MEETING REPORT

Emerging Infections: A Tribute to the One Medicine, One Health Concept

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Impacts

- A symposium on 'Emerging Infections: A Tribute to the One Medicine, One Health Concept' was held on 13 and 14 November 2008 at Kansas State University, Manhattan, Kansas, USA. Kansas State is part of the 'Kansas City Animal Health Corridor', with its universities and some 130 companies controlling one-third of the expenditure on animal health in the world.
- This Meeting Report with summaries of all 32 presentations stresses how human and veterinary medicine are evolving together, sharing research models for emerging pathogens, prion diseases, bunya and influenza viruses and emerging/re-emerging diseases.
- Mitigating zoonoses will require an integrated approach linking effective surveillance with environmental awareness and research to develop antiviral therapy and new vaccines, often within a perspective of systems biology.

Keywords:
Zoonotic disease; pathogens; prions; bunya viruses; influenza viruses; zoonotic infections

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Summary

Events in the last decade have taught us that we are now, more than ever, vulnerable to fatal zoonotic diseases such as those caused by haemorrhagic fever viruses, influenza, rabies and BSE/vCJD. Future research activities should focus on solutions to these problems arising at the interface between animals and humans. A 4-fold classification of emerging zoonoses was proposed: Type 1: from wild animals to humans (Hanta); Type 1 plus: 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 plus: from wild animals to domestic animals to humans, with further human-to-human transmission (Severe Acute Respiratory Syndrome, SARS). The resulting holistic approach to emerging infections links microbiology, veterinary medicine, human medicine, ecology, public health and epidemiology. As emerging 'new' respiratory viruses are identified in many wild and domestic animals, issues of interspecies transmission have become of increasing concern. The development of safe and effective human and veterinary vaccines is a priority. For example, the spread of different influenza viruses has stimulated influenza vaccine development, just as the spread of Ebola and Marburg viruses has led to new approaches to filovirus vaccines. Interdisciplinary collaboration has become essential because of the convergence of human disease, animal disease and a common approach to biosecurity. High containment pathogens pose a significant threat to public health systems, as well as a major research challenge, because of limited experience in case management, lack of appropriate resources in affected areas and a limited number of animal research facilities in developed countries. Animal models that mimic certain diseases are key elements for understanding the underlying mechanisms of disease pathogenesis, as well as for the development and

efficacy testing of therapeutics and vaccines. An updated veterinary curriculum is essential to empower future graduates to work in an international environment, applying international standards for disease surveillance, veterinary public health, food safety and animal welfare.

The symposium, 'Emerging Infections: A Tribute to the One Medicine, One Health Concept', was held on 13 and 14 November 2008 at Kansas State University (KSU), Manhattan, Kansas, USA. The 32 presentations were linked to a general theme, as well as four workshops held consecutively on: (i) research models for emerging pathogens; (ii) prion diseases; (iii) bunya and influenza viruses; and (iv) emerging/re-emerging diseases. In addition, prior to the opening of the symposium, as part of KSU's Provost's Lecture on Excellence in Scholarship, **Dr Robert G. Webster** of St Jude Children's Research Hospital, Memphis, Tennessee, USA, spoke on 'Emergence and Control of Pandemic Influenza'.

Provost's Lecture on Excellence in Scholarship

Dr Webster first pointed out that among the known 16 haemagglutinin (HA) and nine neuraminidase (NA) subtypes of influenza A virus, the HA and NA subtypes were equally important and that the spikes that attach the virus to the respiratory tract were constantly changing. One mating of two different influenza A viruses could lead to 256 different influenza viruses. Inter-pandemic influenza based on H3N2 and H1N1 subtypes together led to 35 000 deaths a year in the United States (Horimoto and Kawaoka, 2005; Salomon and Webster, 2009). Therefore, it was important to prepare, manufacture and utilize effective annual vaccines for influenza. Although last year's vaccine had not been very effective because of the late emergence of a new strain, it was hoped that changes would make this year's vaccine more effective. In seeking to identify and counteract influenza viruses, extra effort needed to be put into surveillance, as demonstrated in Derek Smith's maps of viral evolution (Enserink, 2008; Russell et al., 2008).

The human dimension of an influenza pandemic was evident in the New Zealand experience in 1918, when the Minister of Health declined to quarantine the SS Niagara because it was carrying the Prime Minister and the result was that Auckland became a 'City of the Dead' (Rice, 2005). The origin of that world-wide 1918–1919 influenza pandemic, which probably began at Fort Riley, near Manhattan, Kansas (Barry, 2006), is now better understood because of the work of Taubenberger et al. (2005) who had sequenced the virus. It was found to be closely related to classical swine influenza isolated by Shope. This complete sequencing of the original 1918 virus had been greatly assisted by the retired pathologist, Johan Hultin, who recovered tissues frozen in permafrost in Brevig, Alaska, from the mummified corpse of a woman who had died from influenza and sent them to Taubenberger's team at the Armed Forces Institute of Pathology in Rockville, Maryland (Rozell, 1999/2005). Kobasa et al. (2007) have demonstrated from their work with macaques that atypical host innate immune responses may have contributed significantly to the lethality of that virus.

Figure 1 below describes the ecology of influenza viruses, which begins in the intestinal and respiratory tracts of shorebirds and wild ducks, before spreading to other species. The Ruddy Turnstone is a key bird spreading the virus to other avian species, in the midst of its annual migration from South America to Northern Canada. The complexities of the transmission of influenza viruses requires special consideration not only of H5 which has evolved into 10 different families or 'clades', but also of the global spread of H7, H2N3 and especially of H9N2, 'the sleeper in Asia', which donates six of the eight genes for H5N1. The spread of these different influenza viruses has stimulated influenza vaccine development, which currently indicates that inactivated oil emulsion vaccines are best in poultry while cold-adopted live vaccines and inactivated vaccines are about equally efficacious in humans; however, numerous issues remain to be resolved in the context of MDCK cells, tissue culture vaccines, adjuvants and for novel vaccine formulation (Hoffmann et al., 2000; Belshe, 2007).

The pros and cons for stockpiling human vaccines are finely balanced. The opposition to stockpiling is based on the awareness that any 'pre-pandemic' vaccine is unlikely to match the dominant strain that emerges to cause a pandemic. Furthermore, because of immune potentiation (an aberrant antibody response that increases virus uptake by cells), there is the possibility that a 'pre-pandemic' vaccination could lead to more serious disease signs. In addition, as the first infection in childhood with influenza virus leaves a lifelong immunological imprint (called 'antigenic sin'), later infection with antigenically related viruses can cause recall of immune responses to the original virus rather than to subsequent drift variants. There are also manufacturing issues linked to both the scale of

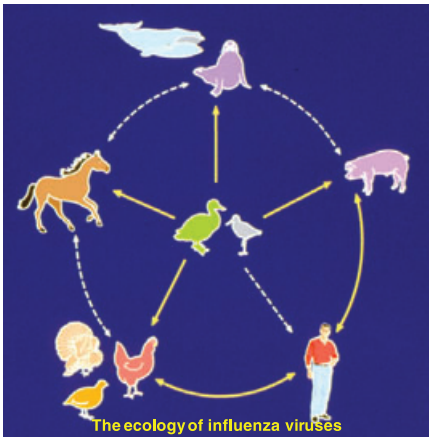

Fig. 1. The ecology of influenza viruses.

vaccine required and the method of its production, as well as the possibility of the vaccine leading to a very small number of cases of Guillian–Barré Syndrome.

The argument for stockpiling 'pre-pandemic' vaccines is based on modelling that suggests that the first wave of a pandemic would be over in 3 months and even with reverse genetics, it would take about 6 months to produce a specific vaccine. Furthermore, the use of a 'pre-pandemic' vaccine could lead to priming for a novel subtype, with the result that a 'pre-pandemic' vaccine could prevent many deaths, but not infection. Dr Webster's view was that a 'pre-pandemic' vaccine for H5N1 and another for H9N2 should be stockpiled, because even with variation in the virus, such vaccines would likely prevent deaths, but not infection.

Other topics covered by Dr Webster included the importance of establishing the antigen content for vaccinating ducks effectively, the emergence of resistance mutants to the drug Oseltamivir (Tamiflu), and the possibility that drug combinations could reduce the emergence of resistance mutants (Ilyushina et al., 2006). Dr Webster concluded that it was still possible to control the H5N1 virus, but only if further work were carried out with antiviral influenza drugs (including the stability of the drugs, new drug targets, and combination chemotherapy), agricultural vaccines (with standardization of antigen content), human vaccines (improving adjuvants, cell-based vaccines, priming for cross-related boosters, determining appropriate original antigen content), and immunotherapy (which was presently under-evaluated).

In the subsequent discussion, attention focused on modern virology. Poultry vaccines had been shown to be very, very effective with a standardized vaccine with sufficient antigen content. There were problems in manufacturing vaccines to the required standard, as well as in delivering vaccines in a timely manner, and in avoiding sub-standard vaccines that could cause virus drift; however, nature itself causes far more virus drift than the occasional sub-standard vaccine. Dr Webster still believed that the H5N1 virus could be stamped out, but it was becoming more and more difficult because of the increasing numbers of epicentres of persistent infection in domestic waterfowl ducks that were leading to epidemics of influenza in a number of species.

Emerging Infections Symposium Opening

The symposium was opened by the co-chairs of the evening session, Drs Jürgen Richt (Kansas State University, Manhattan Kansas) and Adolfo García-Sastre (Mount Sinai, New York). The presentations were to have begun with a paper from **Dr Hilary Koprowski** of Thomas Jefferson University, Philadelphia Pennsylvania, USA, 'How Plant Vaccines Came to Be'. However, the 91-year-old Dr Koprowski, renowned for his earlier work with the live virus polio vaccine (Oshinsky, 2005), was ill; and his paper was presented later by his colleague at Thomas Jefferson University, Dr Bernard Dietzschold. The initial focus was on the plant-derived biomedicals from which the Biotechnology Foundation Laboratories is developing vaccines for 13 diseases. For example, tobacco was being used to develop vaccines for cancer, whooping cough, rabies, anthrax, smallpox, SARS, diphtheria, tetanus and avian flu. A single plant cell could produce multiple monoclonal antibodies. A multivalent pediatric vaccine candidate (DTP) was being produced in transgenic plants. There were five clear advantages of plant products: (i) safety in production; (ii) easy storage and distribution (e.g. florets of cauliflower expressing vaccine products);

(iii) needle-free ease of administering to humans and animals through various orifices; (iv) expression of multiple antigens in one plant; and (v) low cost and speed of production.

Dr Robert G. Webster then tackled the question, 'H5N1 Avian Influenza: Has the Threat Been Overblown?' His basic answer was 'No', although the virus is now off the front pages of news reports. Avian influenza (AI) is caused by a negative sense RNA virus that possesses a segmented genome and since it has no proofreading mechanisms, replication is error-prone, resulting in antigenic variation. Among the HA subtypes, H5 is currently considered to have the highest capacity to cause pandemic influenza. The currently circulating highly pathogenic (HP) H5N1 virus emerged in Southern China in 1996 and was first detected on a goose farm in Guangzhou Province. In May 1997, the H5N1 virus killed six people in Hong Kong and was detected in wet markets in Hong Kong. The avian virus in its highly pathogenic form has now spread to multiple epicentres in over 60 countries and over 500 million poultry have died or been slaughtered.

It would appear that migratory birds are capable of spreading the virus, but are not the primary cause of the spread, and that 10 distinguishable clades are now being detected, largely in infected domestic poultry. However, it should be noted that domestic waterfowl appear to be the primary source of infection for poultry, although unhygienic conditions in commercial poultry farms and within wet markets are also playing an important role in the global spread of the virus. There is considerable dispute about the causes of the spread of the virus, but its high virulence is clear. While the virus is killing chickens often in less than a day, as well as ducks in 1–2 days, large numbers of people are not being killed because the virus does not at this time have the capacity to spread from human to human due in part to failure to reach the *lower* human respiratory tract (Van Riel et al., 2006; Shinya et al., 2006).

There are many different views, but key issues remain unresolved: What is the reservoir? Who are the spreaders? What is driving the antigenic diversity? Ducks, especially domestic ducks placed in rice fields and then moved to other areas, have become a Trojan horse, carrying influenza viruses, but not being harmed by them, while transferring viruses in a form that is virulent in other species (Gilbert et al., 2008). Finkelstein et al. (2007) examined the common amino acids found in the Spanish 1918, Asian 1957 and Hong Kong 1968 pandemic viruses. There were 13 common amino acids, but only one of them has been found in the PB2 in the Asian H5N1 viruses. However, it should be noted that Finkelstein and his colleagues found changes in PB2, PA, NP, M1 and NS1. So far the H5N1 viruses have not yet acquired the amino acids associated with the past three pandemics.

The Asian H5N1 HPAI virus will probably transmit to poultry in the Americas soon, but the precise date and method of transmission cannot be predicted. There is a low probability that the means of transmission will be migratory birds, some probability from frozen meat, but a high probability of the spread of the avian virus from smuggled birds (including fighting cocks).

Malik Peiris (personal communication) has noted that there is currently a low probability of a highly lethal H5N1 human pandemic, but if it does occur its impact will be catastrophic if over 60% of infected people die, as is currently the situation for the limited number of human cases (Salomon and Webster, 2009).

Silvio Pitlik, MD (Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel) presenting 'One Health, One Medicine – A Clinician's Perspective', pointed out that prior to 1981, in the pre-AIDS era, there were only four papers listed in PubMed on emerging infections. However, there were now more than 1000 per year. The AIDS epidemic is the leading paradigm of emerging infections. Based on old literature data and more recently, on viral archaeology, the primal detection and immediate response to the epidemic was delayed by several decades. With the recent endorsement of the American Veterinary Medical Association of the 'One Health' initiative, the time had come for veterinarians, physicians and scientists to work together on many emerging zoonoses, especially HIV/AIDS (AVMA, 2008).

Dr Pitlik proposed a 4-fold classification of emerging zoonoses: 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). The resulting holistic approach to emerging infections links microbiology, veterinary medicine, human medicine, ecology, public health and epidemiology. All of these disciplines were important to understand how wild animals could transmit disease to both domestic animals and humans, as well as how domestic animals could transmit disease to humans.

The recently revived concept of 'one medicine, one health' implicates a multidisciplinary and co-ordinated response which promises to improve our capacity to deal more effectively with new emerging infections. Veterinarians and physicians can increasingly co-ordinate their efforts to cope with zoonotic threats. For example, during the large outbreak of West-Nile virus infections in Israel in 2000, a multidisciplinary team of public health specialists, veterinarians and physicians acted in a co-ordinated

way to cope effectively with the outbreak. Also, in many sporadic cases of various zoonotic infections, a multidisciplinary approach resulted in a successful management of the clinical cases.

In an overview, '*In Vivo* Disease Modeling of High Containment Pathogens' **Dr Heinz Feldmann** (Rocky Mountain Laboratories, Division of Intramural Research Labs, National Institute of Allergies and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA) pointed out that high containment pathogens posed a significant threat to public health systems, as well as a major research challenge, because of limited experience in case management and a lack of appropriate resources. Dr Feldmann had recently moved from the Special Pathogens Program of the National Microbiology Laboratory of the Public Health Agency of Canada to become Laboratory Chief at the new biosafety level 4 laboratory at Rocky Mountain Laboratories (Backus, 2008). He explained that animal models which mimic disease are key elements for the understanding of the underlying mechanisms of disease pathology, as well as for the development and efficacy testing of therapeutics and vaccines. Studies in reservoir species are rare; and animal models which mimic the reservoir host and the transmission of the pathogen to humans are limited.

Small animal models, in particular the mouse, are the most feasible in high containment and they offer the most options for research due to greater access of immunological and genetic tools. However, their mimicry of human disease as well as their predictive value for therapeutic efficacy is often limited thereby making them, at best, valuable initial screening tools for pathophysiology, treatment and vaccine studies.

Future efforts need to focus on finding more relevant small animal models through pathogen adaptation or by the use of genetically altered host or pathogen species. In parallel, much needed immunological and genetic tools for new animal models should be developed. This would help reduce the numbers and use of large animal models in high containment which is associated with ethical issues, as well as higher exposure risks and higher expenses.

Recent research into the highly pathogenic Marburg and Ebola viruses suggests that fruit bats are a reservoir species (Leroy et al., 2005) carrying these viruses at a subclinical level, which with the appropriate stimulation (e.g. pregnancy, starvation or co-infection) could be reactivated and transmitted (Strong et al., 2008). Considerable progress has been made in developing a live, attenuated recombinant vaccine, making use of Cynomolgus macaques as animal models (Jones et al., 2005). Assessment of the recently reconstituted 1918 influenza virus resulted in high virulence for Cynomolgus macaques. Infected animals mounted an immune response, characterized by disregulation of the antiviral response that was insufficient for protection, indicating that atypical host innate immune responses may contribute to lethality (Kobasa et al., 2007).

Dr Albert Osterhaus (Erasmus University, Rotterdam, the Netherlands), presented a virological overview of 'Research on Respiratory Viruses'. As Morens et al. (2004) had pointed out, respiratory diseases and HIV/AIDS were the two most significant causes of deaths from infectious diseases. It would require 'the ultimate virologist' to summarize the many new human respiratory viruses and consider how to control them. As emerging 'new' respiratory viruses are identified with increasing frequency in many wild and domestic animals, issues of interspecies transmission have become of increasing concern. For example, replication of highly pathogenic H5N1 AI virus associated with more or less severe signs and death has been has been documented in several species of wild ducks (Keawcharoen et al., 2008), domestic cats (Kuiken et al., 2006), red foxes (Reperant et al., 2008) and tigers and leopards (Keawcharoen et al., 2004). The development of safe and effective vaccines for humans and animals has become a priority and besides adjuvanted vaccines, recombinant modified vaccinia virus (Ankara-strains) vaccine proved to be a promising alternative vaccine candidate that could be used for the induction of protective immunity against various H5N1 influenza strains (Kreijtz et al., 2007). International collaboration and co-ordination of all stakeholders are essential for the future control of many emerging infections that cross the species barrier.

In a subsequent morning welcome to participants, **Dr Jon Wefald**, President of Kansas State University expressed his belief that the 'One Health, One Medicine, One World' theme is beginning to resonate around the world and that emerging problems require extensive collaboration between individuals and disciplines. **Dr M. Duane Nellis**, Provost of Kansas State University, praised the work of the Homeland Defense Food Safety and Security Team (Brown and King, 2008; Rhodes, 2008) and stressed the university-wide mission of Kansas State University to address the global food security crisis. **Dr Ralph Richardson**, Dean of the College of Veterinary Medicine, Kansas State University, spoke of the convergence of human disease, animal disease and biosecurity, focusing on the 'Kansas City Animal Health Corridor' between Manhattan, Kansas, Kansas City and Columbia, Missouri with its universities and some 130 companies controlling one-third of the expenditure on animal health in the world. The aim of the College of Veterinary Medicine was 'to reach excellent people, to collaborate and to do things of significance' (see http://www.vet.k-state.edu).

Thomas Thornton, President of Kansas Bioscience Authority concluded the welcomes with a challenge to participants to work together on bioscience work, noting that funding for collaboration was available to those who were ready to 'walk the talk'. In his view, research was the foundation of a bioscience economy, especially as we have much to lose from an incident and much to gain from research. Manhattan, Kansas, with its new Biosecurity Research Institute, was on the final short list for the possible relocation of the National Bio and Agro-Defense Facility from Plum Island NY (Jolley, 2008). (Note: Manhattan, Kansas was selected in December 2008 by the Department of Homeland Security as the location of the new Bio and Agro-Defense Facility).

Dr Paul-Pierre Pastoret [World Organisation for Animal Health (OIE), Paris] concluded the Symposium Opening with a presentation, 'One World, One Health: An OIE Perspective'. Dr Pastoret expressed his strong support for the symposium and congratulated Dr Richt on the opening of his new laboratory. However, both OIE and the Food and Agricultural Organization (FAO) preferred the term 'One World, One Health' to 'One Medicine, One Health', because even though human and veterinary medicines are evolving hand-in-hand, there would always remain differences between them, due to philosophical and economic reasons. Two recent joint OIE/FAO publications set out the OIE/FAO vision (Vallat and Mallet, 2006; New Delhi, 2007). Earlier thematic issues of the OIE's *Scientific and Technical Review* have been devoted to zoonoses and wildlife (Brown, 2004; Hugh-Jones, 2006), as well as climate change (de la Rocque, 2008).

The OIE was created in 1924, with the aim of controlling the international spread of infectious animal diseases. From this original mission, a new mandate has emerged, to improve animal health worldwide, in 172 member countries and territories. This mandate can only be fulfilled if new institutional and technical mechanisms are developed at global, regional and national levels. To succeed, the OIE must now provide policy makers with the right information, arguments and tools in order for political will and good governance to be exercised effectively and in a sustainable manner.

Both food-borne and non-food-borne zoonoses are economically significant, as veterinary medicine works increasingly at the interface between human and animal health. A comprehensive review made at the beginning of this century identified 1415 species of infectious organisms known to be pathogenic to humans, including 538 bacteria and rickettsia, 307 fungi, 287 helminths, 217 viruses and prions and 66 protozoa (Cleaveland et al., 2001). Of these infectious organisms, 868 (61%) were classified as zoonotic; and 175 pathogenic species, of which 75% were zoonotic, were considered to be associated with emerging diseases, the vast majority coming from wildlife. In Dr Pastoret's view, the prevention and control of zoonoses would only be possible with effective implementation of OIE standards and guidelines linked to the World Trade Organization (2008) Agreement on Sanitary and PhytoSanitary measures.

Dr Pastoret reflected that even if animal medicine in the developed world seems nowadays more interested in food safety than food security, we should never forget that there are still developing countries where food security is a major problem. With regard to livestock production, world demand for animal protein (milk, eggs, meat) is expected to rise by 50% by 2020. This growing demand for animal protein will be fuelled by both an increase in the world's human population from 7 to 9 billions by 2050, as well as changing dietary habits for hundreds of millions of poor households in emerging countries who are joining the middle classes. Furthermore, livestock production, often threatened by diseases, plays a considerable role in the survival of poor rural communities in developing countries, where a large percentage of human population still depends on livestock breeding to survive. Effective control of animal diseases in developing countries would help provide access to valuable markets, which are currently closed to those countries not yet able to control or eliminate important so-called 'foreign animal' diseases.

Workshop I: Research Models for Emerging Pathogens

Dr James Swearengen (Association for Assessment and Accreditation of Laboratory Animal Care International, Frederick, Maryland, USA) began the workshop with a presentation entitled 'Animal Models of Infectious Disease: Challenges in Biocontainment'. He considered four challenges: (i) oversight of animal care and use; (ii) veterinary care; (iii) handling potential exposures; and (iv) risk assessment. Facing the first challenge begins with obtaining the necessary membership for the Institutional Animal Care and Use Committee (IACUC). The most problematic issue regarding IACUC membership is identifying a non-affiliated member with the necessary security clearances needed to review classified research proposals should the need arise. From a safety perspective, the IACUC can assist in identifying safety concerns during the protocol review process, especially when animals are expected to become ill and require more frequent handling. Furthermore, it is often difficult to define endpoint criteria, especially when clinical signs progress rapidly. Establishing effective endpoint criteria which do not rigidly confine the investigator should be the goal. Pilot

studies may be a useful tool in collecting the information necessary for this purpose. Infectious diseases often result in pain or distress in animals that must be relieved. If analgesics are withheld, there must be a detailed justification for doing so. This justification should provide specific reasons why the analgesics would prevent the necessary data from being collected and the mechanism of their interference. The IACUC should scrutinize these justifications to ensure they have the information necessary to make an informed, science-based decision.

Second, the strict entry and exit requirements of high containment make veterinary care and health monitoring very labor-intensive. Telemetry and video surveillance are often useful adjuncts to direct observations of animals. Extensive training and mentoring are critical to assuring good veterinary care can be provided safely and in a timely manner. Many times, financial incentives are necessary to obtain the highly trained staff needed to work in a high containment environment.

Third, historical data have shown that the two most significant routes of exposure of personnel to infectious agents in a laboratory setting are by aerosol and percutaneous inoculation (Rusnak et al., 2004a). Special attention should be given to the use of sharps (e.g. needles), sharp edges on equipment and caging and bites and scratches from animals. Risk categories and treatment options need to be established in extensive pre-exposure planning. One described method for pre-exposure planning involves developing categories of exposure risk (e.g. negligible to high) and then applying them in context of the route of exposure (Rusnak et al., 2004b). Animal studies bring with them a whole new set of risk factors when dealing with infectious organisms; and exposures to these organisms must be anticipated.

Fourth, risk assessment needs to be conducted frequently, in some cases daily, with recognition that new safety precautions might be required to face unexpected situations. For example, an animal's disposition can change quickly with the onset of clinical signs, requiring daily risk assessment and development of ways to mitigate new risks as they occur.

While new challenges and risks may occur in a high-level biocontainment environment, diligent planning and forethought can substantially reduce these risks, as well as provide assurance of meeting regulatory requirements for using animals in research.

Dr Michael Katze (Department of Microbiology, Washington National Primate Center, University of Washington, Seattle, Washington, USA) asked 'Can Systems Biology and the Computer Save the World from the Next Pandemic' and responded 'Yes, but it better happen soon'. The viruses being investigated in Dr Katze's laboratories are influenza, Ebola, Marburg, Vaccinia, Herpes Simplex, Hepatitis C, SARS, measles, West Nile Virus (WNV), HIV-1, HIV-2, SIV (Simian immunodeficiency virus) and Lassa. A central question that Dr Katze raised was: 'Can we take advantage of systems biology, computational tools and mathematical models to help the world develop an AIDS vaccine, one quarter of a century after the emergence of HIV?' In his view, the failure to defeat AIDS was significant evidence that virologists were not doing a good job developing effective drugs and vaccines.

Admittedly, the host response to any virus infection is highly complex. Systems biology, when properly understood and practised, could tackle that complexity. A helpful definition of systems biology had been given by Denis Noble (2008): 'Systems biology...is about putting together rather than taking apart, integration rather than reduction. It requires that we develop ways of thinking about integration that are as rigorous as our reductionist programs, but different.... It means changing our philosophy, in the full sense of the term'. In Dr Katze's view, virologists could use systems biology and genomics to: (i) study the global impact of virus infection on host gene expression; (ii) discover cellular regulatory pathways targeted by viruses; (iii) identify new cellular targets for antiviral therapy; (iv) develop new vaccines; and (v) make new discoveries (Tan et al., 2007). In his own laboratory, a long range goal was to develop an 'Influenza and Respiratory Virus Compendium', which would ensure the creation of a centralized data base to catalogue all transcriptional events in cells and organs infected by influenza and other virulent respiratory viruses.

A systems biological tool box should contain four key tools: (i) sequencing of genomes (i.e. the complete set of genetic material in the cell of a living organism); (ii) the use of microarrays (i.e. a high throughput technique to examine global gene expression in cells and tissues *at the RNA level*; (iii) the use of proteomics (i.e. a high throughput technique to examine global gene expression in cells and tissues *at the protein level*); and (iv) the use of bioinformatics (i.e. computational biology to help make sense of massive data sets). At times, the challenge could appear overwhelming, but it was now clear that an integrated approach to viral respiratory disease is a complex three-step process: (i) the use of cell culture, mouse and other animal models to study viral infection; (ii) the combination of traditional histophathological, virological and biochemical approaches with functional genomics and proteomics; and (iii) obtaining the signatures of virulence and insights into mechanisms of host defence response, viral evasion and pathogenesis (Katze et al., 2008).

Much had been achieved. We can indeed identify molecular signatures of disease progression. In select

cases, we can predict disease progression and identify prognostic biomarkers. We can distinguish between infections by highly pathogenic and low pathogenic viruses. We can define distinct mechanisms by which viruses evade the host defences.

Much remains to be achieved. The limitations of classical hypothesis-driven approaches should be recognized. The computer needs to be used for data analysis, not just e-mail and PowerPoint. Our work is not simply about the virus and virus replication and viral encoded *inhibition* of host signalling. We need to recognize that a very early and uncontrolled innate and inflammatory host response often causes the pathology associated with virus infection (even in AIDS): kinetics is vital. To develop effective antiviral therapies and vaccines, we should be targeting *host* pathways rather than viral enzymes. It is important that more computer biologists work together with biologists, as virologists and computational types learn to trust each other.

Opening the symposium in place of Dr Koprowski, **Dr Adolfo García-Sastre** (Department of Microbiology, Department of Medicine, and Emerging Pathogens Institute, Mount Sinai School of Medicine, New York, USA) explained his current research on the 'Inhibition of Type 1 IFN Responses by RNA Viruses'. The type I interferon (IFN) system is one powerful first line of innate immune defence against virus infections. However, many, if not all, viruses, have acquired during evolution IFN antagonist proteins that overcome this host antiviral response. For influenza viruses, the viral non-structural NS1 protein prevents IFN production, while for dengue virus and for nairoviruses, the viral polymerase proteins have acquired domains responsible for inhibition of the IFN system. Interestingly, although all three viruses inhibit the IFN system by diverse and distinct mechanisms, all of them target components of the ubiquitination machinery to achieve IFN antagonism. Subversion of ubiquitination pathways appears to be a general means by which RNA viruses can evade innate immunity.

Dr Thomas Geisbert (Boston University Medical School of Medicine, Boston Massachusetts, USA) presented 'Progress in the Development of VSV-Based Vaccines to Prevent and Treat Ebola and Marburg Hemmorhagic Fever'. He explained that there were currently at least five different vaccine systems showing promise in protecting non-human primates against Ebola and Marburg viruses. These five approaches to filovirus vaccines were based on virus-like particles, DNA, two replication-defective viral vector systems and a replicating virus vector. In the context of replicating virus vectors, a vaccine based on recombinant vesicular stomatitis virus (rVSV) has been shown to protect non-human primates against Ebola and Marburg vaccines, eliciting completely protective immune responses, as well as being effective when the vaccine was administered as a post-exposure treatment (Jones et al., 2005; Daddario-DiCaprio et al., 2006a, Daddario-DiCaprio et al., 2006b).

There were specific advantages to using a rVSV as a vaccine vector: (i) Vaccination with a live virus characteristically elicits strong humoral and cellular responses and results in production of long-lasting memory B cells and T cells; (ii) VSVs are able to accommodate large gene inserts and multiple genes in their genomes without dramatically affecting virus growth; no upward limit to the amount of foreign sequence inserted in the VSV genome has been identified; (iii) VSV's single-stranded RNA genome does not undergo reassortment and therefore lacks the potential of reassorting with wild type viruses *in vivo*; (iv) VSV replicates within the cytoplasm of infected cells and does not undergo genetic recombination; and (v) the seroprevalence of VSV antibodies within the general human population is extremely low and there is a lack of serious pathogenicity in humans.

In the midst of this encouraging initial research, certain questions now need to be investigated: Is there interference between vaccine components? What dose confers protection? What is the mechanism of protection? In the context of safety, what happens with individuals with altered immune systems? Regarding post-exposure treatment, what combination approaches would improve protection and extend the therapeutic window?

Dr Chieko Kai (Institute of Medical Science, University of Tokyo, Japan) outlined the 'Molecular Determinants of Nipah Virus Pathogenicity'. Nipah virus (NiV) is a paramyxovirus that was first discovered in 1999 as a zoonotic virus during an outbreak of infection in pigs and humans in Malaysia. More than a million pigs died or were killed. The biological property of NiV to infect a wide range of hosts and to produce a disease causing significant mortality in humans has made NiV infections a public heath concern.

However, the molecular mechanisms for the high pathogenicity have not yet been fully clarified. Dr Kai and her colleagues have established a reverse genetic system that enabled the rescue of replicating recombinant NiVs from a cloned cDNA, and showed that the rescued rNiV retained the severe pathogenicity in an animal model by experimental infection. By generating a recombinant NiV expressing EGFP, they suggested that additional factors besides the cellular receptor are required for full NiV replication (Yoneda et al., 2006).

They further investigated accessory proteins of NiV (V, W and C). These accessory proteins of paramyxoviruses have been suggested to be involved in pathogenicity; and

the P, V and W proteins of NiV have been shown to function as inhibitors of IFN signalling. To study the role of these accessory proteins in NiV infection, they generated recombinant NiV lacking V, W or C protein by the reverse genetics system. All the recombinant viruses replicated well in cell culture, although the maximum titres of them were slightly different compared with the parental rNiV. The virulence of the recombinant viruses in a hamster model is now under investigation. She concluded that the reverse genetics for NiV provides a powerful tool for the analysis of the molecular mechanisms of pathogenicity and cross-species infection.

Dr James Robl (Hematech Inc, Sioux Falls, South Dakota, USA), with his colleagues **Yoshimi Kuroiwa** and **Poothapillai Kasinathan**, presented 'Production of Recombinant Human Polyclonals in Transchromosomic Cattle'. They explained how a genetically modified bovine system had been created for the production of hyperimmune human polyclonal antibodies. Because of respective limitations associated with using humans as donors, many potential applications of human polyclonal antibodies have not previously been realized. Therefore, it was necessary to incorporate into the cow genome the entire unrearranged human heavy and light chain immunoglobulin loci, as well as inactivate the bovine immunoglobulin genes. A cloned embryo was transplanted into a surrogate cow; transgenic calves were born; the genetically modified cows were immunized; and bulk material was collected.

The detailed process for the production of fully human polyclonal antibodies in cows has a number of important steps. The human immunoglobulin genes are introduced into the bovine genome using a human artificial chromosome (HAC) vector constructed from fragments of hChr14 (heavy chain) and hChr 22 (lambda light chain). The HAC vector is introduced into fetal bovine fibroblast cells using a microcell-mediated chromosome transfer approach. Modified cells are used for embryonic cloning to produce live transchromosomic calves. In this process, the HAC is retained at a high rate and immunoglobulin genes undergo rearrangement and express human antibody. Gene inactivation is performed using a sequential targeting system for fetal fibroblast cells to knock out both alleles of the bovine gene encoding immunoglobulin.

In a more general context, it is useful to note that with genetic modification technologies, multiple genetic modifications can be made without breeding. Genes can be targeted (e.g. bovine genes can be knocked out) and microchromosome transfers can be used to transfer large sequences of DNA (Kuriowa et al., 2004). Thus, the prion gene can be knocked out of cattle, eliminating BSE susceptibility and potential transmittance (Richt et al., 2007). The immunogloblulin heavy chain gene can also be knocked out of cattle, eliminating competition with human antibody production (Kuriowa et al., 2002).

The practicalities of gene modification are straightforward. The gene targeting approach requires two and a half months for one targeting. There is successful targeting of both active and silent genes, with allele-specific targeting. Calves are successfully produced following three rounds of sequential cloning and there is rapid generation of multiple targeting events without germ line transmission. A full health evaluation of each cow is made at planned intervals covering growth rate and a physical examination, blood chemistry, haematology, histopathology, immune system function, brain function and reproduction. It takes about 18 months to produce a live calf.

The HAC vectors used in this process are autonomous replicating vectors, which can carry Mb of DNA and there is no requirement for integration into endogenous chromosomes. The three essential components are a centromere, two telomeres and origins of replication.

The outcome of this on-going research has been that live homozygous knockout calves have been produced by embryonic cloning. Successful application of these technologies will be critical for the development of a bovine system for production of hyperimmune human polyclonal antibodies. These bovine-derived human polyclonal antibodies have therapeutic applications for infectious disease, immune deficiency, organ rejection, toxin/venom neutralization, autoimmune diseases, cancer and biodefense.

Workshop II: Prion Diseases

Dr Cristina Casalone (National Reference Centre of BSE, Istituto Zooprofilattico di Piemonte, Liguria e valle d'Aosta, Italy) presented 'BSE and BASE: An Update', based on her research with her colleagues **Maria Caramelli**, **Cristiano Corona** and **Chiara Porcario**. Bovine spongiform encephalopathy (BSE or Mad Cow Disease) had long been thought to be caused by a single prion strain. Starting from 2004, however, approximately 40 atypical BSE cases, referable to two different strains, defined as H- and L-type, were identified worldwide. Dr Casalone described the two types of surveillance that the Reference Centre in Italy conducts: passive and active. To date, Italy has reported 142 positive cases of BSE from 5 million tested cases with most cases being reported before 2003. In 2004, Dr Casalone's group in collaboration with Dr Gianluigi Zanusso (Department of Neurological and Visual Science, Verona) and Dr Fabrizio Tagliavini (Istituto Neurologico 'Carlo Besta') discovered a different type of BSE very unlike previously reported classical BSE (cBSE) cases. The 'new' BSE was termed Bovine Amyloidotic Spongiform Encephalopathy (BASE) and described as an L-type since the unglycosylated

protein part migrated lower. Dr Casalone noted that BASE localizes in the forebrain, whereas cBSE appears to affect mainly brainstem. (Casalone et al., 2004).

In vivo studies indicate low transmission rates for BASE in wild-type mice. C57/BL mice only became infected after a second passage and then the infection appears to be cBSE-like (Capobianco et al., 2007). In cattle, BASE is transmissible after the first passage and shorter incubation times were reported, suggesting that BASE may be more virulent than cBSE in the natural host (Lombardi et al., 2008). Dr Casalone concluded that BASE is either a sporadic disease or a totally different form of BSE. Distinct molecular phenotypes have been identified in bovine prion diseases (Biacabe et al., 2004) leading to the description of C- and H-type.

In more recent studies, it has been shown that BASE is transmissible to humanized transgenic mice (Kong et al., 2008), whereas Comoy and colleagues (2008) used macaques to illustrate the point. Dr Casalone concluded that BASE has at least the same human health implications as those of cBSE. The good news is that presently both the Italian and US protocols are able to detect/identify both cBSE and atypical cases. As of 30 October 2008, there were 41 atypical BSE cases identified worldwide; 21 L-type, 3 BASE and 17 H-type. Therefore, the preventive measures currently in place for human and animal health protection should be maintained indefinitely.

Dr Michael Clawson (US Department of Agriculture, Agricultural Research Service, US Meat Animal Research Center, Clay Center, Nebraska) discussed the 'Association of a Bovine Prion Gene Haplotype with Atypical BSE' on the basis of his research with various colleagues (Clawson et al., 2008). Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, are a class of fatal neurodegenerative disorders that occur in humans, ruminants, cats and mink. Three distinct TSEs afflict cattle: cBSE, atypical H-type BSE and atypical L-type BSE. Classical BSE was identified in the 1980s and is acquired by cattle through the consumption of feed contaminated with the infectious prion agent. As noted in the previous presentation, atypical BSEs have only recently been recognized as distinct cattle prion diseases and are extremely rare. The full extent of genetic susceptibilities to atypical BSEs remains unknown; however, one atypical H-type case identified in the United States in Alabama in 2006 was most likely caused by a genetic mutation in the prion gene, E211K (Richt and Hall, 2008).

Dr Clawson's group has recently sequenced the prion gene (PRNP) of 192 cattle (including 96 beef and 96 dairy cattle), first, to identify polymorphisms and secondly, to test PRNP variation for an association with BSE (Clawson et al., 2006). They identified 382 polymorphisms in beef cattle and 158 in dairy cattle and subsequently identified an association of a bovine prion DNA sequence (haplotype) with atypical BSE that is independent of E211K. Despite the low frequency of this haplotype among general cattle populations, it was present in a majority of H- and L-type atypical BSE cases from Canada, France and the United States. This result indicates that there is a genetic component to atypical BSE susceptibility in addition to E211K. Dr Clawson concluded that cattle PRNP variation is complex, that there are distinct bovine PRNP alleles associated with both classical and atypical BSE susceptibility and that a portion of BSE can be managed through genetics.

Dr Qingzhong Kong (Department of Pathology, Case Western Reserve University, Cleveland, Ohio, USA) presented 'Chronic Wasting Disease (CWD) and Bovine Spongiform Encephalopathy (BSE): Public Health Risk Assessment' in the light of his research with 18 colleagues. Transmissible spongiform encephalopathy is a family of fatal, transmissible neurodegenerative diseases that affect humans and a variety of animals. The human forms of the disease include Creutzfeldt–Jacob disease (CJD), Gerstmann-Straussler-Scheinker disease, Fatal Familial Insomnia and Kuru. Among animals, the disease takes the form of BSE in cattle, scrapie in sheep and goats and CWD in deer, elk and moose (Collinge, 2001).

It has been shown that TSE results from the misfolding of the highly conserved prion protein (PrP) and that the cellular PrP (PrP^c) is essential for both prion replication and prion pathogenesis. However, the detailed mechanisms of prion replication and pathogenesis remain to be solved. There are neither treatments for prion diseases nor reliable diagnostic tests for early-stage TSE.

Chronic Wasting disease was first discovered in Colorado in 1967 and is endemic in 14 US States and two provinces in Canada. CWD has been experimentally transmitted to other animals, including cattle, sheep, ferrets, mice, hamsters and squirrel monkeys; however, the transmissibility of CWD to humans was unknown. The species barrier for prion transmission is determined by the PrP sequence homology between the donor and the recipient as well as the prion strain. The research by Kong and his colleagues with humanized and cervidized transgenic mouse models indicates the current natural CWD strain has a very low possibility of direct transmission to humans with PrP-129MM; however, novel cervid strains highly infectious to humans could emerge.

Dr Kong then explained his studies with both cBSE and atypical BSE (BASE) cases (Kong et al., 2008). Atypical BSE has been found in eight European countries, as well as in the USA, Canada and Japan. His on-going research with humanized Tg mice demonstrates that both L- and H-type atypical BSE strains are transmissible to humans and the L-type seems to be much more virulent

than the cBSE strain in humans. No transmission of the E211K mutant BSE has been observed thus far.

Dr Alan Young (Department of Veterinary Science, South Dakota State University, Brookings, South Dakota, USA), supported by six collaborators, continued the study of TSEs in Workshop II with his study, 'Fixed and Migratory Leukocytes in the Pathogenesis of TSEs'. To better understand the role of the immune system with regard to prions, they sought to answer three questions: (i) By what mechanism is PrP^{res} delivered to the germinal centres? (ii) What are the molecular events occurring in PrP^{res}-infected germinal centers? and (iii) How is PrP^{res} disseminated systemically throughout the lymphoid system?

In their initial experiment, Dr Young and his lab team cannulated sheep afferent lymphatic vessels to define the transport of PrP^{Sc} from the local tissue to the regional lymph node. They demonstrated that prion entry is via the afferent lymphatics within 24 h of injection. Transport was achieved through cell-free delivery in the first 15 min and through cell associated delivery pathway peaking 12–24 h following injection.

In the second set of experiments, Dr Young's group cannulated the efferent lymphatics of lymph nodes infected with sheep scrapie prions to determine the potential role(s) of circulating lymphocytes in prion pathogenesis (Young et al., 1997). Lymph was collected from the efferent duct, and changes in the leucocyte subsets were monitored. They concluded that measurable changes in the output of B cell subsets following a scrapie infection suggested a specific local response to prion infection.

In the final series of experiments, Dr Young's group tracked the kinetics of PrP^{Sc} appearance in the lymph node germinal centres following injection. They observed the immediate localization of PrP^{Sc} in the germinal centers within 30 min of inoculation. Interestingly, highly susceptible sheep (Q171Q genotype) could maintain PrP^{res} in the node for up to 2 months post-injection, whereas sheep with a Q171R genotype only maintained the PrP^{res} in the node for 1–2 weeks. This result points towards susceptibility and resistance to scrapie.

Dr Young concluded that the data from these experiments suggest a two-phase pathogenesis for prion disorders. In the first phase, prions are transported to regional germinal centers similar to other antigens; and in the second phase, prions appear to replicate within the lymphatic tissue of susceptible but not resistant animals, thus establishing long-term infection of affected lymph nodes (Catron et al., 2004; Pape et al., 2007).

Dr Anumantha Kanthasamy (Department of Biomedical Sciences, Iowa Center for Advanced Neurotoxicology, Iowa State University, Ames, Iowa, USA) ended the Workshop II presentations with a study supported by six colleagues, 'Divalent Metals Stabilize Cellular Prion Proteins and Alter the Rate of Proteinase-K Dependent Limited Proteolysis' (Kanthasamy et al., 2008). The key biochemical event in the pathogenesis of prion diseases is the conversion of normal cellular prion proteins (PrP^{c}) to the proteinase K (PK) resistant, abnormal form (PrP^{sc}); however, the cellular mechanisms underlying the conversion remain enigmatic. Binding of divalent cations such as copper to the octapeptide repeat regions of PrP has been shown to be important for the stability of the protein (Choi et al., 2006). Nevertheless, the roles of other divalent cations in the normal processing of cellular PrP^{c} are not well understood.

Dr Kanthasamy and his team examined the role of divalent metals (Mn^{2+} and Cu^{2+}) on PrP^{c} expression and degradation in cell culture and brain slice models. Neuronal cells expressing mouse prion proteins with a genetically altered novel epitope (mAb 3F4) and brain slices were exposed to Mn^{2+} and Cu^{2+} over 24 h. Levels of PrP^{c} protein and mRNA were measured. Limited proteolysis, mRNA stability, proteasomal activity and pulse-chase experiments were conducted. The objectives of these experiments were: (i) to determine the effect of cellular prion on mitochondrial dysfunction and oxidative damage, (ii) to evaluate whether or not divalent metals influence the stability of the prion protein, and (iii) to understand the role of such metals in the pathogenesis of prion diseases.

Interestingly, his studies concluded that manganese enhances the stability of the prion protein. During metal exposures, cellular prion protein levels increase without an increase in mRNA levels or a decrease in protein degradation. The effect of manganese on the prion protein is different from other metals such as cadmium. Additionally, the normal prion protein protects neuronal cells against manganese induced acute neurotoxic response. These experiments support the hypotheses that certain divalent metals may play a critical role in the pathogenesis and progression of prion diseases.

Workshop III: Bunya and Influenza Viruses

Dr Richard Elliott, working with his colleague **Dr Xiaohong Shi**, both of the Centre for Biomolecular Sciences, University of St. Andrews, of St. Andrews, Scotland presented 'Engineering the Bunyavirus Genome'. Bunyaviruses are characterized by a tripartite, negative sense RNA genome and impinge on (i) human health by causing diseases such as encephalitis (e.g. La Crosse virus) and haemorrhagic fever [e.g. Crimean–Congo haemorrhagic fever virus and Rift Valley fever (RVF) virus]; (ii) animal health by causing diseases such as RVF, Cache Valley and Akabane viruses or (iii) crop plants by causing diseases such as tomato spotted wilt virus. This family contains

prime examples of emerging and re-emerging viruses, including the hanta viruses presented next.

Dr Ellott's group developed a highly efficient reverse genetics system to recover the prototype bunyavirus, Bunyamwera (BUNV), from cloned cDNA (Lowen et al., 2004) and this system was subsequently adopted by others to recover La Crosse and RVF viruses. They exploited this technology to engineer the BUNV genome to create viruses carrying specific marker tags in different viral proteins. Thus far they have recovered recombinant BUNV expressing epitope-tagged L protein (Shi and Elliott, 2009) and NSm (Shi et al., 2006) and Gc proteins (X. Shi, J. van Mierlo, A. French and R.M. Elliott, unpublished) fused to green fluorescent proteins. These recombinant viruses are useful tools to monitor the time course of BUNV infections, as well as illustrating the plasticity of the Bunyamwera virus genome.

Dr Ramon Flick, Chief Scientific Officer of the BioProtection System Corporation, Ames, Iowa and formally the Director of the BioScience Level 4 Facility of University of Texas Medical Branch, Galveston, Texas, explained how 'Hantavirus Reverse Genetics Challenges Replication Dogma'. Hantaviruses belong to the *Bunyaviridae* family of viruses, are transmitted by aerosolized rodent excreta or rodent bites and are a major international public health concern. Their ability to produce serious, often fatal, human disease underlines the possible use of these viruses as biological weapons and the need for a system that allows manipulation of the viruses. Basic research empowers the identification of antiviral targets, the screening of relevant antiviral compounds and the creation of subunit vaccines. Furthermore, as set out in the first three presentations of Workshop III, the advent of reverse genetics of negative-stranded RNA viruses has made it possible to manipulate these viruses at will and to evaluate the effects of these changes on the biology and pathogenesis of many viruses, as well as the efficacy of antiviral and prophylactic measures (Walpita and Flick, 2006).

A reverse genetics technology for Hantaan (HTN) virus, the prototype of the genus *Hantavirus*, has now been established (Flick et al., 2003). This system consists of HTNV genome segment-based minigenomes containing different reporter genes. The viral proteins necessary for minigenome replication are provided by either HTNV-superinfection (helper virus-driven system) or by co-transfected HTN-L/N expression plasmids (plasmid-driven system). Overall, the surprising results question previously accepted dogmas in the negative strand RNA virus field: (i) the highly conserved parts of the genome segment ends are described as the putative promoter region for segmented negative-stranded RNA viruses; however, minigenomes lacking these regions are still able to serve as a template for efficient viral replication and (ii) the viral nucleoproteins described as absolutely essential for viral replication processes (the naked RNA genome cannot serve as a template for the viral RNA polymerase) are not required as *trans*-acting factors for efficient minigenome transcription and replication. This knowledge is an important step towards the understanding of the special features of the hantaviral replication cycle and for the development of targeted antiviral drug strategies.

Dr Stuart T. Nichol, Chief of the Molecular Biology Laboratory, Special Pathogens Branch, National Center for Zoonotic, Vector-borne and Enteric Diseases, Centers for Disease Control and Prevention (CDC) Atlanta, Georgia, USA set out a study of 'Rift Valley Outbreak Dynamics and Reverse-Genetics Generated Vaccine' in which he and 18 colleagues had collaborated (Bird et al., 2008a,b). Rift Valley fever virus is a mosquito-borne human and veterinary pathogen associated with large disease outbreaks throughout Africa, Madagascar and the Arabian Peninsula. Infection of livestock can result in sweeping 'abortion storms' and high mortality among young animals. Human infection results in self-limiting febrile disease that in ~1–2% of patients progress to more serious complications including hepatitis, encephalitis, retinitis or a haemorrhagic syndrome with high fatality.

The most recent large RVF outbreak occurred in eastern Africa in 2006–2007, particularly Kenya, southern Somalia and Tanzania. Following initial RVF virus laboratory confirmation, a high-throughput RVF diagnostic facility was established at the Kenyan Central Veterinary Laboratories in Kabete, Kenya, to support the real-time identification of infected livestock and to facilitate outbreak response and control activities. Detailed analysis of 3250 livestock and wildlife specimens in Kenya showed evidence of RVF infection in almost 10% of animals tested across 23 Districts. The complete S, M and/or L genome segment sequence was obtained from 31 representative RVF virus specimens. Analyses revealed the concurrent circulation of multiple virus lineages, gene segment reassortment and common ancestry of the 2006–2007 outbreak viruses with those from the 1997–1998 eastern Africa RVF outbreak. Evidence of recent increases in virus genomic diversity and gene pool size 2–4 years prior to the 2006–2007 outbreak was also found, indicating ongoing RVF virus activity and evolution during the inter-epizootic/epidemic period.

Such findings highlight the need for safe and effective vaccines. Reverse-genetically produced double-deleted vaccine was tested. All vaccinated animals subsequently challenged with a high dose of virulent RVF virus survived infection and could be serologically differentiated from naive experimentally infected animals by the lack of NSs antibodies. These rationally designed marker RVF

vaccine viruses show great promise for use in combating this significant veterinary and public health threat.

Dr Ruben Donis of the Molecular Virology and Vaccines Branch, Influenza Division, Center for Disease Control (CDC) Atlanta, Georgia, USA presented an analysis of 'Influenza Pandemic Preparedness: Early Detection and Vaccination'. He began by underscoring the possibly catastrophic impact of a severe and unmitigated pandemic in the United States: 1.9 million deaths, 9.9 million hospitalizations, 40 million people in outpatient care and a total of 90 million people infected (33% of the US population). Worldwide, between 175 and 350 million deaths are possible (Meltzer et al., 1999; Fedson, 2005). Seven steps could help mitigate such a pandemic: (i) medical care; (ii) community intervention; (iii) social distancing; (iv) personal protective equipment; (v) targeted use of antiviral drugs; (vi) pre-pandemic vaccine; and (vii) pandemic vaccine. Within this framework of possible mortality mitigation programmes, the CDC has set out five influenza pandemic preparedness goals and objectives: (i) prevent or delay a pandemic; (ii) detect and report a pandemic, seeking to decrease the time needed for detection; (iii) investigate the course of a pandemic, seeking to decrease the time needed to determine risk factors and appropriate interventions; (iv) control a pandemic, decreasing the time needed to provide countermeasures; and (v) recover from a pandemic (CDC, 2008).

The two inherent viral risk factors that might cause a pandemic are influenza virus reassortment and genetic drift. Genetic changes caused by either reassortment of a particular virus or mutations in the HA or other genes could lead to increased pathogenicity and the possibility of sustained human-to-human transmission. Of particular concern is the possible reassortment of human H3N2 viruses with avian H5N1 viruses (Chen et al., 2008). Mutations in HA adapt the avian virus to bind sialic acid receptors in human respiratory cells (Stevens et al., 2006). Structural differences near the HA receptor binding pocket alter the effect of mutations in receptor binding specificity. For example, when comparing A/Vietnam/1203/2004 with A/Indonesia/05/2005 viruses, there are multiple amino acid differences near the pocket that may affect receptor binding. Research is continuing, but there is some evidence that recent avian H5N1 viruses are exhibiting an increased propensity for acquiring human receptor specificity by mutations in the receptor binding site (Stevens et al., 2008).

The evolution of influenza A viruses has considerable impact on vaccine production and distribution. Protection for both human and animal hosts is mediated by neutralizing antibodies to HA. Protective immunity is strain-specific and the vaccine antigen must match circulating virus strains. The efficacy of pre-pandemic stockpile is compromised by the rapid change in the antigenic properties of the viruses (antigenic drift). Three H5N1 candidate vaccines for Clades 1, 2.1 and 2.3 have been distributed by CDC to manufacturers; some clinical trials have been completed; others are underway to determine their efficacy. Multiple genetically distinct H5N1 clades co-circulate in birds and any of them could emerge as a pandemic, especially as the H5 evolution in birds leads to rapid diversification of these viruses. Therefore, permanent surveillance and updating of the CDC vaccine seed library is necessary to maximize antigenic match and the efficacy of an eventual pandemic vaccine.

Dr Hana Weingartl of the National Centre for Foreign Animal Disease (NCFAD) of the Canadian Food Inspection Agency and the Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada presented a paper on 'Human 1918 Pandemic Influenza in Experimentally Infected Swine' (Weingartl et al., 2009). The aim of the paper was to increase understanding of the natural history of the pandemic influenza virus during the twentieth century and its interspecies transmission. A better understanding of the biology of the 1918 virus will encourage recognition of the risk potential of circulating animal viruses to initiate human pandemics, leading to novel targets for therapeutic and prophylactic intervention.

It was only in the early 1930s that the 1918 influenza virus was clearly identified as a viral disease in both people and swine (Shope, 1931; Smith et al., 1933; Laidlaw, 1935). Genomic sequence data suggest that 1918 genes are all avian-like and that a change of receptor specificity in 1918 from avian to mammalian required only a few amino acid changes in the HA. Speaking on behalf of the 11 co-authors, Dr Weingartl explained the methodology of determining the virulence of the rescued 1918 (r1918) pandemic human influenza H1N1 virus, which was related to the A/swine/Iowa/15/1930 rescued virus (r1930). Both above viruses were rescued using the plasmid-derived reverse genetic technology. Mice, ferrets and macaques infected with the r1918 virus died, but pigs did not develop severe respiratory distress or become moribund. All work with the 1918 pandemic influenza virus was performed in the NCFAD biosafety level 4 facility. The clinical disease of r1918 was similar to the disease introduced by the r1930 H 1N1 virus.

The resulting data support the hypothesis based on phylogenetic analysis that the human pandemic influenza virus which began in the spring of 1918 is likely an ancestor of the classical swine H 1N1 influenza virus. The human 1918 pandemic influenza virus might have originated directly from an unknown avian or another source (e.g. via swine), rapidly acquiring the minimal adaptive changes required to start a pandemic, possibly

just before or quickly after the cross over to humans. Precisely how and when the human virus went into swine, or the swine virus went into humans, is not fully understood. However, it is clear that improved virus surveillance in humans, other mammals and birds is critical to prevent inter-species transmission in the future.

Dr Gregory Gray MD of the Center for Emerging Infectious Diseases, University of Iowa College of Public Health, Department of Epidemiology, Iowa City, Iowa, USA set out 'Evidence for Swine, Avian and Equine Influenza Virus Infections in Man'. In a series of epidemiological studies among workers with intense exposure to animals (Gray et al., 2008), Dr Gray and his colleagues found considerable evidence that swine workers (Myers et al., 2006; Gray et al., 2007), poultry workers (Ortiz et al., 2007), hunters (Gill et al., 2006) and veterinarians (Myers et al., 2007) have experienced zoonotic influenza A virus infections. While a majority of these infections were either mild or subclinical, these epidemiological data suggest that animal workers have the potential to contribute to the generation of novel viruses and serve as a bridging population for influenza virus between animals and humans.

Mathematical modelling has demonstrated that such workers may accelerate the spread of pandemic viruses (Capuano et al., 2007). The body of research indicates that public health interventions are essential to mitigate the public health risks. The highest priority is to focus resources on country-specific epidemiological studies targeted at understanding both disease and transmission in animals and humans. Studies are required to identify risk for humans, linked with surveillance in industrial settings, village farms and markets (Planning Group, 2005). Cross-species virus transmission has clearly caused the emergence of new epidemic diseases (Parrish et al., 2008). The transfer of viruses to new hosts may take many years, but is of great significance, as, for example, in the recent efficient transmission of equine influenza virus to dogs (Crawford et al., 2005; Enserink, 2005).

Dr Wolfgang Garten of the Institute of Virology, Phillips-Marburg University, Marburg, Germany spoke of 'Viral Glycoproteins Cleaved by Cellular Proteases'. Dr Garten's presentation focused on glycoproteins of viruses that are contained within lipid envelopes of enveloped viruses (Klenk et al., 1984). He began by pointing out that glycoproteins of many lipid-enveloped viruses are proteolytically cleaved by host cell proteases (i.e. proteolytic enzymes) to gain their biological functionality. Most viral glycoproteins are cleaved at a single distinct peptide motif within the ectodomain to attain fusion capacity. For example, in the context of the influenza virus, the level of receptor-dependent restriction of an AI virus in the human airway epithelium can be estimated; and these estimates support a theory that alteration of the receptor specificity of an avian virus could facilitate its human-to-human transmission (Matrosovich et al., 2007). Some viral proteins acquire additional properties, for example, a selective incorporation into the virus envelope.

Within this framework of how viral proteins acquire additional properties, Dr Garten explained that additional cleavages may also occur. Therefore, it is necessary to investigate further the cleavage sites of viral glycoproteins, the responsible cellular proteases, the localization of the cleavage events, the roles of glycoprotein cleavage and the inhibition of cleavage. It is essential to understand how the different protease inhibitors interfere with the proteolytic processing of viral glycoproteins and thus with virus replication. For HIV-1, the role of furin and PC/LPC (a serine endoprotease of the subtilisin family, expressed in most mammalian cells) is of particular importance in initiating infection (Hallenberger et al., 1992, 1997).

Further work at the Institute of Virology by Dr Garten and his colleagues considered influenza virus pathogenicity (Garten and Klenk, 1999, 2008), proteolytic processing of Marburg virus glycoprotein (Volchkov et al., 2000) and how the Lassa virus matrix protein is the driving force for virus particle release (Strecker et al., 2003). Much of this basic research has considerable practical implications in developing new strategies to combat virus infection (Boettcher et al., 2009). Dr Garten reported the tree-dimensional structure of the matrix protein of Borna disease virus (BDV), which is a tetramer and possesses the capacity for binding of an RNA of about 16 nucleotide length as well as lipid (Neumann et al., 2009).

Workshop IV: Emerging/Reemerging Diseases

Dr Bernhard Dietzschold of the Department of Microbiology and Immunology at Thomas Jefferson University, Philadelphia, Pennsylvania, USA, presented 'Borna Disease and Rabies: Two Old Reemerging Zoonotic Virus Infections' about two of the oldest infectious diseases on record that are now reemerging in many parts of the world. BDV or a BD-like virus was recently detected in both wild and domesticated psittacine birds (Honkavuori et al., 2008, Kistler et al., 2008), while canine rabies is rapidly spreading throughout Southern and Eastern Africa with devastating effects on public and animal health. Both BDV and rabies virus (RV) are highly neurotropic in nature, negative strand viruses and are able to infect a large variety of mammals, probably including birds.

Professor Dietzschold pointed out that BDV and RV have huge differences in their respective pathogenesis even though these two diseases share many common features. For example, RV replicates in the cytoplasm, whereas BDV replicates in the nucleus. Secondly, immu-

nopathological lesions in the central nervous system (CNS) are the major pathogenic mechanism in Borna disease; yet lesions are not significant for the pathogenesis of RV. Thirdly, RV causes an acute encephalomyelitis that may be transmissible to humans, whereas Borna disease is characterized by a chronic/persistent infection that appears to be non-transmittal to humans. And lastly, T_H1 cells play a predominant role in BDV pathogenesis as well as in the immune defence against BDV. With RV, neutralizing antibodies were also thought to be the major player in the immune response against rabies, but recent data obtained from mouse studies have suggested that a complex interaction of both innate and adaptive immune responses seem to be responsible for RV lesions in the CNS.

While human rabies has become a worldwide public health problem, it is questionable whether BDV is a pathogen for humans. With both diseases, there is a need to develop new antivirals and vaccines (Bajramovic et al., 2002; Cenna et al., 2008).

Dr Christiane Herden, from the Institute of Pathology of the University of Veterinary Medicine in Hanover, Germany and the Institute of Veterinary Pathology of the Justus-Liebig-University in Giessen, Germany, working with her colleagues, **Dr Dirk Schaudien** and **Prof. Wolfgang Baumgärtner**, as well as **Prof Ulrich Eisel** of the Department of Molecular Neurobiology, University of Groningen, the Netherlands, presented a talk titled 'Experimental Borna Disease Virus Infection of Mice with Neuronal TNF Overexpression' regarding the work they had recently completed. Borna Disease Virus was first recognized over 200 years ago, it is named after an infectious outbreak among cavalry horses in 1894–1896 in the town of Borna in Saxony, Germany. BDV belongs to the family *Bornaviridae* and is an enveloped, negative-sense, single stranded RNA virus that both replicates and transcribes in the nucleus of cells. BDV has a linear (non-segmented) genome containing an overlap of ORFs and transcription units. BDV uses read through and extensive RNA splicing during its life cycle.

There has been a long debate whether or not BDV could infect humans. Early findings indicated that the sera of psychiatric patients contained high levels of BDV antibodies (Rott et al., 1985; Van de Woude et al., 1990), but upon further investigation, the human sequences were almost identical to laboratory strains of BDV indicating sample contamination and not human exposure (Richt et al., 1997; Lieb and Staeheli, 2001; Schwemmle, 2001; Dürrwald et al., 2007). Dr Herden and her colleagues believe that the successful isolation of virus or nucleic acids from human samples has not been confirmed.

Dr Herden and her colleagues hypothesized that elevated levels of tumour necrosis factor α (TNF) in the CNS had a significant impact on the clinical signs of BDV as it has already been documented that elevated TNF levels play a role in various other CNS diseases such as Alzheimer, AIDS, dementia and MS. To test their theory, they used TNF-transgenic mice that expressed TNF under the control of the NR2B-subunit of the NMDA-glutamate receptor. They concluded that neuronal overexpression of TNF leads to enhanced CNS inflammation when exposed to BDV and somehow modifies the neuromodulatory pathways resulting in spontaneous epileptic seizures.

Dr Konstantin G. Kousoulas, of the Division of Biotechnology and Molecular Medicine at the School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana, USA, presented 'A New Genetic Vaccine against West Nile Virus' which summarized his recent research with 10 collaborators (Iyer et al., 2009a). West Nile virus was first isolated more than 70 years ago from a patient in the West Nile province of Uganda (Smithburn et al., 1940). Public, equine and animal health became threatened when WNV emerged in regions of Europe and North America (Lanciotti et al., 1999). The most serious manifestation of WNV is encephalitis (inflammation of the brain) which can be fatal to humans and equine and sometimes birds. WNV is a positive sense RNA virus belonging to the family *Flaviviradae* (Lindenbach et al., 2007) and its transmission cycle uses the mosquito as a vector.

Vesicular stomatitis virus has been extensively utilized as a viral vector system for the induction of protective immune responses against pathogens. In this study, Dr Kousoulas and his colleagues constructed a recombinant VSVs expressing either the Indiana or Chandipura virus G glycoprotein and the WNV envelope (E) glycoprotein, as this protein is the major antigenic determinant involved in virus binding and fusion (Ledizet et al., 2007). Mice were vaccinated with the new construct, challenged with virulent WNV-LSU-AR01 isolated in Louisiana (Iyer et al., 2009b) and extensive immunological analyses were performed using polychromatic flow cytometry staining. The results suggest that VSV-vectored vaccines administered intranasally can efficiently induce protective humoral and cellular immune responses against WNV infections in mice.

Dr Barbara Sherry of the Department of Molecular Biomedical Sciences, College of Veterinary Medicine at North Carolina State University, Raleigh, North Carolina, USA presented 'The Cardiac Innate Response to Viral Infection' discussing her work with collaborators **Zurney, Li, O'Donnell, Sevinsky, Stephenson**, and **Dermody**. Viral myocarditis occurs in 2–20% of the human population, although generally it is fatal only in infants (Esfandiarei and McManus, 2008). Investigations of virus–host

interactions for emerging pathogens are often carried out in immortalized 'generic' cell lines. However, Dr Sherry and her colleagues have found that in a mouse model of viral myocarditis, the host innate protective response determining disease outcome is both cell type- and organ-specific.

Three reovirus studies were conducted. First, in their mouse model, Dr Sherry found that the IFN-β response determines protection against myocarditis (Sherry et al., 1998). Interestingly, cardiac myocytes express high basal levels of IFN-β which pre-arms this vulnerable, non-replenishable cell type. In contrast, adjacent cardiac fibroblasts express high basal levels of proteins in the IFN response pathway, making these cells highly sensitive to IFN and preventing viral replication and spread within the heart. This suggests evolution of an integrated network of cell type-specific innate immune components for organ protection (Zurney et al., 2007).

Second, reovirus was found to induce increased myocarditis but decreased encephalitis in mice lacking NF-κB. The increased cardiac damage resulted from impaired viral induction of IFN-β in these mice. This study demonstrates an organ-specific role for NF-κB in the host response to viral infection (O'Donnell et al., 2005).

Third, a proteomic approach was used to find proteins involved in the cardiac response to viral infection. As a result, heat shock protein 25 (HSP25) was identified as a potential antiviral factor relevant to cardiac myocytes. HSP25 is anti-apoptotic and is known to be modulated by members of seven different virus families. The range of viruses that induce HSP25 expression or phosphorylation, together with the new observation that reovirus induces HSP25 degradation in cardiac myocytes support a possible cell type-specific antiviral function.

Together, these three reovirus studies demonstrate that the innate immune response to viral infection in mice is both cell type- and organ-specific, suggesting that results from studies of emerging pathogens in cell lines should be interpreted with caution.

Dr Raymond R. R. Rowland of the Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine at Kansas State University, USA, speaking on behalf of his colleague **Dr Richard Hesse**, as well as **Dr Ying Fang** of the Center for Infectious Disease Research and Vaccinology of South Dakota State University, gave a presentation titled 'PCV2 and PRRSV: Emerging and Re-emerging Viruses in Pigs: New Paradigms in Infectious Disease'. Over the past thirty years, as the swine industry has moved from small farms to large multi-site confinement operations, new viruses have emerged (Rowland, 2007; Tian et al., 2007). Two important diseases for pigs in confinement are porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Each of these diseases possesses two geographically distinct genotypes and are persistent and robust, because these diseases are able to maintain a low-level infection and remain resistant within a population. Therefore, pigs are usually infected with both viruses.

The study was conducted by constructing phylogenic trees of 20 North American PRRSV-1 isolates over a 5-year period. All but two of the isolates possessed the same 51 nt deletion in nsp2, which suggests a clonal origin. Similar results were found with PCV2. This finding will allow the use of deletions in the nsp2 as a marker for the evolution of PRRSV (Kim et al., 2007). Experimental studies incorporating dual infections show that PRRSV infection increases PCV2 viraemia. In understanding the interaction of viruses with their hosts, it is important to take into consideration polymicrobial interactions.

Detection, control, remediation and prevention of food animal infectious disease are essential for animal health, food safety and the prevention of bioterrorism. Dr Rowland expressed strong support for the One Health Initiative, a worldwide strategy for expanding interdisciplinary collaborations and communications in health care for humans and animals (See: http://www.onehealthinitiative.com/about.php). This approach seeks to identify emerging diseases with zoonotic potential, connect syndromes in animals and humans with aetiological agents and then develop appropriate diagnostic and therapeutic tools for veterinary and human medicine. In this context, work has recently begun at Kansas State University, with Professor Jürgen Richt as Project Director, to co-ordinate the activities of faculty in the Colleges of Veterinary Medicine, Arts and Sciences, Engineering and Agriculture.

Dr Bernd Kitze of the Department of Neurology, Goettingen University, Goettingen, Germany presented 'T-cell/B-cell Cooperation and Chronic Inflammatory CNS Diseases'. Chronic inflammatory neurological diseases result from immune reactions within the CNS that are caused by either chronic viral infections or by autoimmune mechanisms or both. Viral agents causing inflammatory CNS diseases include measles, rubella and Human T-Lymphotropic (HTLV-1) viruses, while autoimmune inflammatory CNS diseases include multiple sclerosis and CNS vasculitis.

In this particular presentation, the focus was upon HTLV-1 associated diseases (Kitze and Brady, 1997) and Multiple Sclerosis. For both diseases, there is a great deal of cooperation of B- and T-cells. This cooperation requires small amounts of antigens to maintain an intracompartment immune response. The attack of the immune system eventually results in bystander damage of the CNS cells as well as clinical signs and symptoms of disease (Kitze and Usuku, 2002).

Whether viral or autoimmune, CNS diseases are restricted to individuals who carry certain immune

response genes, especially those from the HLA complex which have been studied in detail. The immune response can be monitored by analysing the cerebrospinal fluid. At present, the standard therapies available for these diseases are Type 1 IFNs and steroids. However, in the near future, more sophisticated immunological therapies will become available.

Dr Klaus Osterrieder of the Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, New York, USA presented a study of 'Emergence of Lethal Herpesvirus Disease Caused by a Mutation of the DNA Polymerase'. Equine Herpesvirus Type 1 (EHV-1) causes respiratory disease, abortion and neurological symptoms (Nugent et al., 2006). More specifically, this disease affects the ciliated respiratory epithelials and nasal mucosa. The disease first enters the horse's respiratory tract, spreads to the lymph nodes and establishes latency. A single nucleotide polymorphism resulting in an amino acid variation is associated with the neuropathogenicity of naturally occurring strains (Goodman et al., 2007; Van de Walle et al., 2009). The key question is: Does this single amino acid exchange (N752/D752) by itself influence neuropathogenicity?

In this study, infectious clones of different EHV-1 strains were used. A previously natural D752 variant, strain Ab4, was rendered into an N752 mutant virus. Also, a natural N752 virus, strain NY03 was mutated into a D752 virus. A blinded challenge of EHV-1 was performed on a group of seronegative mixed-breed horses. Neurological examinations were performed and resulted in no symptoms present in the N752 group; however, in the D752 group, fever, inflammation of the CNS, ataxia and serous nasal discharge were present. This study also found that N752 mutants are highly sensitive to Aphidicolin, a drug which targets viral polymerases.

The results of this study indicate that D752 has a significant replicative advantage when compared to the N752 strains. Moreover, this single amino acid variation (N752D) in a herpesvirus replicase is not only necessary but also sufficient for expression of the neuropathogenic potential, without having a major effect on virus shedding from infected animals. This is important when looking at the spread of the disease within a population. The D752 genotype is underrepresented amongst EHV-1 isolates in the United States at about 15% and has been stable over the past 50 years. Therefore, the emergence of a more virulent pathotype is unlikely.

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