remain unanswered: (1) Is systems biology too complicated and too expensive to become the pre-eminent approach in virology and immunology? (2) Are mathematicians and computer scientists up to the challenges? (3) How will new technologies like Next Generation Sequencing impact virus systems biology research, especially in the context of RNA sequencing? and (4) How can new principal investigators best be identified and appointed? (ViroLab, 2012).

2. The Role of Wildlife in Emerging Zoonoses

It has long been recognized that the emergence of any zoonoses is a complex process involving 'ecological interactions at the individual, species, community and global scale' (Childs et al. 2007, p.2). This topic began with a presentation from Professor A. A. Aguirre that focused on the ecological framework in which any zoonotic disease should be considered. The role of bats as an important reservoir host for many dangerous zoonotic pathogens was then considered in some detail (cf. Daniels et al., 2007; Field et al., 2007; Gonzalez et al., 2007; Wang and Eaton, 2007).

Professor A. A. Aguirre (Department of Environmental Science and Policy, George Mason University and Executive Director, Smithsonian-Mason Global Conservation Studies Program, Front Royal, Virginia, USA) presented **Emerging Zoonotic Diseases of Wildlife: Developing Global Capacity for Prediction and Prevention**. He began by explaining that Conservation Medicine and more recently EcoHealth have emphasized the need to bridge disciplines, thereby linking human health, animal health, and ecosystem health under the paradigm that 'health connects all species in the planet' (Aguirre et al., 2002). In his view, the recent convergence of global problems such as climate change, biodiversity loss, habitat fragmentation, globalization, infectious disease emergence and ecological health demands integrative approaches breaching disciplinary boundaries. The International Union for Conservation of Nature (IUCN) maintains a Red List of threatened species—an important initiative in view of the 869 animal extinctions that have already occurred, of which 3.7 percent were caused by disease (Smith, K. F. et al., 2006).

Professor Aguirre noted that the U.S. Agency for International Development (USAID) has been a major leader in the global response to the emergence and spread of Highly Pathogenic Avian Influenza (HPAI). Since mid-2005, it has programmed approximately $500 million to build capacities in more than 50 countries for monitoring

the spread of HPAI among wild bird populations, domestic poultry, and humans, and to mount a rapid and effective containment of the virus when it is found. Recent analyses indicate that these efforts have contributed to significant downturns in reported poultry outbreaks and human infections and a dramatic reduction in the number of countries affected. Furthermore, the USAID Bureau for Global Health, Office of Health, Infectious Disease and Nutrition (GH/HIDN) recently funded two cooperative agreements, PREDICT and RESPOND, under its Avian and Pandemic Influenza and Zoonotic Disease Program to continue and expand this work. The goal of PREDICT is to establish a global early warning system for zoonotic disease emergence that is capable of detecting, tracking, and predicting the emergence of new infectious diseases in high-risk wildlife (e.g. bats, rodents, and non-human primates) that could pose a major threat to human health. The goal of RESPOND is to improve the capacity of countries in high-risk areas to respond to outbreaks of emergent zoonotic diseases that pose a serious threat to human health. The geographic scope of this expanded effort is directed to zoonotic 'hotspots' of wildlife and domestic animal origins (Jones et al., 2008).

PREDICT includes a program of SMART (Strategic, Measurable, Adaptive, Responsive and Targeted) surveillance which focuses on preventing the "spilling over" from wildlife to humans or to halt these diseases rapidly after that spillover by understanding what factors induce emergence and rapidly identifying ways of prevention, control, and mitigation. The overall aim of SMART is to promote an integrated, global approach to emerging zoonoses. This integration requires commitment from a broad coalition of partners and stakeholders including government agencies, universities and non-governmental organizations, collaborating for specific purposes and to generate in the future new international structures able to respond to these emerging zoonoses. With 1.5 billion animals being imported into the United States each year, as well as an extensive international trade in illegal animal exports (Smith K. F. et al., 2009) and some 75 percent of emerging zoonoses worldwide having wildlife origins, Professor Aguirre stressed that EcoHealth has become a necessity, not an optional policy goal.

Dr. G. A. Marsh and his colleague Dr. L.-F. Wang (Australian Animal Health Laboratory [AAHL], Geelong, Victoria, Australia) began their presentation, **Bats: A Mixed Bag of New and Emerging Viruses**, by pointing out that the "old" bat viruses were represented by many zoonotic pathogens, including Rabies virus, Yellow fever virus, St Louis and Japanese encephalitis viruses, and West Nile virus. Now bats have been identified as natural reservoirs for a number of new and emerging viruses—Ebola virus, Marburg virus, Hendra virus and SARS-like coronaviruses. There are some 1,000 different bat species; and they often roost in high density colonies of over one million flying mammals, which have, in a very real sense, been travelling for millions of years, exposing themselves to many pathogens; therefore, the resulting complexity is not surprising. Key research questions include: (1) Why do bats seem to be able to co-exist with a great diversity of viruses without showing disease signs? (2) What triggers the spillover of bat viruses into other animals? (3) Do bats control viral infection differently from other mammals?

Attempts to isolate viruses from bats have generally been unsuccessful. Therefore, in an effort to improve the success rate for virus isolation, Dr. Marsh and his team have recently developed primary cell culture lines from numerous different species of bats (Crameri et al., 2009). The use of these bat cell lines, in combination with improved sampling techniques, has lead to recent isolation of Hendra virus from a number of bat

urine samples collected in several locations across Queensland, Australia, including those associated with human and horse virus spill-over events (Smith, I. et al, 2011). Furthermore, this henipavirus surveillance program has led to the isolation of a number of novel viruses from two different virus families, whose zoonotic potential is not yet known. In an attempt to understand virus/host interactions, as well as to provide insight into the key factors involved in future spill-over events, AAHL has launched a number of international collaborative projects in Southeast Asia and Ghana, West Africa.

C. Kohl and her colleagues M. Sonntag, A. Nitsche and A. Kurth (Robert Koch Institute, Berlin, Germany), B. Harrach (Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary), K. Mühldorfer and G. Wibbelt (Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany) presented **Adenovirus with Bat Origin: Possible Intra-Species Transmission**. They reported that since bats have been reported as reservoir hosts of emerging pathogens, research interest in their inhabiting viruses has significantly increased. A novel bat Adenovirus (bat AdV-2) was isolated by cell-culture screening from organ tissue of free-ranging vespertilionid bats (*Pipistrellus pipistrellus*) in Germany (Sonntag et al., 2009). The phylogenetic analysis of the genome sequence of bat AdV-2 demonstrated a close relationship to canine adenovirus 1 and 2 (CAdV-1 and -2) (Kohl et al., 2012). The very similar genome organization supported the hypothesis of a shared ancient ancestor. Interestingly, both CAdVs are presenting untypical pathological features within the family *Adenoviridae*. These adenoviruses were found to have an unusually broad host range and are causing a rather higher pathogenicity in a variety of carnivore hosts. The untypical pathological features might be understood as signs of a missing adaptation host and could provide a model to study ancient inter-species transmission events.

3. Cross-Species Transmission of Zoonotic Pathogens

This section of the conference addressed cross species transmission of selected pathogens. In addition to the summaries below of three presentations on this topic, this Special Supplement includes an article, **Epidemiological Survey of *Tryanosoma cruzi* Infection in Domestic Owned Cats from the Tropical Southeast of Mexico** by Dr. M. Jiménz-Coello et al. setting out how a significant public health problem in Mexico has been caused by the cross-species transmission of American Trypanosomiasis (AT) from triatomine bugs to domestic cats, representing a potential risk to humans.

Speaking on behalf of an extensive team of collaborators from a number of institutions—C. Osborne, P. Cryan, T. J. O'Shea, L. M. Oko, C. Ndaluka, C. H. Calisher, A. Berglund, M. L. Klavetter, R. A. Bowen and K. V. Homes—Dr. S. R. Dominguez (Section on Pediatric Diseases, The Children's Hospital, University of Colorado School of Medicine, Aurora, CO, USA) began by noting that the first pandemic of the 21st century, the deadly SARS virus, had its natural reservoir in bats. In his presentation, **Alphacoronaviruses in New World Bats: Prevalence, Persistence, Phylogeny and Potential for Interaction with Humans**, he suggested that bat coronaviruses (CoVs) may well be the ancestors of all group 1 and 2 CoVs. Today bats had become a primary species encountered by humans in terms of potential exposure to significant disease agents. Their research was tackling three important unanswered questions: (1) What is the prevalence and diversity of bat CoVs in New World bats? (2) Do bat CoVs persist in bat populations and/or individual bats? and (3) What are the potential interactions of infected bats with the human population?

A three-year study (Osborne et al., 2011) had collected clinical and environmental samples from bats at 16 rural sites and 5 urban sites throughout

Colorado, as well as bat carcasses obtained from various counties throughout the state from the Colorado Department of Public Health and Environment. Of the 1,002 fecal or anal swab samples, 75, that is 7 percent, were positive for CoV RNA. The highest prevalence of the virus was in juvenile bats; and although rates of prevalence varied from year to year, late spring was the time when the virus peaked. Although bat CoVs persisted within bat populations and their roosts, individually tagged CoV-infected bats cleared their infections within 6 weeks without apparent illness. New world bats of the same species in geographically distinct locations and over the course of several years harbour similar CoVs; and some New World bat CoVs may be able to infect bats of different genera. Strikingly, bats, which had known or potential contact with humans had a high prevalence of 10 to 20 percent of CoV infection. It is clear that significant opportunities exist for zoonotic transmission of coronaviruses from bats to humans and vice versa, especially as more than 95 viruses have already been isolated from or detected in bat tissues.

Noting that many mammalian and avian species in addition to bats are susceptible to coronavirus infection, Professor K. V. Holmes and her colleagues, K. Guo, Z. Qian and S. Wennier (Department of Microbiology, University of Colorado School of Medicine, Aurora, CO, USA) and G. Peng and F. Li (Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN, USA) presented the **Emergence and Evolution of Alphacoronaviruses: Insights from Spike and Receptor Analysis.** Some coronaviruses can only infect a single host species, while others can infect multiple species, because coronavirus host range is determined, in part, by specific interactions of the viral spike protein, S, with cellular receptor proteins that include ACE2, APN and CEACAM1. The recent emergence of SARS coronaviruses from civets, bats and/or other reservoir species into humans depended upon a few amino acid substitutions in the receptor-binding domain (RBD) of S from the animal viruses that allowed them to recognize human ACE2 instead of, or in addition to, receptors of their natural hosts (Li, 2008).

Alphacoronaviruses of pigs, cats, dogs and human coronovirus 229E use APN receptors of the host species, and all four viruses recognize feline APN (Tusell et al., 2007). In contrast, for human alphacoronavirus NL63, the receptor-binding motif (RBM) with its three loops in the RBD binds specifically to human ACE2. In the RBDs of the cat virus, FIPV, Professor Holmes and her research team predicted 3 loops structurally similar to the NL63 RBMs; and they constructed chimeric FIPV RBDs containing one, two or three RBMs from NL63. Receptor binding assays using enzyme-linked immunoassays (ELISA), flow cytometry and co-immunoprecipitation identified 3 loops (RBMs) in FIPV RBD that are required for binding to feline APN. Furthermore, substitution of only a few key amino acid residues within the RBMs of FIPV altered APN specificity and viral host range. Thus the emergence of alphacoronaviruses into new host species can occur when spontaneous mutations arise in the RBMs that permit binding to variants of the APN receptor protein expressed by different host species.

Considering the interaction between human and swine H1N1 viruses since 1900, Professor H. D. Klenk (Institute of Virology, Philipps University, Marburg, Germany) presented the **Mechanisms of Pathogenicity and Host Adaptation of Influenza Viruses in the Light of the New H1N1 Pandemic**. He explained that there was now a clear scientific consensus that wild aquatic birds are the natural hosts for a large variety of influenza A viruses. Occasionally these viruses are transmitted from this reservoir to other species, such as chickens, pigs and humans, leading to devastating outbreaks in

domestic poultry and the possibility of human influenza pandemics. By the end of February 2010, there had been 15,921 deaths, with the World Health Organization later confirming cases in 171 countries and territories, with deaths in at least 139 countries and territories before the spread of the H1N1 virus diminished. However, Professor Klenk set out the evidence to support his view that the pathogenic and pandemic potential of this new H1N1 virus is not yet exhausted.

The host range and pathogenicity of any virus are polygenic traits depend on the interaction of different viral proteins with specific host factors. It has long been known that proteolytic activation and receptor specificity of the hemagglutinin (HA) are important determinants for pathogenicity and interspecies transmission, respectively. There is now considerable evidence that HA mutations altering receptor specificity and cell tropism of the 2009 pandemic influenza A virus (H1N1v) are linked to the D222G amino acid substitution and are associated with a particularly severe outcome of infection (Liu et al., 2010).. It should be remembered that the viral polymerase has to enter the nucleus of the infected cell in order to promote replication and transcription of the viral genome. Adaptive mutations in polymerase subunits of avian viruses improve binding to importin Alpha, a component of the nuclear pore complex in mammalian cells. As a result, nuclear transport of these proteins and efficiency of replication are enhanced. Thus, the interaction of the viral polymerase with the nuclear import machinery is an important determinant of host range.

Some of the structural features typical for avian viruses have been preserved in the polymerase of the 2009 pandemic influenza A virus (H1N1v) suggesting that this virus has the potential to further adapt to humans. Recent studies have shown that the NS1 protein, another important determinant of pathogenicity and host range, is SUMOylated, and that this modification enhances virus growth. Interestingly, NS1 of H1N1v is not SUMOylated (Xu et al., 2011). Taken together, these observations support the view that the pathogenic and pandemic potential of the new virus is not yet exhausted. Furthermore, because of the firm evidence of HA polymorphism in position 222, mutants and other mutations with altered receptor specificity will have to be closely monitored.

In the subsequent discussion it was noted that when a virus becomes highly pathogenic, this might block its spread if additional hosts are not readily available. Furthermore, the role of co-infection with bacterial inflection was highly relevant in the 1918-1919 influenza pandemic and might well be relevant in a future pandemic.

4. Emerging and Neglected Influenza Viruses

There have been at least three influenza pandemics every century since 1700, with some evidence of earlier epidemics and pandemics after 1500. In *The Cambridge World History of Human Disease,* A. W. Crosby (1993; p. 810) has noted that although the black death and World Wars I and II killed higher percentages of the populations at risk, the 1918-1919 influenza pandemic was possibly ‘in terms of absolute numbers, the greatest single demographic shock that the human species has ever received’. The summaries below of seven presentations on this topic highlight the diversity of influenza viruses in North America (cf. Nelson et al., 2011), while other relevant research has been published with respect to Swine Influenza Viruses (SIVs) in Europe (Kyriakis et al., 2009)

Considerable research has now been done into how the Highly Pathogenic H5N1 Avian Influenza virus spreads from wild birds and ducks to chickens and other species, including humans (Rabinowitz et al., 2009; Ma et al, 2008). The studies of how influenza viruses can be genetically altered to become more transmissible have become

a matter of much controversy (Palese and Wang, 2012; L. H. Kahn, 2012). In addition to the summaries below, this Special Supplement includes an article, **Lessons from Emergence of A/goose/ Guangdong/1996-like H5N1 Highly Pathogenic Avian Influenza Viruses and Recent influenza Surveillance Efforts in Southern China**, in which Dr. X.-F. Wan has considered the emergence and ecology of Influenza A Viruses in Southern China, especially the highly pathogenic H5N1 virus.

Backed by an extensive team of collaborators, Professor A. D. M. E. Osterhaus (Head, Department of Virology, Erasmus Medical Centre, Rotterdam, The Netherlands) began his presentation, **Emerging and Neglected Influenza Viruses**, by explaining the complex aetiology of the Influenza A, B and C viruses. While humans can serve as host species for all three viruses, Influenza A can also be present in other mammals and avian species, Influenza B in seals, and Influenza C in pigs. The severity of the disease is relatively high with Influenza A, moderate with Influenza B and low with Influenza C, with the prevalence in humans high with both Influenza A and B viruses, but lower with Influenza C. Furthermore, a clear distinction needs to be made between seasonal influenza, avian influenza and pandemic influenza. There are two different mechanisms of host adaptation—sequential mutations and genome reassortment. Most recently, the new H1N1 swine flu pandemic outbreak of 2009 drew attention to the speed with which an influenza virus could move around the world. However, the fact that this particular virus was not as virulent as first anticipated proved crucial in confronting the virus, even though it spreads rapidly among humans, unlike the much more virulent H5N1 avian flu virus, from which more than 300 people have died from more than 500 verified cases from 2003 to 2011 (WHO, 2012).

Although clinical evidence of H5N1 avian influenza appears predominantly in diving ducks, a number of dabbling duck species—Mallard, Teal, Wigeon and Gadwall—appear to spread H5N1, generally acquired from wild birds, without showing major signs of disease. The likelihood of a major pandemic linked to H5N1 has not decreased in the last five years, even though publicity has certainly decreased. Furthermore, Professor Osterhaus pointed out that the recent H1N1 pandemic influenza outbreak indicated that the scientific community was wrong in its earlier belief that ‘a pandemic strain could only arise from a subtype that had not previously been widely disseminated in humans [because] the H1N1 virus has shown that human varieties characterized by different hemagglutinin (HA) molecules may follow separate lines of evolution and may generate potentially pandemic strains within an existing human HA subtype. Hence, it is essential to develop methods for estimating how many antigenically different subtypes may reside within each HA type’ (cf. Rappuoli et al., 2009).

In the light of the continuing prevalence of many subtypes of influenza, there is a critical need for improved monitoring, especially in Asia and Africa, as part of a move from a reactive to a proactive approach, with greater research into the possibility of developing a universal vaccine. Although there are increasing opportunities for virus infections to emerge and spread rapidly in our global society, new tools are being provided by research in molecular biology, epidemiology, genomics and bioinformatics. Already early warning systems based on state of the art virus detection techniques, as well as targeted intervention strategies based on data about the mutual virus-host interaction have been instrumental in dealing with numerous viral threats, including SARS and avian influenza.

The extensive research of the Department of Virology at Erasmus Medical Centre in Rotterdam was highlighted by a further presentation, **Influenza Pneumonia: The Role**

of the Alveolar Macrophage, given by Dr. D. van Riel. Highly pathogenic avian influenza (HPAI) H5N1 virus causes severe, often fatal, pneumonia in humans. The pathogenesis of HPAI H5N1 virus is not completely understood, although the alveolar macrophage (AM) is thought to play an important role. The AM resides in the pulmonary alveolus, the primary site of HPAI H5N1 virus replication in humans. It had been shown previously that HPAI H5N1 virus attaches abundantly to these AM (van Riel et al., 2006). The aim of this study was to determine the response of primary human AM to HPAI H5N1 virus, seasonal H3N2 virus or pandemic H1N1 virus, and to compare these responses with that of macrophages cultured from monocytes.

HPAIV H5N1 infection of AM compared to that of macrophages cultured from monocytes resulted in a lower percentage of infected cells (up to 25% versus up to 84%), lower virus production and lower TNF-alpha induction. Infection of AM with H3N2 or H1N1 virus resulted in even lower percentages of infected cells (up to 7%) than with HPAI H5N1 virus, while virus production and TNF-alpha induction were comparable. In conclusion, this study revealed that macrophages cultured from monocytes are not a good model to study the interaction between AM and influenza viruses. Furthermore, the interaction between HPAI H5N1 virus and AM could contribute to the pathogenicity of this virus in humans, due to the relatively high percentage of infected cells rather than virus production or an excessive TNF-alpha induction (van Riel et al., 2011).

Dr. E. A. Govorkova presented the study, **Fitness of Highly Pathogenic H5N1 Influenza Viruses in Ferrets**, on behalf of a research team at St. Jude Children's Research Hospital Center of Excellence for Influenza Research and Surveillance (St. Jude CEIRS), Memphis, TN, USA which included N. A. Ilyushina, B. M. Marathe and R. G. Webster. She began by pointing out that while the neuraminidase (NA) inhibitors are currently our first line of defense against a pandemic threat, the potential emergence of virulent and transmissible drug-resistant H5N1 viruses has important clinical implications (Writing Committee, 2008; White et al, 2009).

The St Jude's CEIRS research team used reverse genetics techniques and generated two pairs of H5N1 recombinant viruses: A/Vietnam/1203/2004-like (HA clade 1) and A/Turkey/15/2006-like (HA clade 2.2). One virus of each pair was wild-type, while the other carried the H274Y NA mutation conferring resistance to NA inhibitor oseltamivir. Within each pair, the wild-type and oseltamivir-resistant virus caused disease of equal severity in ferrets and replicated to comparable virus titers in the upper respiratory tract. Then, in order to assess the fitness of drug-resistant H5N1 influenza viruses, the research team considered virus-virus interactions within the host by co-inoculating ferrets with mixtures of the oseltamivir-sensitive and oseltamivir–resistant H5N1 viruses in varying ratios (e. g. 100/0; 80/20; 50/50; 20/80; 0/100). Using this novel approach, they demonstrated that the proportion of A/Vietnam/1203/2004-H274Y clones tended to increase while the proportion of A/Turkey/15/2006-H274Y clones tended to decrease. Their findings suggest that the H274Y NA mutation can affect the fitness of two H5N1 viruses differently and is dependent on background NA sequence. Dr. Govorkova pointed out that antigenic and genetic diversity, virulence, the degree of NA functional loss of H5N1 virus and differences in host immune response can also contribute to such differences. Therefore, the risk of emergence of drug-resistant influenza viruses with uncompromised fitness should be monitored closely and considered carefully in pandemic planning.

In a collaboration with C. Corzo, K. Juleen and M. Gramer (University of Minnesota Veterinary Diagnostic Laboratory, St Paul, MN, USA) and J. Lowe (Carthage Veterinary Services, Carthage, IL, USA), Dr. R. Webby and his colleagues at St Jude

Children's Research Hospital in Memphis, M. Ducatez, E. Stigger-Rosser, D. Wang and D. Darnell sought to answer the question: Is disease surveillance collected from diseased animals giving a true picture of swine flu activity in the United States?

They initiated an active surveillance program in healthy pigs in multiple sites in 2009, during a period coincident with the emergence of the H1N1 pandemic in humans. Their study, **Active Surveillance for Influenza Viruses in North America**, presented an analysis from 12 months of data which indicated that similar viruses can be detected in both active and passive surveillance schemes, and that there has been an explosion of diversity in swine influenza viruses (SIV) in the United States. Not only were a number of pandemic H1N1 infections in swine detected, but a number of pandemic/endemic swine virus reassortants were found, albeit from healthy animals (Ducatez et al., 2009). Virologically, the pattern of disease surveillance grounded in the activities of state diagnostic laboratories collecting information from diseased animals is representative; however, epidemiologically this data from diseased animals is not representative. Reverse zoonoses have had a huge impact on SIV in the United States (Vincent et al, 2008) and the pandemic virus is now endemic. However, in considering whether any particular reassortment causes alarm, it must be acknowledged that there is not yet a good model of risk, so H3, like H1, is going to be found in pigs for some time to come, but the consequences of this diversity in SIV are not yet clear.

The extensive collaboration now taking place in the study of swine influenza was evident in the presentation by Dr. K. M. Lager (Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, US Dept of Agriculture, Agricultural Research Service [USDA-ARS], Ames, IA, USA) on behalf of his colleagues P. C. Gauger, L. C. Miller and M. E. Kehrli, Jr., as well as other members of the research team, several with multiple institutional affiliations including D. A. Senne and D. L. Suarez (National Veterinary Services Laboratories, USDA, Veterinary Services, Ames, IA), D. E. Swayne (Southeast Poultry Research Laboratory, USDA-ARS, Athens, GA), and J. A. Richt and W. Ma (Kansas State University, Manhattan, KS, USA). Their consideration of **The Mixing Vessel Pendulum** began by explaining the three elements of how swine could be considered as a mixing vessel for influenza A viruses as formally proposed by Scholtissek et al (1985): (1) Swine are susceptible to infection with influenza A viruses from avian and human viruses; (2) The avian viruses can adapt within the pig, producing novel reassortants; and (3) These reassortants can then be shed and are infectious to man. The goal of this presentation was to test the first part of the mixing vessel hypothesis, concerned with the susceptibility of swine to avian and human influenza viruses, making use of both mixing vessel studies in pigs and genetic markers to investigate adaptation.

Dr. Lager noted that the emergence of the H5N1 highly pathogenic avian influenza virus that can transmit from avian species directly to man, and the presumption that the 1918 H1N1 influenza jumped from birds to man has expanded our understanding of the swine mixing vessel hypothesis as a potential, but not exclusive, source of human pandemic viruses (Taubenberger et al, 2005). Moreover, the emergence of the 2009 pandemic H1N1 virus has re-emphasised swine as a potential source of pandemic virus. In this study, all of the challenge viruses (avian H5, H7, H9) induced a similar effect in pigs; challenge viruses did replicate in pigs; the infections were subclinical with mild pneumonias; most infections resulted in seroconversion; and none of them transmitted to contact controls. This series of studies suggests pigs could be easily infected with avian viruses; however, an adaptation step is needed to generate fit viruses that transmit among swine. Parallel studies are currently underway testing the susceptibility of pigs to human seasonal influenza viruses. Future studies using reverse genetics could

investigate potential genetic markers for adaptation of avian viruses to swine which may provide insight into the interspecies transmission of influenza viruses.

A further study of swine influenza viruses, ***In vitro* and *in vivo* Characterization of Viral Reassortment between North American Triple Reassortant and Eurasian H1N1 Swine Influenza Viruses**, was presented by Dr. W. Ma (Dept of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA) on behalf of his colleagues Q. Liu, J. A. Richt and C. Qiao, as well as G. del Real (Department of Biotechnology, Instituto Nacional de Investigaciones Agrarias [INIA], Madrid, Spain), A. Garcia-Sastre (Department of Microbiology, Mount Sinai School of Medicine, New York City, USA) and R. J. Webby (St. Jude Children's Research Hospital, Memphis, TN, USA). The 2009 pandemic H1N1 virus (pH1N1) was derived through the reassortment of a North American triple reassortant swine influenza virus (SIV) and an avian-like Eurasian SIV. However, to date, the exact mechanisms by which the pH1N1 arose are not understood.

In this study an attempt was made to recreate the 2009 pandemic virus by co-infecting cells (in vitro) or a group of pigs (in vivo) with Eurasian (SP04) and North American triple reassortant (KS07) SIVs (Ma et al., 2010a). Infected pigs were co-housed with two groups of sentinel animals to investigate virus maintenance and transmission. The origin of each gene segment of viruses was determined which were isolated from supernatants collected from co-infected cells or nasal swabs and bronchioalveolar fluid samples collected from infected and sentinel animals. Different reassortant viruses were identified from co-infected cell lines; however no virus with the genotype of pH1N1 was found. Less reassortant viruses were found in the lungs of co-infected pigs in contrast to those in co-infected cells. Interestingly, only the intact KS07 was detected from nasal swabs from the second group of sentinel pigs. These results demonstrated multiple reassortant events can occur within the lower respiratory tract of the pig; however, only a specific gene constellation is able to be shed from the upper respiratory tract. However,, in this study it was not possible to generate the pH1N1 constellation using co-infection with the techniques described above and previously (Ma et al., 2010b).

In a collaboration among four institutions, Dr. S. E. Belisle (Department of Microbiology and Washington National Primate Research Center, University of Washington, Seattle, WA, USA) presented a **Systems Biology Approach to Understanding Influenza across Species** on behalf of her colleague, M. G. Katze (of the same Center), W. Ma and J. A. Richt (Dept of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, USA), T. M. Tumpey (Influenza Division, Centers for Disease Control and Prevention, Atlanta, GA, USA) and H. Feldmann (Rocky Mountain Laboratories, Hamilton, MT, USA). She began by reflecting on the ability of swine to act as a reservoir for many influenza viruses, becoming infected with low mortality, regardless of influenza virus strain. The objective of the study was to further understand the porcine response to influenza and to compare this response to other animals infected with the same virus.

To accomplish this objective, they used statistical and functional analysis of global gene expression to compare host transcriptional response during acute infection by a contemporary H1N1 pandemic influenza virus (A/California/04/2009) in swine, non-human primates and mice. Using their data, they compared and contrasted the biological pathways most significantly associated with gene expression changes during acute infection across these species. Their goal was to leverage data collected in their previous studies (Ma et al., 2011; Safronetz et al., 2011) to better understand influenza

virus pathogenesis through a cross-species analysis that considered three crucial questions: (1) Which genes change over the course of acute infection? (2) What are the top functions altered during infection? (3) How does functional response compare across the three species?

Despite challenges to data integration and interpretation, including the differences in transcript representation and annotation on the microarrays for the different species, the researchers found notable differences in response to influenza in the lungs of the three species. Although similar functional groups of genes changed with infection in all three species, the nature of that response was species-specific. Swine exhibited an elevated transcriptional response that tapered by resolution of influenza. Mice exhibited a decrease in many acute phase and immune response genes quickly followed by a steady increase in expression. Host response in macaques was most pronounced and maintained over time. In considering the transcription of immune-related genes in swine, mice and nonhuman primates, they found that although the number of immune-related genes changing in each species was similar, the precise genes changing were very different, with only 14 immune response genes commonly differentially expressed across all three species. This suggested that the nature of immune response within each species may be quite different.

In response to the perennial question after any scientific experiment, "Where do we go from here?" they offered four ideas: (1) Time series analysis could reveal unique response kinetics across species, thereby leading to targeted analysis; (2) Data integration across multiple data types, including transciptomics, proteomics, miRNA and NGS could generate a more complex, multidimensional view of response; (3) As annotation of the different species-specific genomes improves, this information could be integrated into future analyses, making a better understanding of the biological responses to infection possible; and (4) The gathering of this additional information could empower more precise analysis on what makes each species uniquely susceptible or resistant to influenza. In the firm view of these particular six researchers, studies such as this are necessary for a deeper understanding of influenza pathogenesis and demonstrate the utility of systems biology in the study of emerging viruses.

5. Hemorrhagic Fever Viruses

Three relevant articles on this topic have been published below, highlighting the global dimensions of both infection and treatment, no matter where the virus first emerges. The need for geographical comparative studies of the emerging hantavirus, Puumala hantavirus (PUUV), has already been indicated by Professor Henttonen and his team in their presentation summarized above in the opening topic of this Meeting Review. In a further investigation into the same hantavirus, Dr. Eckerle and her colleagues have presented an article within this Special Supplement entitled **Atypical Severe Puumala Hantavirus Infection and Virus Sequence Analysis of the Patient and Regional Reservoir Host**. In this article they focus on the difficulties in the diagnosis and treatment of a single patient and performed virus sequence analysis showing regional clustering in reservoir and host. In their more wide-ranging conference presentation they investigated cytokine expression in a cohort of patients hospitalized with acute severe hantavirus infection during an epidemic in Germany in 2010 (cf. Faber et al, 2010). Elevated pro-inflammatory cytokines during the early phase of disease compared to healthy controls and increase of immunsuppressive TGF-β from early to later phase of disease supported the hypothesis of an immune-mediated pathogenesis of Puumala hantavirus (Sadeghi et al, 2011). This finding indicates that the immune status of the host for old-world hantaviruses plays an important role, not only the virus itself.

In a further article published in this Special Supplement, **How Ebola Virus Counters the Interferon System**, A. Kühl and S. Pöhlmann have reviewed which components of the innate immune system could be effective against the zoonotic transmission of Ebola virus (EBOV) to humans, which results in severe hemorrhagic fever and high case-fatality rates. Their focus is on how the interferon (IFN) system, as a key innate defense against viral infections, is targeted by distinct EBOV proteins, and on how specific effector molecules of the IFN system could form a potent barrier against the spread of EBOV in humans.

Finally, in **Lassa Fever in West Africa: Evidence for an Expanded Region of Endemicity,** Dr. N. Sogoba and his colleagues H. Feldmann and D. Safronetz, have stressed the importance of increased surveillance for Lassa virus across West Africa.

The seven presentations summarized below cover a number of hemorrhagic fever viruses. For example, an important example of a highly contagious and life threatening hemorrhagic fever virus is Crimean-Congo hemorrhagic fever virus (CCHFV), caused by a tick-borne virus of the *Bunyaviridae* family (Elliott. 1990) First recognized in the Crimea in 1944, with an identical virus isolated in the Congo in 1956, the incidence and geographical spread of this disease with its high human fatality rate have increased significantly in the past ten years. However, the causes of this increase are not yet clear (Maltezou and Papa, 2011). In the light of the need to develop new therapies and effective, safe vaccines, the next seven research presentations could prove to be of considerable significance, not only for CCHFV, but also for the Hendra, Nipah, Lujo and Ebola viruses. Although these viruses have certain common features in their causes and consequences, each hemorrhagic fever virus needs to be carefully studied as a distinct entity.

Dr. R. Rodrigues and her colleagues G. Paranhos-Baccalà, and G. Vernet (all Emerging Pathogens Laboratory, Fondation Mérieux, Lyon, France), J.-M. Crance (Virology Laboratory, Institut de Recherche Biomédicale des Armées [IRBA], Grenoble, France) and C. N. Peyrefitte (both institutions) presented **Crimean-Congo Hemorrhagic Fever Virus Infects Human Hepatocytes and Induces Apoptosis and IL-8 Secretion**. She began by explaining that the knowledge of Crimean-Congo hemorrhagic fever virus (CCHFV) pathogenesis is improving, as recently new target cells have been identified such as antigen presenting cells (Peyrefitte et al., 2010). Moreover, it has already been shown, that CCHFV causes liver damage in infected patients and in the animal model (Bereczky et al., 2010). The research objectives were to consider: (1) How does CCHFV affect hepatocarcinoma cell lines? (2) Is CCHFV able to enter and replicate into these cell lines? and (3) Does CCHFV modulate the in vitro cellular response?

To better understand the CCHFV pathogenesis in liver cells, they analyzed in vitro the host response induced after CCHFV infection in Huh7 (unable to produce IFN-Beta) and Hep-G2 (capable of producing IFN-Beta) cell lines. They noticed that while in Huh7, CCHFV infection elicited at day 3 a cytopathogenic effect, no visible effect was seen in CCHFV-infected HepG2. This intriguing feature led them to analyse the viral parameters expecting a differential cellular response. Both cell lines were shown to be permissive to CCHFV and with a high viral yield as monitored by plaque titration assay, genomic and anti-genomic strand quantification. These CCHFV- infected hepatocarcinoma cell lines induced only IL-8 secretion. In addition, a pro-apoptotic effect was observed in Huh7 but not in HepG2. Interestingly, no type-I IFN was detected for Hep-G2 during the kinetic study, suggesting a strong inhibition of IFN secretion. They concluded that CCHFV does enter and replicate in hepatocytes and that hepatocytes could be involved in CCHF pathogenesis associated with antigen presenting cells for CCHFV dissemination. While CCHFV did not induce IFN-Beta

secretion in hepatocyte cell lines, CCHFV did induce the secretion of IL-1 in hepatocyte cell lines. Furthermore, CCHFV induced a higher secretion of IL-8 in the apoptotic Huh7 cell line than in the nonapoptotic Hep-G2 cell line. Thus this research indicated that IL-8 production and apoptosis seemed to be markers of CCHFV pathogenesis in hepatocyte cell lines.

Professor T. W. Geisbert (University of Texas, Medical Branch, Galveston, TX, USA) presented an **Evaluation of Countermeasures against Hendra and Nipah Viruses in Nonhuman Primate Models.** He pointed out that the henipaviruses, Hendra virus (HeV) and Nipah virus (NiV) are enigmatic emerging pathogens that can cause severe and often fatal neurologic and/or respiratory disease in both animals and humans. Guinea pigs, hamsters, ferrets, and cats have been evaluated as animal models of human HeV infection. A research team led by Professor Geisbert recently evaluated African green monkeys as a nonhuman primate model for henipavirus infection and discovered that they are the first consistent and highly susceptible nonhuman primate models of HeV and NiV infection (Geisbert et al., 2010; Rockx et al, 2010). The severe respiratory pathology, neurological disease and generalized vasculitis manifested in both HeV- and NiV-infected African green monkeys provides an accurate reflection of what is observed in henipavirus-infected humans. These nonhuman primate models were then employed to evaluate several post exposure treatments including ribavirin (which did not work) and a human anti-henipavirus monoclonal antibody (which was successful).

Dr. M. Faber, with his colleagues B. Dietzschold, J. Li, D. Curtis and C. Arbuzzese (all Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA, USA), B. Rockx and H and F. Feldmann (Laboratory of Virology, National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA), H. Weingartl (National Centre for Foreign Animal Disease, Canadian Food Inspection Agency; Department of Medical Microbiology, University of Manitoba, Winnipeg, MB, Canada) and B. Horvat (INSERM, Lyon, France), considered the Nipah virus (NiV), a zoonotic paramyxovirus that can infect multiple species including humans, where it causes a lethal encephalopathy. Their research on **A Single Immunization with Live-Attenuated Recombinant Rabies Viruses Expressing the Nipah Recombinant Rabies Viruses Expressing the Nipah Virus (NiV) Glycoprotein Causes a Strong Anti-N_iV Memory Response** constructed live-attenuated recombinant rabies viruses (RVs) expressing the NiV G protein and tested them for their ability to trigger a primary or secondary virus-neutralizing antibody (VNA) response against NiV in mice.

The research was motivated by the awareness that neutralizing antibodies are probably the major effectors against this viral infection. The rationale of using RV vectors for the development of a NiV vaccine was four-fold: (1) RV-vectored vaccines are not pathogenic regardless of the route of administration or the immune status of the host; (2) RV-based vaccines are very efficacious even after a single immunization by the oral route; (3) RV-based vaccines have the ability to target macrophages and dendritic cells, to induce TH1 T cell response and are capable of inducing long-lasting immunity; and (4) Post-exposure prophylaxis using recombinant RV vaccines is very effective, even when the CNS is already infected (Faber et al., 2009a; Faber et al., 2009b).

The NiV G gene was inserted into the non-pathogenic RV vectors SPBAANGAS or SPBAANGAS-GAS, resulting in SPBAANGAS-NG or the double GAS variant SPBAANGAS-NG-GAS, respectively. Further research led to four significant conclusions: (1) There are no detectable amounts of NiV G present in recombinant NiVG-RV particles; (2) The presence of an NiV G gene does not increase,

but rather decreases the pathogenicity of the recombinant viruses; (3) Priming with NiVG-RV triggers a strong NiV G-specific memory response which correlates inversely with vaccine concentration used for the priming; and (4) a single immunization with NiVG-RV is probably sufficient to protect against a NiV challenge infection.

Arenaviruses are rodent-borne bisegmented ambisense RNA viruses, which include Lassa fever virus, lymphocytic choriomeningitis (LCM) and Tacaribe viruses. Dr. E. Bergeron and his research team, A. K. Chakrabarti, C G. Albariño, L. K. McMullan, B. B. Bird, C. F. Spiropoulou and S. T. Nichol (all Viral Pathogens Branch, Centers for Disease Control and Prevention, Atlanta, GA, USA) and M. L. Khristova (Biotechnology Core Facility Branch, Centers for Disease Control and Prevention) presented the **Reverse Genetic Generation of Lujo Virus, a Novel and Highly Pathogenic Arenavirus**. The index case for this acute febrile illness virus was a travel agent living on a farm during 2008 in Lusaka, Zambia, who infected a local cleaner, as well as a paramedic and a nurse in Johannesburg, South Africa, all of whom died, with the paramedic infecting a further nurse who was treated with ribavirin and survived (Paweska et al, 2009). The name of the virus originated from the first two letters of the two key cities, LUsaka and JOhannesburg. Four of the five infected persons died of hemorrhagic fever-like symptoms (Paweska et al, 2009; Briese et al,2009)

Viral genome sequencing revealed that this virus differed from other arenaviruses by at least 36% and is highly pathogenic, with a case fatality rate (CFR) of 80% (Paweska et al, 2009; Briese et al, 2009). In view of the uniqueness and high virulence of Lujo virus (LJV), the research team developed a reverse genetics system to study the molecular characteristics of this novel arenavirus. This system will facilitate studies of LJV biology, development of antiviral screening assays and pathogenesis studies in animal models.

T. Cutts (National Microbiology Laboratory, Public Health Agency of Canada, Canadian Science Centre for Human and Animal Health, Winnipeg, Manitoba, Canada) with his colleagues S. Theriault (Chief, Applied Biosafety Research Program, same Centre) and G. Kobinger (Chief, Special Pathogens Program, same Centre) presented **Cytofix^{TM} Inactivation of VeroE6 Cells Infected with Zaire Ebola Virus (ZEBOV) both *in vitro* and *in vivo***. First, it was pointed out that removing infected tissues from high containment laboratories requires implementation of a number of different decontamination techniques to render the organism inert and is subject to flexibility according to the laws of the country in which the laboratory is located. According to the Canadian Biosafety Guidelines 4th edition, an organism may be removed from containment once it has been rendered inert, but no procedure is in place to validate these biosafety guidelines, and it is up to individuals to implement the relevant guidelines (Public Health Agency of Canada, 2004, p. 28. Chap. 3.1.4). Methods such as gamma irradiation, formalin fixation, acetone and methanol permeation, plus the use of various other chemical agents are common practices to preserve cellular tissue or blood components and to inactivate organisms (Villinger et al., 1999; Elliott et al., 1982; Mitchell and McCormick, 1984; Sanchez et al., 2007; Preuss et al., 1997). Such methods still raise questions as to their effectiveness or their redundancy. Furthermore, these inactivation steps can lead to the alteration of the target organism possibly affecting the qualitative and quantitative results. The focus of the Applied Biosafety Research Program was to evaluate and develop technologies and procedures relevant to biocontainment in the context of the laboratory, as well as to prevent unintentional and intentional release of dangerous organisms into the environment.

Using the commercial product, Cytofix/Cytoperm™ from BD Biosciences, this research sought to inactivate Vero E6 cells which had been infected with the deadly Zaire Ebola Virus (ZEBOV). The aim of the research was to determine the effectiveness and duration of Cytofix/Cytoperm for fixing the cellular material infected with ZEBOV. The VeroE6 cells were infected with the wild-type ZEBOV and a mouse adapted ZEBOV(MAZEBOV) and assayed after a 5 minute and 20 minute exposure to Cytofix™ followed by neutralization. Samples of blood from a non human primate infected with ZEBOV were drawn at 7 dpi and assayed for effectiveness in the same manner as the in vitro studies with Cytofix™. In addition, Vero E6 cells infected with MAZEBOV were treated in the same manner and injected into BALB/c mice to compose the in vivo studies. Cytoxicity and neutralization assays were used to determine the effect (if any) the treatment had on both the virus and the health of host cells.

Results of the tissue culture TCID50 assay showed that a 5 minute exposure to Cytofix™ inactivated a large portion of the cells containing infectious virions, while after a 20 minute exposure no detectable levels of virus were observed. Blood samples from the non-human primates showed similar results to the cell culture assay having no detectable virus from infected cells after 20 minutes of exposure. In vivo studies with mice showed that both a 5 minute and 20 minute exposure time to Cytofix™ had a 100% survival rate after 28 days post infection while the positive controls succumbed after 4 to7 dpi. Because laboratories differ in their preferences of technique, the time of inactivation also varies. What this research demonstrated was the effectiveness of a quick procedure of 20 minutes for inactivating viruses within cells infected with ZEBOV, thereby rendering organisms safe to remove from containment.

The presentation at the Cancun Conference, **Functional Analysis of the Ebola Virus Glycoprotein in Cell Lines from Potential Reservoir Bat Species** by A. Kühl, K. Grinβ, M. Kienne, T. S. Tsegaye (all Institute of Virology, Hanover Medical School, Hanover, Germany), M. Hoffmann and G. Herrler (Institute of Virology, University of Veterinary Medicine, Hanover), M. Müller and C. Drosten (Institute of Virology, University of Bonn Medical Center, Bonn, Germany) and S. Pöhlmann (Institute of Virology, Hanover and Department of Infection Biology, German Primate Center, Göttingen, Germany) has now been expanded and published in The Journal of Infectious Diseases (Kühl et al., 2011). Their focus was on how the EBOV-glycoprotein (EBOV-GP) facilitated viral entry and promoted viral release from human cells. They compared EBOV-GP interactions with human cells and cells from African fruit bats, leading to the finding that GP displayed similar biological properties in human and bat cells. The only exception was GP localization, which was to a greater extent intracellular in bat cells as compared to human cells. Collectively their results suggested that GP interactions with fruit bat and human cells are similar and do not limit EBOV tropism for certain bat species.

The presentation by Dr. E. de Wit with her colleagues, V. J. Munster and H. Feldmann (all from the Laboratory of Virology, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Hamilton, MT, USA) and S. Metwally (FAO Reference Center for Vesicular Diseases, US Department of Agriculture, Animal and Plant Health Inspection Service, Foreign Animal Disease Diagnostic Laboratory, National Veterinary Service Laboratories, Plum Island Animal Disease Center, Greenport, NY, USA), **Assessment of Rodents as Animal Models for Reston Ebolavirus** has now been revised and published in *The Journal of Infectious Diseases* (de Wit et al., 2011). Although Reston Ebolavirus

(REBOV) has not yet been linked with disease in humans, the presence of antibodies against REBOV in people working closely with infected macaques and swine indicates that humans can be infected with this virus (Miller et al., 1990; Miranda et al., 1991; Barrette et al., 2009). However, research has been hampered by the fact that the only available disease model for REBOV to date has been cynomolgus macaques.

Seeking new REBOV disease models, the research team assessed various rodent models—the Balb/c mouse, Hartley guinea pig, Syrian hamster and $STAT1^{-/-}$ mouse that lacked the signal transducer and activator of transcription 1 (Durbin et al., 1996). Although virus replication occurred in guinea pigs and hamsters, progression to disease was only observed upon inoculation of $STAT1^{-/-}$ mice. Despite certain drawbacks set out in the journal article, the $STAT1^{-/-}$ mouse can be used to investigate the determinants of differences in pathogenicity in various REBOV strains, as well as to assess vaccination and antiviral therapies (de Wit et al., 2011; Barrette et al., 2009; Durbin et al., 1996; Miranda et al., 1991; Miller et al., 1990).

6. Emerging Bacterial Diseases

The unity of human, animal and ecosystem health outlined by Professor Aguirre, as well as the interactions among multiple tick-borne pathogens in a natural reservoir host set out by Professor Fish and his research team, both summarized in Topic 1 above, highlight the necessity of cross-disciplinary collaboration in studying zoonotic bacterial diseases (Daszak et al., 2007, pp. 470-471). Such collaboration is especially important in studying tick-borne infectious disease, which emerged so extensively in the United States during the last three decades of the twentieth century (Paddock and Yabsley, 2007, p. 290).

Now, in an article published in this Special Supplement, **Beyond Lyme: Etiology of Tick-Borne Human Disease with Emphasis on the Southeastern United States**, Drs. Stomdahl and Hickling have explained that tick distributions are in flux, especially in the southeastern United States, requiring health providers to think 'beyond Lyme' in order to identify the specific tick species that bite humans and the different pathogens these ticks carry. In an international context, Drs. Wood and Artsob have set out the increasing importance of travel-associated rickettsioses in their article, **Spotted Fever Group Rickettsiae: A Brief Review and a Canadian Perspective**. In a third article published in this Special Supplement Drs. Verma and Stevenson present an article on epidemiology of leptospirosis with its one million cases worldwide. In **Leptospiral Uveitis – There's More to It Than Meets the Eye!** They hypothesize in detail about how the eye inflammation uveitis is triggered and stress the impact that 'understanding how this bacterium is able to induce this inflammatory process will be a key to the better management and prevention of the disease'. This continuum of basic research leading to understanding a disease and then to managing that disease and finally to preventing it offers a pattern of scientific discovery that is relevant to many other emerging zoonotic diseases.

Supported by the work of eight collaborators, L. Joens (University of Arizona), C. Parker (US Dept of Agriculture), M. Hook (Texas A & M University) and D. Call, M. Hunzicker-Dunn, C. Kang, D. Shah and S. Simasko (all of Washington State University), as well as seven graduate students and post-docs, Professor M. E. Konkel (School of Molecular Biosciences, Washington State University, Pullman, Washington, USA) investigated the behaviour of Campylobacter jejuni. C. jejuni is one of the most common causes of bacterial gastroenteritis worldwide, causing some 400 to 500 million cases each year. Opening his presentation, **The Foodborne Pathogen *Campylobacter jejuni* Exploits Mammalian Host Cell Receptors and Signaling Pathways**, Professor

Konkel noted that the percent of *C. jejuni* isolates that are resistant to antibiotics is continuing to increase, and that *C. jejuni* infections are frequently associated with serious sequelae, including Guillain-Barré Syndrome. It is well understood that infection with *C. jejuni* is often a consequence of eating foods contaminated with undercooked poultry. However, *C. jejuni* pathogenesis is a highly complex process that is dependent on many factors including motility, adherence, cell invasion, protein secretion, intracellular survival, and toxin production. Acute illness, characterized by the presence of blood and leukocytes in stool samples, is specifically associated with *C. jejuni* invasion of intestinal epithelial cells. Dissecting bacteria-host cell interactions are critical to understanding the infection caused by *C. jejuni*.

Previous work has shown that maximal invasion of host cells by *C. jejuni* is dependent on synthesis of the *C. jejuni* CadF and FlpA fibronectin (Fn) binding proteins and requires the secreted *Campylobacter* invasion antigens [Cia(s)] (Larson et al., 2008). To test the hypothesis that maximal cell invasion requires specific signaling events, binding and internalization assays were performed in the presence of numerous inhibitors of cell signaling pathways. The research team found that *C. jejuni* cell invasion utilizes components of Focal Complexes (FCs), as invasion is significantly inhibited by wortmannin (an inhibitor of PI-3 kinase) and PP2 (a c-Src inhibitor). They further demonstrated that a wild-type strain of *C. jejuni* results in the activation of the Rho GTPase Rac1. These observations are consistent with the proposal that *C. jejuni* binding to host cell-associated Fn and secretion of the Cia proteins trigger integrin receptor activation, which in turn promotes intracellular signaling and actin cytoskeletal rearrangement. Based on these data, they concluded that *C. jejuni* utilizes a novel mechanism to promote host cell invasion. The research findings Professor Konkel presented were recently published in *Cellular Microbiology* (Eucker and Konkel, 2012).

Simple, fast and specific tests for pathogen identification are essential for epidemiological investigation of numerous diseases. Within the field of immunodiagnostics, a quantitative determination of either antibody or antigen by antigen-antibody interaction can be made by lateral flow tests (also known as a dipstick or rapid tests). Dr. E. Baranova and her colleagues P. Solov'ev, N. Kolosova and S. Biketov (all State Research Center for Applied Microbiology, Obolensk, Russia) began the presentation, **Development of Lateral Flow Tests for the Fast Identification of Zoonotic Disease Agents**, by pointing out that lateral flow (LF) tests can be used in the field, as a diagnostic tool that produces results that can be read visually by the naked eye within 20 minutes after sample application. The creation of an algorithm for the development of an appropriate LF test to identify biopathogens requires the development of a target antigen, obtaining specific antibodies (Biketov et.al., 2010) and then creating a LF-test formulation to be trial tested. The target antigens must have the ability to induce species-specific antibodies, as well as be characterized by surface localization with multiple epitope presentation on the surface. The antibodies need to have a specificity and sensitivity sufficient for application in the LF detection format, as well as the capacity to be preserved after labelling with gold particles and after immobilization on a surface.

Over a period of 22 months, the research team developed and tested in the field LF-tests for the detection of *Bacillus anthracis* which causes anthrax , *Yersinia pestis* which causes bubonic plague and *Francisella tularensis* which is the causative agent of tularaemia (or rabbit fever). All three of these LF-tests have now been made available as commercial products and are being used throughout Russia for the rapid identification of these dangerous pathogens.

Drs. J. D. Trujillo and P. L. Nara (Center for Advanced Host Defences, Immunobiotics and Translational Comparative Medicine, Iowa State University, Ames, Iowa, USA) have developed and validated a new approach to the diagnosis of infectious agents. Dr. Trujillo explained that they are employing novel Polymerase Chain Reaction (PCR) based methods for the detection and differentiation of current and emergent Mycoplasma species relevant to human and animal medicine and biodefense. Their presentation, titled **Novel SYBR® Real-time PCR Assay for Detection and Differentiation of Mycoplasma Species in Biological Samples From Various Hosts**, began by explaining the relevance of Mycoplasma species, which are endemic, strict or opportunistic pathogens in human and animal medicine. Moreover, Mycoplasma species are important re-emerging pathogens and foreign animal diseases. Importantly, Mycoplasma species are difficult to culture or are un-culturable, and thus are difficult to impossible to detect by conventional diagnostic methods. Moreover, current PCR methods have limited breath of species detection and differentiation, requiring the use of species specific assays which are costly and time consuming. Their goal was to develop a pilot Mycoplasma genus diagnostic assay to validate the novel application of High Resolution Melt (HRM) methodology for rapid, sensitive and cost effective detection and differentiation of various pathogenic mycoplasma species.

Dr. Trujillo presented the validation and utilization of SYBR® green dye in real-time PCR (qPCR) Mycoplasma detection and differentiation assay (PanMYCO qPCR). This PCR assay utilizes primers specific for this genus (modified from S. C. Baird et al., 1999). This PCR assay results in the generation of small DNA fragments of various base pair lengths called PCR amplicons. Each amplicon has a melt temperature (TM) that is determined following qPCR. Sequence of amplicon representative of the Mycoplasma species present, defines the melt temperature (TM) and allows for the use of amplicons TM in species identification with limited resolution and excellent sensitivity. The PanMYCO qPCR assay has similar sensitivity to a conventional nested PCR assay for *Mycoplasma bovis* with a linear detection range of one colony forming unit (Trujillo et al, 2009).

Additional work presented described increasing species resolution of this assay, by defining unique melt profiles for each Mycoplasma species amplicon utilizing Precision Melt software from Biorad, CA, USA to perform HRM analysis. Greater than 30 different species of Mycoplasma found in bovine, caprine, ovine, avian and porcine hosts have been characterized with the PanMYCO qPCR and HRM analysis. Occasionally, this testing has resulted in the detection of multiple species in a single sample or discovery of novel or emergent Mycoplasma species. This data analysis method allows for the sensitive detection and rapid differentiation of numerous Mycoplasma species in many different hosts.

Dr. Trujillo concluded that this novel real-time PCR assay can detect and potentially differentiate all known Mycoplasma species. Moreover, this presentation demonstrated the novel use of genus specific SYBR green PCR and HRM analysis for the detection, differentiation and discovery of medically important pathogens. Several additional translational research projects have been launched to demonstrate the importance and utility of the PanMYCO qPCR assay in the context of infectious disease surveillance. One translational research project focuses on validation of this novel molecular methodology for field detection assays.

7. Outbreak Responses to Zoonotic Diseases

There is increasing awareness of the need for improved laboratory investigation, risk assessment, contingency planning and simulation exercises in order to respond

effectively to zoonotic diseases (Lipkin, 2008; Westergaard, 2008a and 2008b; Escorcia et al., 2012). In view of the need to research into and respond to so many emerging zoonoses, it is relevant to note the four-fold classification of emerging zoonoses proposed earlier by Silvio Pitlik: Type 1: from wild animals to humans (Hanta); Type 1+: from wild animals to humans, with further human-to-human transmission (AIDS); Type 2: from wild animals to domestic animals to humans (Avian flu); and Type 2+: from wild animals to domestic animals to humans, with further human-to-human transmission (SARS) (R. E. Kahn et al., p. 410, 2009). Confronting outbreaks of these emerging zoonoses is often possible with an imaginative combination of laboratory investigation and extensive fieldwork (Borchert et al., 2011; Robinson, 2011).

Three distinctive articles appear below on outbreak responses to zoonotic diseases, highlighting the importance of linking together basic research, practical action and an integrated One Health-oriented approach. In **Virus-like Particle-based Countermeasures against Rift Valley Fever Virus**, Dr R. Koukuntla and his colleagues Dr. R. B. Mandell and Dr. R. Flick have outlined their pioneering work to create, develop and produce a virus-like particle (VLP)-based vaccine against Rift Valley fever virus (RVFV)—a dangerous arbovirus for which there is at present no US Food and Drug Administration (FDA) or US Department of Agriculture (USDA) approved vaccine. In **Flexibility of Mobile Laboratory Unit in Support of Patient Management during the 2007 Ebola Zaire Outbreak in Democratic Republic of Congo**, Dr. A. Grolla and nine co-authors from eight different institutions in five different countries have explained how two mobile laboratories were set up and capable of running within less than 24 hours of arrival, providing safe, accurate, rapid and reliable diagnostic services as the Ebola Zaire outbreak began in the Democratic Republic of the Congo. Finally, in **Emerging and Exotic Zoonotic Disease Preparedness and Response in the United States: Coordination of the Animal Health Component,** Dr. R. L. Levings has set out the integrated approach of Emergency Management and Diagnostics, Veterinary Services, Animal and Plant Health Inspection Service, United States Department of Agriculture in the prevention of, the preparedness for, the response to and the recovery from a zoonotic disease outbreak. In all three of these areas—basic research, practical action and an integrated One Health-oriented approach—much has been achieved in recent years, but much also remains to be achieved as soon as possible. Even when those diseases are not transmitted to humans there are substantive challenges, as highlighted in the next case study by Woods on combating brucellosis in cattle in Zimbabwe.

In a practical, problem-oriented presentation, Dr. P. S. A. Woods (Veterinary Public Health Section, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, South Africa and University of Reading) with R. S. Beardsley (Pharmaceutical Health Services Research, School of Pharmacy, University of Maryland, Baltimore, Maryland, USA) and N. M. Taylor (Veterinary Epidemiology and Economics Unit, School of Agriculture, Policy and Development, University of Reading, Reading, United Kingdom) asked **Can We Increase Farmers' Perception of Their Brucellosis Susceptibility to Improve Adoption of Preventive Behaviors Amongst Small-Scale Dairy Farmers in Zimbabwe?** She explained the background to the problem, presented a model that was used to develop a strategy to confront the disease, and then set out the results and recommendations of the research team.

Brucellosis is an extremely infectious bacterium which causes abortion in cows, different syndromes in other animal species and malaria-like undulant fever, arthritis, depression and epididymitis in people. However, it had been controlled in Zimbabwe until 2001 when financial constraints forced the government veterinary services to

curtail disease surveillance and discontinue free vaccinations. Small-scale farmers did not seek vaccination from other sources, partly because they were unaware of the necessity of vaccination; and also at that time brucellosis was absent from small-scale farming areas. However, uncontrolled cattle movements from 2000 to 2009 linked to invasions of large-scale farms resulted in dispersal of possibly brucella-positive cattle and movement of the disease into small-scale herds. The result was that brucellosis became a potential problem in these herds and now presents a serious zoonotic threat.

Preventing brucellosis requires movement control to stop brucella-positive cattle entering an area, as well as live vaccine for female calves. Although there is no human to human spread of the disease, it is essential that people do not handle new-born calves or abortions from brucella-positive cows, nor drink unpasteurized milk from brucella-positive cows (Arimi et al., 2005). In essence, reducing the risk of brucellosis requires that farmers adopt appropriate preventive behaviors, with these control efforts and changes in behavior being community-directed in order to be sustainable. It was this stress upon community direction that formed the basis for funding by the Wellcome Trust to investigate the hypothesis that the level of a farmer's knowledge about brucellosis would influence subsequent preventive behaviour. The approach, based partly on the 'Health Belief Model' (Rosenstock et al., 1988) was grounded in the expectation that each small-scale farmer would make health behavior choices according to individual perceptions about the disease and personal beliefs about their abilities and the costs required to change the risks of their cattle and families acquiring the disease. In this project the independent variable was the level of an individual farmer's knowledge about brucellosis, while the dependent variables were two key preventive behaviors—decreasing cattle disease by calfhood vaccination and preventing zoonotic disease by milk pasteurization.

The research was carried out in partnership with a national network of small-scale dairy cooperatives with all activities conducted with existing local personnel. The aim was to tailor the educational program to the initial knowledge or awareness of each community of farmers, recognizing the considerable difference in knowledge levels between and within communities. Local teams, not outsiders, developed appropriate educational materials, targeting those with the lowest levels of knowledge. Completed survey questionnaires indicated a significant relationship between the initial level of farmers' knowledge about brucellosis and their calf brucellosis vaccination practices. The range of brucellosis knowledge among some 210 small-scale farmers in Southern Zimbabwe was considerable, with 38 percent of farmers being unaware of the disease, 12 percent having limited knowledge and 50 per cent having good knowledge. However, even amongst those farmers with a relatively high level of knowledge, 78 per cent of farmers had not vaccinated their calves at the time of the survey. Furthermore, there was a disappointingly low uptake of milk boiling despite a significant increase in knowledge about raw milk as a mode of infection for humans. Although the information sessions did increase farmers' awareness of the dangers of zoonotic brucellosis, an exaggerated perception of the effectiveness of calf vaccination decreased the likelihood of safe milk practices. This outcome indicated the importance of reaching the women who were responsible for milk and food preparation.

Ongoing research is investigating if increasing the role of nurses and environmental health technicians to emphasize human infection and to reach different family members, within a research paradigm which combined veterinary and human medicine would increase the uptake of milk hygiene practices.

8. Food-borne Zoonotic Diseases

There is increasing awareness of the need to balance transparency with carefully designed information disclosure strategies in the face of sudden outbreaks of food-borne diseases (National Research Council, 2011; Taylor, 2011). Both consumers and producers must be rapidly informed of any significant dangers with specific food products; however, considerable misinformation can be spread if laboratory results are incomplete or inconclusive (Palm et al., 2012). Recent experience with E.coli-infected sprouts in Germany and listeria-infected cantaloupes the United States has highlighted the difficulties in identifying the original source of a disease outbreak, as well as the swiftness with which an unexpected food-borne disease can cause sickness and death (Blaser, 2011; Buchholz et al., 2011; Frank et al, 2011; Armour, 2011). It should be noted that that there was no easily identified zoonotic link in either of these two food-borne diseases derived from bacteria, which killed 29 people in the United States and 50 throughout Europe during 2011; however, as Professor C. Kastner points out below, a significant number of these food-borne diseases do have a zoonotic origin (Parker et al., 2011).

Two articles linked to this topic are published in this Special Supplement. First, there is **Emerging Antimicrobial Resistance in Commensal *E. Coli* with Public Health Relevance** by Dr. A. Käsbohrer and her colleagues. Their aim was to assess the prevalence of and trends in antimicrobial resistance through active monitoring programs along the food production chains for poultry, pigs and cattle, as well as to collect isolates for resistance testing and then select certain isolates for further phenotypic and genotypic characterisation. The research team found alarming rates of resistance to antimicrobials in zoonotic bacteria and commensals, as set out in their article, which could compromise the effective treatment of human infections. This work provides a basis on which to improve both risk assessment and risk mitigation strategies in the face of the increasing antimicrobial resistance to zoonotic bacteria and parasitic organisms within both humans and animals. Second, in **American Trypanosomiasis Infection in Fattening Pigs from the South-East of Mexico,** M. Jiménz-Coello and her colleagues have investigated the extent to which the protozoa *Trypanosoma cruzi* (*T. cruzi*) is presenting in fattening pigs in Yucatan, Mexico, threatening parasitic infections in animals destined for human consumption.

Tackling the question of how to refine national and international strategies to combat food-borne zoonotic diseases, Professor C. Kastner (Food Science Institute, Kansas State University**,** Manhattan, Kansas, USA) considered the public health and economic impact of **Food-borne Zoonotic Diseases**. He began by noting that each year in the United States, according to statistics from the Centers for Disease Control, 48 million people become sick from food-borne diseases, 128,000 are hospitalised, and 3,000 die. A significant portion of these diseases have a zoonotic origin, with extensive product recalls and domestic as well as international trade disruptions (Fung et al., 2001). Therefore, more than 20 years ago the US Department of Agriculture established a Food Safety Consortium (2011) which focuses on food-borne zoonotic diseases involving beef in Kansas, pork in Iowa and poultry in Arkansas. The continuing aim of that consortium is to develop long-term control strategies which identify the critical control points and control technologies, as well as short-term strategies to address incidental contamination, whether accidental or intentional.

The US livestock industry in general and Kansas in particular are vulnerable to food-borne zoonotic diseases. For example, in Kansas, sources of contamination include feed, feedlots (which vary in size from 1,000 to 150,000 head per lot) and packing plants (which vary in size from 3,000 to more than 5,000 head per day per plant). Beef processing points where mixing of different ingredients occurs are the most critical

points for both incidental and intentional contamination. In the light of these challenges, a Biosafety Level 3 research facility, the Biosecurity Research Institute (2011) has been built on the Kansas State University campus, to evaluate strategies to detect and control food-borne zoonotic diseases from production through processing.

Furthermore, in Minneapolis, Minnesota, NCFPD (National Center for Food Production Defense, 2011) has been operational since 2004 as a Department of Homeland Security Center of Excellence. NCFPD has adopted a systems approach whose goals include to: (1) ensure significant improvements in supply chain security, preparedness and resiliency; (2) develop rapid and accurate methods to detect incidents of contamination and to identify the specific agent(s) involved; (3) apply strategies to reduce the risk of food-borne illness due to intentional contamination in the food supply chain and to develop the tools to facilitate recovery from contamination incidents; (4) deliver appropriate and credible risk communication messages to the public; and (5) develop and deliver high-quality education and training programs to develop a cadre of professionals equipped to deal with future threats to the food system. These research centers are essential to minimize the threat of food-borne zoonotic diseases.

T. Cutts (National Microbiology Laboratory, Public Health Agency of Canada, Canadian Science Centre for Human and Animal Health, Winnipeg, Manitoba, Canada) presented **Comparative Inactivation Studies of *Listeria Monocytogenes* at Room and Refrigeration Temperatures** on behalf of a research team which included B. Carruthers, C.-L. Cross, S. Theriault (Chief, Applied Biosafety Research Program, same center) and himself. Listeria monocytogenes, a non-sporulating, gram-positive bacillus, is found chiefly in ruminants, but can affect all species and causes listeriosis, an infrequent but serious illness that affects the central nervous system of humans and domestic animals (Bortolussi, 2008; Chan and Weidmann, 2009). Listeriosis can be acquired from the consumption of contaminated foods and has an incubation period ranging from 2 to 70 days (Bortolussi, 2008; Chan and Weidmann, 2009). Because of this variable incubation period and the fact that listeriosis leads to a mortality rate of 20 to 30 percent, the Applied Biosafety Research Program at the National Microbiology Laboratory of The Public Health Agency of Canada considered the significance of proper decontamination of listeria in food processing environments (Chan and Weidmann, 2009). The importance of this work is indicated by the fact that somewhere from 1 to 10 percent of ready-to-eat foods are thought to be contaminated with listeria (Health Canada, 2004).

Recently, Listeria monocytogenes has gained notoriety because of its ability to grow at the low temperatures, high salt and low pH used in food processing plants (Bortolussi, 2008). Therefore, a study was undertaken to determine the bactericidal efficacy of various liquid disinfectants and the effect that low temperatures have on the ability of these disinfectants to inactivate L. monocytogenes at conditions found in food processing plants.

At both room and refrigeration temperature (4^0), ethanol, Javex, SU393 and Peracetic acid (PAA) products outperformed all others. Surprisingly, there was no significant variation in performance at room temperature compared with refrigeration temperature. However, since some organisms undergo changes during a temperature shift, it is crucial to test each disinfectant at the temperature at which it will be employed. Bleach was found to be effective but is toxic, corrosive and residue forming, while the PAA and ethanol compounds do not form residues and are not corrosive. As a result of these studies major Canadian food processing plants have changed their decontamination procedures and are no longer using Quaternary Ammonium Compounds (Quats), which were previously used extensively. Positive relations have

been built up between companies and laboratories, leading to more relevant laboratory studies and industrial applications (Public Health Agency of Canada, 2012).

9. Prion Diseases

A prion (proteinaceous infectious particle) has been defined as a 'malformed version of a normal cellular protein which apparently "replicates" by recruiting normal proteins to adopt its form, [thus becoming] capable of infecting other cells of the same, or a different organism' (Thain and Hickman, 2004, p. 573; Prusiner, 2003). Although two Nobel Prizes in Medicine have been awarded for prion research, to Carleton Gajusek in 1966 and to Stanley Prusiner in 1997, the precise nature of the infectious agent remains unclear to such an extent that controversy continues about whether a prion is solely protein (Brooks, 2011, pp. 75-100). Whatever the cause, prion diseases are fatal chronic neurological diseases that affect the brains and nervous systems of many mammals, including humans (Imran and Mahmood, 2011).

Prions can be detected in tissues by a number of research techniques, including infective bioassay, animal inoculation, Western blot and immunochemistry. It is clear that prions can cause spongiform encephalopathies within both humans and animals (e.g. Creutzfeldt-Jakob disease, kuru, scrapie, transmissible mink encephalopathy, feline spongiform encephalopathy, and bovine spongiform encephalopathy) (Blood et al., 2007, p. 1456). Summaries of the three presentations below offer further insights into the nature of prion diseases.

In **Prion Diseases**, Professor J. J. Badiola and Dr. C. Akin (University of Zaragoza, Zaragoza, Spain) focused on the 1986 outbreak of bovine spongiform encephalopathy (BSE) ("mad cow disease") in the United Kingdom, which led to a better understanding of the epidemiology and molecular characteristics of the disease. Epidemic BSE affected mainly the United Kingdom, with a total of 184,615 positive animals compared to 5,765 in all other member states of the European Union (OIE, 2012). Control and eradication of Transmissible spongiform encephalopathies (TSEs) became a priority, not only in Europe, but throughout the world.

In 2000, a reinforcement of the passive surveillance program and the establishment of an active one were established by the European Commission for all the European Union member states (European Commission, 2001). Passive surveillance, focused on animals with clinical signs of the disease, and active surveillance was carried out in the following target groups: healthy slaughtered; fallen stock; emergency slaughtered; and animals culled under BSE eradication. Apart from these measures, specific risk materials (e.g. tonsils, intestines, spleen, spinal cord and skull, including the brain and eyes) were defined and prohibited from being included in the human food chain. Moreover, a banning of all meat and bone meal for animal feed was established (European Commission, 2009).

The result of these powerful eradication measures has been a rapidly decreasing number of new BSE cases, with less than 50 cases detected worldwide in 2010, 45 of which were in the European Union (OIE, 2012). The impressive containment of BSE in the United Kingdom from 35,090 reported cases in 1993 to 11 in 2010 is testimony to the determination with which scientists, politicians, civil servants and farmers have worked together to bring the disease under control.

Professor C. I. Lasmézas (Dept of Infectology, The Scripps Research Institute, Scripps, Florida, USA) began her presentation, **Zoonotic Potential of New Animal Prion Diseases: Assessment in Non-Human Primates**, by noting that the first demonstration of the transmissibility of a prion disease to Non-Human Primates (NHPs) was made in

1966 by Carleton Gajdusek when he transmitted kuru to chimpanzees. Since then, animal and human prion diseases have been transmitted to a range of NHPs. Cynomolgus macaques have shown the highest selectivity with regards to the prion strain by which they can be infected and therefore seem to be the species of choice to assess the risk that any given animal prion strain can infectibe pathogenic for humans (Lasmézas, 1996).

Prions were thought to be very difficult to transmit from one species to another; however, the experience of studying scrapie highlights the difficulties inherent in studying prion diseases in the lab. Scrapie had been transmitted orally to other ruminants (goats) but only intracerebral inoculations had successfully transmitted scrapie to monkey, mouse or mink. However, the oral transmission of bovine spongiform encephalopathy (BSE) to domestic cats in 1990 forced a revision of this earlier belief. Transmissions of BSE have now occurred orally to sheep, goat, monkey, mink, cheetah, puma, cat and mouse. Intracerebral transmission of BSE has also occurred to pig. Furthermore, intraspecies oral transmission of BSE has taken place within numerous species— monkey, mink, sheep, goat, cow, hamster and mouse. vCJD (variant Creutzfeldt-Jakob Disease) is a new human disease, which was caused by eating ruminant-derived food products contaminated with BSE. vCJD poses a public health problem because of the absence of preclinical diagnostic test, the long incubation periods of prion diseases in humans (possibly extending up to 50 years) and the transmissibility of the disease by blood transfusion.

The research team at the French Commissariat a l'Energie Atomique (CEA) demonstrated that bovine spongiform encephalopathy (BSE) was transmissible to macaques within 3 years with a 100% infection rate and caused a disease indistinguishable from the human variant of Creutzfeldt-Jakob disease (Lasmézas et al., 1996). This provided a model to study carefully the peripheral pathogenesis of vCJD, the oral infectious dose of BSE, and evaluate the risk of human-to-human transmission of vCJD by blood transfusion (Herzog et al., 2004). Further, the research team used the macaque model to assess the zoonotic potential of emerging forms of BSE called L-or H-type. The L-type BSE presents with higher pathogenicity to macaques than classical BSE (Comoy et al, 2008). Therefore, continued precautionary measures remain necessary to protect the human food chain. Experiments are ongoing at the National Institute of Allergy and Infectious Disease, Hamilton, Montana, to assess the risk linked to chronic wasting disease that is spreading throughout the USA. The closing acknowledgements of Professor Lasmézas to 35 other researchers indicated both the complexity and importance of continuing work in prion diseases. Furthermore, since the Cancun Meeting further important research has been published (Hamir et al, 2011).

Infectivity distribution studies of animals infected with BSE prions animals are a matter of considerable importance in seeking to elucidate the route of infectious prions from the gut to the central nervous system (CNS) in preclinical infected animals. Prof M. H. Groschup and his colleagues, A. Balkema-Buschmann, M. Kaatz, U. Ziegler and C. Hoffmann (all Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany) along with A. Oelschlegel (Scripps Institute Florida, Jupiter, Florida, USA) and L. McIntyre (University of Calgary, Calgary, Canada) investigated the **Early Spread of BSE Prions from the Gut via the Peripheral Nervous System to the Brain**. There are a number of open questions about this lethal journey from the gut to the brain, including where in the gut the disease begins, the initial steps of the neuronal BSE pathogenesis, the ascension of BSE prions to the brain, the haematogenous spread and the centrifugal contamination of the periphery (Buschmann and Groschup, 2005; Hoffmann et al., 2007). The scale of the

research task was indicated by the fact that 1400 samples were collected from per animal autopsy, leading to some 200,000 frozen samples collected and archived at the Friedrich-Loeffler-Institut.

Tissue samples were collected from the gut, the central and autonomous nervous system (ANS) of the challenged bovines and then examined for the presence of pathological prion proteins (PrP^{Sc}). There was some variation among different animals. However, a distinct accumulation of PrP^{Sc} was observed in the distal ileum, confined to follicles and/or the enteric nervous system, in almost all animals (Hoffmann et al., 2011). BSE prions were found in the sympathetic nervous system starting from 16 months post inoculation (mpi) on as well as in the parasympathetic nervous system from 20 mpi on (Kaatz et al., 2012). A clear dissociation of prion infectivity and detectable PrP^{Sc} deposition was obvious in tongue (Balkema-Buschmann et. al., 2011). The earliest presence of infectivity in the brainstem was detected at 24 mpi, while PrP^{Sc}-accumulation was detected first after 28 mpi. In summary, these results deciphered for the first time the centripetal spread of BSE prions along the ANS to the CNS starting already half way during the incubation period. BSE prions spread in cattle from the gut to the brain along the sympathetic, parasympathetic and spinal cord routes, possibly in that order of importance. Spinal cord involvement may even not be necessary at all, but BSE infectivity in the form of PrP^{Sc} spills over into the periphery already in the pre-clinical phase.

10. Modeling and Prediction of Emergence of Zoonoses

The modeling and prediction of emerging zoonoses is a fast growing field of considerable complexity. Of the five papers relevant to this topic, two have been published in full below in this Special Supplement. Dr. G. Zanella and her colleagues consider **Modelling Transmission of Bovine Tuberculosis in Red Deer and Wild Boar in Normandy, France**. Their mathematical model of the *Mycobacterium bovis* infection within and between species takes into account the transmission of *M. bovis* through infected offal—the viscera of animals killed by hunters and left behind. When an animal was hunted in the Brotonne Forest in Normandy prior to 2002 it was eviscerated *in situ* and only the carcass taken away, with the raw viscera left behind. Since 2002, offal disposal has been required in Brotonne forest ; however, the regulation has not always been observed by hunters (Unpublished correspondence with G. Zanella, December 16-17, 2011) An important benefit of mathematical modelling is that it permits consideration of all the elements involved in disease transmission within a population, thereby complementing field data, as well as testing the effects of control measures. Thus the direct transmission of the *M. bovis* infection within the red deer and wild boar populations can be distinguished from indirect transmission through contaminated offal. The model indicates that offal destruction is the key factor in infection control for both red deer and wild boar. The authors conclude that, in principle, the structure of this model is relevant to situations where dead animals play an important role in disease transmission between two or more species.

In a further article published in this Special Supplement, **Constructing Ecological Networks: A Tool to Infer Risk of Transmission and Dispersal of Leishmaniasis,** Dr. C. González-Salazar and Professor C. Stephens set out the role of ecological networks as a powerful tool for understanding and visualizing inter-species ecological and evolutionary interactions. Taking the example of leishmaniasis in Mexico, they show that such networks can be used not only to understand potential ecological interactions between species involved in the transmission of the disease, but also to identify the potential role of the environment in disease transmission and dispersal.

Strikingly, they show how potential interactions can be inferred from geographic data, rather than by direct observation. Their findings have led to the prediction of additional reservoirs in Mexico of many new species, including bats and squirrels. The resulting model can be used to understand and map potential transmission risk, as well as construct risk scenarios for the dispersal of leishmaniasis from one geographic region to another. Such a risk assessment tool for leishmaniasis will be especially useful in the light of the Bill and Melinda Gates Foundation decision in January 2012 to join with 13 major pharmaceutical companies and the World Health Organization in targeting leishmaniasis as one of the neglected tropical diseases to receive improved drugs, diagnostics, vector control strategies and vaccines (Bill and Melinda Gates Foundation, 2012; Boseley, 2012). However, the possibility of new reservoirs suggests it is hard to imagine that Leishmaniasis can be completely eradicated. Nevertheless, it is increasingly clear that leishmaniasis has a disturbing capacity to jump from species to species, so efforts to control the disease must be given a high priority (Unpublished correspondence with C. Stephens, February 1, 2012; cf. Flanagan et al, 2011).

It is difficult to model and predict the distribution and impact of a new emerging virus. For example, the emergence in November 2011 in Europe of a midge-borne virus member of the *Bunyaviridae* family, named Schmallenberg virus after the location in Germany where it was first detected, has caused serious birth defects in lambs, goats and cattle (ECDPC, 2011). Scientists, farmers, veterinarians, public health officials and consumers are all confronted with the uncertainty inherent in facing a new animal pathogen (Farmers Weekly, 2012). Appropriately, at the same time as this new virus has emerged, the Animal Health and Veterinary Laboratories Agency (AHVLA) has set up a new independent advisory group to evaluate veterinary surveillance in England and Wales, although their original intent was in part to consider funding reductions (Trickett, 2012).

Modeling risk factors for zoonotic influenza infections is challenging because the infections are often rare; the laboratory assays are often difficult and imprecise; and the most definitive studies require intensive resources. This was the view of Professor G. C. Gray (Emerging Pathogens Institute and College of Public Health and Health Professions, University of Florida, Gainesville, Florida, USA) in his presentation, **Modeling Risk Factors for Zoonotic Influenza Infections in Man: Challenges and Strategies for Success.** In particular, serologic detections of these infections in humans may be confounded by cross-reacting antibody, waning antibody from the infection of interest, inaccurate matching of the enzootic pathogen and the laboratory strain, laboratory errors, and weakly powered statistical comparisons.

The underlying question which Professor Gray and his research team is tackling is: Which human, animal and environmental factors predict disease? These three factors can be viewed as a Venn diagram with its intricate interactions. Like understanding cardiovascular disease, how a person acquires a zoonotic influenza infection is a complex process, and predictive laboratory assays are imprecise. For example, with avian influenza viruses (especially H5N1, popularly known as 'Bird Flu'), poultry veterinarians, turkey workers, hunters and people without indoor plumbing may be at increased risk of AIV infection but infections are rare. Subclinical or mild infections do occur; and occasionally AIV causes severe disease in persons exposed to sick birds. Although AIV transmission from human-to-human seems rare, further cohort studies and more sensitive serological assays are needed.

A basic scientist often tests hypotheses by: (1) carefully setting up an experimental setting; (2) isolating confounding factors; and (3) looking for statistically

significant associations with an outcome. Such a process is not possible for a number of emerging disease problems such as human infections with swine influenza virus (SIV). Experimental studies are not possible. Hence, epidemiologists must perform observational studies of people most likely to be infected with SIV and by looking at possible risk factor associations, infer causality. One must first determine settings where the prevalence of SIV in expected to be high and then study those workers. For example, SIVs are often endemic in large-scale modern production facilities. Risk factors for sow-herd SIV seropositivity involve pig density, whether there is an external source of breeding pigs, the total animals on the site and the closeness of barns. Similarly, risks factors for finisher-herd SIV positivity must be considered—the number of SIV positive sows, size of herd, pig farm density and farrow-to-finish type of farm (Poljak et al., 2008). However, SIV surveillance in pigs is largely passive and voluntary, so recognizing which pig workers to study is a challenge. Detection of SIV infections in man often requires a sentinel event (e.g. human illness with pig exposure or sick pigs). As pigs do not always have clinical signs of novel virus infection; and often there is no compensation system to protect pig farmers, the pork industry is reluctant to permit the study of their workers for SIV infection (Gray and Baker, 2011). Therefore, these observational studies are currently very difficult.

Professor Gray concluded by pointing out that although there are numerous challenges in conducting epidemiological studies for zoonotic influenza, there are six substantive ways to control confounding variables: (1) design every study carefully; (2) use non-animal-exposed controls; (3) employ validated laboratory assay using zoonotic influenza strains; (4) use multivariate modeling to examine cross-reacting serologic responses due to human viruses and vaccines; (5) consider proportional odds modeling; and (6) consider employing a second unique serologic test (See GPL, 2012).

With the support of 26 co-authors from 21 different institutions, Dr. K. J. Linthicum (United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural & Veterinary Entomology, Gainesville, FL, USA) presented two case studies about **Forecasting Emerging Vector-Borne Diseases**. Dr. Linthicum began by pointing out that global climate variability, often linked to El Niño conditions, can be used to forecast emerging vector-borne disease spread in local areas (Linthicum et al., 1999). These forecasts are possible because specific pathogens, their vectors, and hosts are sensitive to temperature, moisture, and other ambient environmental conditions. With consistent and reliable satellite observations, global sea temperatures, climate and vegetation can be observed.

First, temperature plays a major role in its impact on *Aides aegypti* mosquitoes transmitting dengue hemorrhagic fever virus in Southeast Asia (Linthicum et al., 2008) and possibly also on how *Ae. aegypti* transmits chikungunya virus in Africa and Asia (Anyamba et al., 2012), as well as on how *Anopheles* species mosquitoes transmit *P. vivax* malaria in the Republic of Korea. Vectorial competence is dependent upon the Extrinsic Incubation (EI) period in the mosquito vector. The EI represents the time from ingestion of the virus while feeding on a viremic host to the virus arriving in the salivary glands. The shorter the EI period, which occurs during higher ambient temperatures, the greater the vectorial competence (Garrett-Jones and Shidrawi, 1969). If data are available for a specific local area on the daily human-biting rate (ha) of the mosquitoes, the daily rate of blood feeding (a) and the length of the EI cycle (n), it is possible to calculate vectorial capacity (Rattanarithikul et al., 1996).

Second, accurate measurements and understanding of how exceptionally heavy rainfall and flooding affects *Aides* and *Culex* mosquitoes and the introduction of virus infected mosquitoes into susceptible vertebrate hosts enables forecasts to be made about

when and where Rift Valley fever (RVF) will develop in sub-Saharan Africa and Middle East (Anyamba et al., 2009). Outbreaks of Rift Valley Fever are known to follow periods of widespread and heavy rainfall associated with the development of a strong inter-tropical convergence zone over Eastern Africa (Davies et al., 1985). During periods of elevated transmission there is a significantly increased risk of globalization of these and other arboviruses; however, the forecasting methods described provide 2.5 to 5 months early warning before an outbreak and provide ample time for disease mitigation before the first cases appear (Anyamba et al., 2010).

Furthermore, the emergence and expansion of a number of disease vectors (e .g. mosquitoes, mice, locust) often follow the trajectory of the green flush of vegetation in semi-arid lands. The ability to predict periods of elevated risk enables better prevention, containment, or exclusion strategies to be drawn up to limit globalization of emerging pathogens. Thus it has been possible for the Food & Agricultural Organization (FAO) to create a system of alerts—the Emergency Prevention System for Transboundary Animal and Plant Pests and Diseases (EMPRES, 2012).

Subsequent to Dr Lithicum's presentation, significant further work has been done to provide a genome-scale overview of gene expression in the malaria-transmitting mosquito Anopheles gambiae (McCallum et al, 2011), as well as to expand the VectorBase website with regularly updated genome information on two other mosquito species, Aedes aegypti and Culex quinquefasciatus and numerous other organisms, including the tick species Ixodes scapularis (Lawson et al, 2009; NIAID, 2012). The ultimate aim of this research is to create a database that will facilitate a systems-level view of gene expression for many different organisms.

Reflecting on the numerous types of statistical analysis that are used to estimate confidence intervals for proportions in scientific studies, Dr. S. Guillossou and his colleagues Professors H. M. Scott and J. A. Richt (Dept. of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, Kansas, USA) utilized the final presentation of the conference, **Estimates of Low Prevalences and Diagnostic Test Estimates: What *Confidence* Do We Really Have?** to illustrate the differences, limits and sometimes chaotic behavior of different statistical approaches. Dr. Guillossou pointed out that there were more than 15 different methods for determining a 95 percent confidence interval of a proportion. He stressed that it is always important to report the method of statistical analysis being utilized. In his view, the Agresti-Coull interval approach presents a satisfactory compromise between computational requirements and coverage probability (Newcombe, 1998; Brown et al, 2001). Ideally, the effects of coverage probability should be estimated and the most appropriate method chosen before reporting the findings or using proportions as inputs in any epidemiological study.

Conclusion

What did this 6th International Conference on Emerging Zoonoses achieve? There was the opportunity to meet old friends and make new friends, to share one's academic work and to reflect on what lies ahead with emerging zoonoses. It is now clear that human medicine, veterinary medicine and environmental challenges are a unity which must be considered under the umbrella of 'One Health' (One Health Initiative, 2012).

Viruses are continuing to jump from animals to people with unexpected consequences, because the evolution of any virus is impossible to predict. Even the recent relatively mild swine flu virus infected 10 percent of the human population and killed some 100,000 people globally—far less than would have been the case if the

virus had mutated to a more deadly form, as might easily have happened. The reality is, as Professor Nathan Wolfe, Professor in Human Biology at Stanford University, has commented: 'As a species, we're not that focused on the things that have the most potential to be devastating to us as a global population, such as viruses. Unless people take these things seriously, we're going to look back and say we had all the tools necessary to try to address these risks, and we basically ignored them because they weren't dramatic like a car accident or a hurricane' (Geddes, 2011; Wolfe, 2011; L. H. Kahn, 2011). This conference, many others and the 7th International Conference on Emerging Zoonoses to be held in 2014 in Berlin are aimed at creating, improving and using the tools essential to address the risks of viral contagions in a global society.

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Note: A weblink to the full text of the 16 articles noted above can be found at http://onlinelibrary.wiley.com/doi/10.1111/zph.2012.59.issue-s2/issuetoc

References to the Introduction and the Meeting Review are available here in associated documents.

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