Journal of Veterinary Science & Technology

Gale et al., J Veterinar Sci Technolo 2014, 5:4
DOI: 10.4172/2157-7579.1000195

Review Article Open Access

Future Risk Assessments for the Impact of Climate Change on Vector-Borne Viruses of Livestock: Potential of Genomics, Transcriptomics and Proteomics Approaches – A Review

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Abstract

Climate and habitat change, together with globalisation, are important factors in the emergence of arthropod-borne viruses (arboviruses). Changes in the range and abundance of the virus, vectors and hosts may create new combinations of host-pathogen-vector interactions for which data are not available. This paper reviews the genomics data currently available for arboviruses, their vectors and hosts, and assesses the level of understanding of the genetic factors affecting their adaptation to climate. It is anticipated here that genomics, transcriptomics and proteomics approaches may enable the breakdown of the traditional risk assessment pathways into the individual biochemical steps for each of the three interfaces between the virus, vector and host such that future risk assessments could be based on looking for certain gene combinations and their resulting expression profiles. Differences in the virus interaction with the arthropod midgut have been implicated in specifically affecting the extrinsic incubation period for some arboviruses, while differences in viral replication and dissemination through the arthropod may affect vector competence for other arboviruses. Genomics approaches to identify the proteins involved will enhance our understanding of vector competence and may explain why some arbovirus genotypes are more efficiently replicated in the vector at elevated temperatures. Such studies may also reflect the diversity present in real-world systems to a greater extent than experimental systems involving a single combination of vector and virus genotypes. Understanding the genetic basis for tissue tropism will facilitate prediction of new routes of transmission.

Keywords: Vector-borne; Livestock; Genomics; Transcriptomics; Proteomics

Introduction

The emergence of vector-borne viruses of livestock is driven by various combinations of events including environmental and climatic changes together with increased globalisation and transport of goods and persons [1]. Climate change may directly affect vector-borne epizootic viruses through its impact on the geographical distribution and abundance of arthropod vectors and other types of wildlife which serve as host reservoirs or routes for introduction. Indirect effects include the potential impact of climate change on land use, agriculture and farming practice (livestock production and introduction of different breeds) and also its influence on human and animal behaviour.

A major problem encountered in the development of risk assessments for the impact of climate change on the emergence of arthropod-borne viruses (arboviruses) is the lack of data for those unique and novel combinations of vectors, virus, habitats, climate conditions and vertebrate hosts that will be encountered [2]. A key question is whether the indigenous vector populations can transmit an arbovirus which has entered a new region, for example, through an infected livestock animal or exotic vector. The ability of the virus to evolve and adapt to new niches and vectors is of great importance in this respect. Temperature itself has a direct effect on viral replication rates and vector competence [3,4] and serves as a selective criterion for emergence of viral genotypes with increased vectorial capacity, for example West Nile virus (WNV) in the United States [5,6].

Over the last decade the development of next generation sequencing techniques has spawned a huge increase in the amount of genomics information available. The objective of this review is to consider the potential for the genomics and related transcriptome and proteome information gathered for livestock, vectors and vector-borne viruses to be used to fill data gaps in future risk assessments. The focus is on risk assessments for the impact of climate change and particular reference is given to adaptation to climatic factors.

Current Approaches: Genomics, Transcriptomics and Proteomics

The emerging field of functional genomics is concerned primarily with understanding how DNA sequence and gene-expression variation is linked to, or determines, observable, biologically relevant phenotypes [7]. RNA transcripts and their expression levels link an organism's genotype and phenotype. RNA interference (RNAi) is one means to test the link between gene expression and phenotype [7] and transient RNAi-mediated gene-silencing has become the method of choice for functional characterisation of candidate innate immune genes in arthropod vectors, for example. Microarrays are used to map which mRNA molecules (transcriptome) are present, and in what quantities, in certain cells under specific conditions and at different, measured time points. By comparing the transcriptomes, for example, in uninfected and infected vector cells, those cellular genes and proteins which are selectively expressed during the infection process

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Received May 19, 2014; **Accepted** September 20, 2014; **Published** September 22, 2014

Citation: Gale P, Ulrich RG, Wilson A (2014) Future Risk Assessments for the Impact of Climate Change on Vector-Borne Viruses of Livestock: Potential of Genomics, Transcriptomics and Proteomics Approaches – A Review. J Veterinar Sci Technol 5: 195. doi:10.4172/2157-7579.1000195

may be identified. Proteomics approaches are used for the identification of differences in the amounts of specific proteins produced in infected and noninfected cells mostly through mass spectrometry methods. Caporale et al. [8] have established an experimental platform using reverse genetics for investigating the molecular determinants of bluetongue virus (BTV) virulence in murine models.

Genomic Resources Currently Available

Vector-borne viruses

Arbovirus sequences are generally available by direct sequencing soon after isolation of a virus. Unlike the arthropod vectors and livestock hosts, the arbovirus genomes are diverse and rapidly evolving due to mutations. Most arthropod-borne viruses of livestock have RNA genomes. Most RNA viruses mutate rapidly (yellow fever virus (YFV) being an exception) due to the absence of proof-reading activity in the viral RNA-dependent RNA polymerase (RdRp) [9]. The mutation rate is not constant and, for example, differs in Crimean-Congo haemorrhagic fever virus (CCHFV) with African strains seeming to evolve faster than strains from other geographic regions [10]. The rapid mutation rate of RNA viruses gives rise to quasispecies. Many livestock arboviruses including BTV, Rift Valley fever virus (RVFV), Akabane virus (AKAV) and CCHFV have segmented RNA genomes. The coinfection of a host or vector with two or more strains of the same segmented virus may lead to the production of progeny strains combining aspects of each parental strain through reassortment of the segments.

Arthropod vectors

The availability of genomics resources for arthropod vectors reflects their importance as vectors of human disease with the most information available for mosquitoes. Genome sequences have been published for the mosquitoes *Anopheles gambiae* (principal vector of *Plasmodium* parasites), *Aedes aegypti* (YFV and dengue virus (DENV) vector), *Culex pipiens* (WNV vector) and *Cx. quinquefasciatus* (vector for WNV and St Louis encephalitis virus) [11,12]. Genetic linkage maps are available for the North American vector *Cx. tarsalis* (WNV vector) and *Ae. albopictus* [13]. The web-based VectorBase [14] currently gives downloads for a number of expressed sequence tags (ESTs) and genome data for mosquito species.

The current genomic resources available for ticks are limited [15], being predominantly ESTs available at VectorBase [14]. So far, most sequencing has been done for *Ixodes scapularis* (the principal vector in North America for *Borrelia burgdorferi*) although this is highly fragmented and some sequencing has been done for *Rhipicephalus* (formerly *Boophilus*) *microplus* and *Dermacentor variabilis* [15]. The substantial size of tick genomes is a significant obstacle. The genome size of *R. microplus*, a vector of *Babesia* and *Anaplasma* parasites, is estimated at 7.1 billion base pairs (Gb) in length, more than twice as long as the entire human genome [16] and that of *I. scapularis* is 2.1 Gb [15]. The genome size of *Ixodes ricinus* has not been determined yet [15]. The transcriptomes (sialomes) from salivary glands of fed and unfed ticks *I. scapularis, Amblyomma variegatum* and *Rhipicephalus sanguineus* have been described [17].

Little molecular data are available for *Culicoides* biting midges, and even the size of the *Culicoides* genome remains unknown despite the economic importance of the livestock viruses e.g. BTV and African horse sickness virus (AHSV), which they transmit.

Livestock

There are extensive resources available for cattle, *Bos taurus* [18]. Genome sequences are now available at a range of coverages for horse, *Equus caballus,* and domestic pig, *Sus scrofa domestica*, as well as a draft version of the genome for sheep, *Ovis aries* [19].

Interfaces for functional genomics approaches

Since the pathogen, livestock host and vector interact in a "three-way triangle" functional genomics studies may be broken down to three levels [16] namely the vector-pathogen interface, the host-pathogen interface, and the host-vector interface. Each interaction can be viewed as a "molecular battlefield" involving receptor proteins, proteolytic enzyme cascades, cells and proteins of the immune system and even nucleic acids in the form of small RNA regulatory pathways (SRRPs).

The vector-virus interaction

Vector competence is the intrinsic permissiveness of a vector to infection, replication and transmission of a virus [20]. Understanding the competence of local vector populations for arboviruses is central for risk assessment. Laboratory experiments to measure vector competence are time-consuming and expensive and require special biocontainment laboratories. Genomics approaches and transcriptome analysis of the midgut tissues and salivary glands, in particular, will enable the identification of those genes and proteins required for the component steps of the virus-vector interaction including binding of the virus to its receptor in the vector midgut, infection of midgut cells, the arthropod antiviral and immune responses, virus assembly, cell-to-cell spread and infection of the vector salivary glands. These steps are completed within the extrinsic incubation period (EIP) which must be within the lifetime of the vector for successful transmission. The EIP is the time interval between when a vector imbibes an infectious bloodmeal (and is infected) and when it first becomes capable of transmitting the virus to a new host. Considerable genetic variation exists among populations of *Ae. aegypti* in terms of its competence to be a vector for DENV [21]. Mercado-Curiel et al. [22] identified a midgut protein of molecular weight 67 kDa (called R67/R64) in *Ae. aegypti* mosquitoes that binds DENV and may be the receptor as it is related to vector competence. The heat shock protein (Hsp) 70, is the putative receptor for mediating Japanese encephalitis virus (JEV) infection in human hepatocytes [23]. It is tempting to speculate that Hsp70 serves as receptor for JEV in the mosquito midgut, where the heat from a blood meal prompts its synthesis, although the constitutively expressed heat-shock cognate (Hsc) 70B suppresses replication of the arbovirus, o'nyong-nyong virus, in *An. gambiae* [24]. Colpitts et al. [25] identified 203 genes in the *Ae. aegypti* mosquito that were >5-fold differentially up-regulated and 202 genes that were >10-fold differentially down-regulated during infection with one of three flaviviruses studied including WNV and DENV. Furthermore the virus-regulated gene expression was found to be tissue-specific in terms of relative rates of expression in the midgut, abdomen and salivary gland [25]. Recently, gene silencing has been used to identify some endosomal pathway proteins involved in Sindbis virus assembly and cell-to-cell spread in mosquitoes [26]. Over the last decade, the molecular mechanisms that regulate the insect immune response have been increasingly elucidated. SRRPs control key aspects of development and anti-viral defence in mosquitoes [26] and have implications for arbovirus vector competence in mosquitoes. Mosquitoes generate viral small-interfering RNA (siRNA) when infected with RNA arboviruses.

Given their compact genomes, DENV and other flaviviruses probably require an extensive number of host factors. Interestingly there appears to be conservation of host factors for DENV replication in dipteran and human hosts [27]. Although arboviruses have to infect

two genetically diverse metazoans, namely the arthropod vector and the vertebrate host reservoir, genomic plasticity is surprisingly high for some arboviruses [28]. The complete genomes of 13 geographically and temporally diverse isolates show CCHFV strains are highly variable with 20% (8%), 31% (27%) and 22% (10%) of nucleotide (amino acid) differences detected among the virus S (nucleocapsid), M (glycoproteins) and L (RdRp) genome segments, respectively [28]. The finding of CCHFV in nature in a range of hard ticks, including *I. ricinus* in Turkey [29], in addition to *Hyalomma* spp. ticks (the main vector for CCHFV), is of significant interest in relation to genetic variability and adaptation to new tick vectors. Indeed, it should be noted that the remarkable genetic variability of CCHFV isolate CT9 in Iran was attributed to amplification of the virus in *R. sanguineus* [30]. Genomics information that could demonstrate why in molecular terms CCHFV could not be transmitted by *I. ricinus* would be invaluable in our understanding of the limits of range expansion of this virus [31].

The livestock (host)-virus interaction

Understanding the molecular mechanisms for heritable variability in host resistance to viral infection is important and underpins the dose-response relationship in traditional risk assessments. A potential application of genomics approaches with great promise is not only in being able to estimate the dose-response for the more susceptible individuals in a host population but also in determining the proportion of the population that they represent. This has previously been demonstrated for infection in humans by Norwalk virus, which is not vector-borne, by Lindesmith et al. [32]. CCHFV infection in adult mice missing the type 1 interferon receptor ($IFNAR^{-/-}$) resulted in acute, fatal disease compared to asymptomatic infection in wild-type mice. Of interest for risk assessment was that $IFNAR^{-/-}$ mice had up to 1,000-fold higher levels of CCHFV RNA in their blood and other organs [33]. Knowledge of the magnitude of the titres of CCHFV in the blood of a viraemic host is important for understanding the probability of infection of feeding ticks. For example, the overall CCHFV infection rate of 4.4% recorded by Logan et al. [34] for larval *Hyalomma truncatum* ticks after engorging on viraemic newborn (wild-type) mice is presumably related to CCHFV titres in the blood. Thus, the percentage of *Cx. tarsalis* females infected increased with the log of the titre of WNV in the donor birds at the time of blood-feeding [4].

Understanding the molecular determinants affecting BTV-host interactions and pathogenesis is currently incomplete [8]. The strain of BTV-8 in northern Europe in 2006 was unique in causing disease in cattle. Traditionally BTV causes disease in sheep. Even within individual sheep breeds, there are major differences in the severity of clinical signs of bluetongue displayed by individual animals [8]. Bluetongue outbreaks occur more frequently at the edges of areas where BTV is endemic because the populations are more immunologically naïve. Genetic differences between distinct BTV serotypes can also influence the virulence of these viruses and the BTV-8 that spread across Europe recently is extremely virulent. Grant et al. [35] demonstrated a direct relationship between viral loads for DENV and pathogenesis in immunocompromised mice. Moreover phenylalanine at residue 52 in the DENV NS4B RdRp protein confers virulence of DENV through enhancement of viral RNA synthesis in mammalian cells but not in mosquito cells [35].

Reassortment of CCHFV genome segments may affect pathogenicity of the virus to humans [36]. Point mutation-driven amino acid exchanges in the variable regions of the envelope protein of flaviviruses in the tick-borne encephalitis group probably reflect adaptation to different host species [37]. Thus the closely-related louping ill virus, Turkish sheep encephalitis virus and Greek goat encephalitis virus may have escaped their ancestral rodent transmission pathway in forests to infect sheep and goats which served, on nearby upland grazing areas, as amplification hosts for the tick vector, *I. ricinus*. Direct adaptation to a new host species was central in the emergence of Venezuelan equine encephalitis virus (VEEV) where a single amino acid change in the E2 envelope glycoprotein appears responsible for the adaptation of rodent viruses to horses [9].

The adaptation of arboviruses to replicate at elevated temperatures facilitates utilisation of new host species, for example birds [6], which may be important in range expansion. Thus, replication of a WNV strain from Kenya in cell culture was reduced 6,500-fold at 44 °C relative to levels at 37°C, while replication of the strain of WNV originally introduced into North America (NY99) was only reduced by 17-fold [38]. Kinney et al. [38] suggest that the ability of the natural temperature-resistant strain, NY99, to replicate at high temperatures could be important in the increased avian virulence of the NY99 genotype. Thus mean body temperatures of WNV NY99-infected American crows (*Corvus brachyrhynchos*) ranged between 42 and 44°C [38].

Different strains of virus may exhibit different tissue tropisms in mammal hosts. For example, African horse sickness can be classified into pulmonary, cardiac, mixed or febrile forms, with pulmonary disease associated with the highest mortality. AHSV strains isolated from the lungs of infected horses are more virulent than isolates from the spleen [39]. Similarly BTV-8 originating from the 2006 European outbreak can cross the ovine placenta and infect the foetus during early and mid-gestation [40] and transplacental transmission has been demonstrated in cattle [41]. In addition, the field strain from the 2006 European outbreak was unique among field strains in crossing the placenta with relative ease compared to other wild type BTV strains [40].

The vector-livestock (host) interaction

Recent progress in transcriptome research has shown that hard ticks have hundreds of proteins expressed in their salivary glands [42], the majority of which have no known function, and include many novel protein families. Some of the 3,500 putative salivary proteins catalogued undoubtedly assist blood feeding by overcoming the host barriers which include blood coagulation, complement activation and inflammation (which initiates host defensive behaviour). These host defense pathways involve proteolytic cascades that are regulated by serine protease inhibitors called serpins and must be overcome to complete feeding. Mulenga et al. [43] identified at least 45 tick serpin genes in the *I. scapularis* genome of which 40 are differentially expressed in salivary glands and/or midguts. A tick salivary protein called subolesin helps tick survival on the host [44] and an antiserum to the recently discovered mosquito ortholog of subolesin reduced mosquito survival by 11 to 29% [45]. Lombardo et al. [76] have shown that the *An. gambiae* salivary gland protein 6 (gSG6) is secreted with the saliva while the female mosquito probes for feeding. Reducing the expression of gSG6 in salivary glands by adding siRNA resulted in increased probing time and reduced blood feeding ability. The mosquito's body temperature increases dramatically when it takes a blood meal from a warm-blooded, vertebrate host. A common protective response in mosquitoes is synthesis of Hsp70 in the midgut [47]. Indeed RNAi-mediated suppression of expression of *hsp70* impairs digestion of the blood meal.

There is evidence for heritable differences in the attractiveness of livestock to arthropods. Haematophagous arthropods locate their hosts using visual, thermal and chemical cues. The attractiveness of hosts

has been shown to vary between and within livestock populations as a result of size [48] and semiochemical production [49] and different vector populations vary in their response to semiochemicals [50]. The susceptibility of cattle to ticks is heritable and even after repeated infestations, susceptible breeds harbour significantly higher numbers of ticks than do resistant breeds [51]. Several bovine major histocompatibility complex class II alleles have been associated with tick resistance with an association between lower tick numbers and certain BoLA-DRB3.2 alleles [52]. Gasparin et al. [53] have mapped the quantitative trait loci (QTL) controlling resistance to the tick *R. microplus* on bovine chromosomes 5, 7 and 14. Understanding the genetic basis for host attractiveness and host resistance to ticks would help assess vector-livestock host contact rates in risk assessments.

Understanding the genetic bases for adaptation to climatic factors

Adaptation of the virus

Arboviruses are predisposed to be sensitive to temperature due to the small size, ectothermic nature and short life-cycle of the arthropod vectors. Well-documented examples of the effect of increasing temperature on shortening the EIP of livestock viruses include BTV in *Culicoides* midges [3] and WNV and Western equine encephalitis virus (WEEV) in *Cx. tarsalis* [4], although transmission of WEEV decreased at 32°C due to modulation [54]. Of considerable interest is the increased replication efficiency of different WNV strains at elevated temperatures. Thus while replication of NY99 was more efficient at warmer temperatures than that of a South African strain [4], the genotype WNV02 which was first detected in 2001 and spread across the USA, was more efficient than the genotype NY99. Kilpatrick et al. [5] demonstrated that the proportion of *Cx. pipiens* mosquitoes transmitting virus could be modelled with a degree-day term with temperature raised to the fourth power, and that almost double the proportion of *Cx. pipiens* mosquitoes transmit WN02 compared to NY99. Thus warmer temperatures increased the advantage of the WN02 genotype over the NY99 genotype virus with transmission by *Culex* mosquitoes accelerating sharply with increasing temperature.

Environmental temperatures are typically less than those encountered by the RdRp during virus replication within the mammalian or avian host and differences in the rate of RdRp activity at those lower temperatures experienced in the arthropod could directly affect the EIP. The relationship between temperature and activity may be determined by the structure of RdRp. Thus, temperature sensitive mutants of DENV map to the NS5 RdRp protein [55] and a single amino acid substitution in the RdRp of AKAV conferred temperature sensitivity such that activity was reduced by 80% at 37°C and by 99% at 40 °C compared to wild type virus [56]. However, other viral components in addition to RdRp may be involved. Thus, NS4B is a small non-structural protein coded for by WNV that is hypothesised to participate both in viral replication and evasion of the host innate immune system. Wicker et al. [57] reported that a single amino acid exchange (cysteine to serine) in the NS4B protein of WNV was associated with a temperature sensitive phenotype at 41 °C as well as attenuation of the neuroinvasive and neurovirulence phenotypes in mice.

Adaptation of the vector

Ae. albopictus is an important vector for a number of human pathogenic arboviruses, and populations exhibit extreme variation in adaptive traits such as egg diapause, cold hardiness, and autogeny (ability to mature a batch of eggs without blood feeding). The genetic basis of some of these traits has been established, and Sutherland et al. [13] have produced a high-resolution linkage map which may allow in-depth genetic analyses of the genes underlying these complex traits. The evolution of late season reproductive arrest (diapause) among female *Cx. pipiens* mosquitoes allows them to overwinter in temperate climates, while females of the sibling species *Cx. quinquefasciatus* do not exhibit the diapause phenotype [58]. QTLs for diapause and three life history traits were identified and compared for genome positions in the *Cx. pipiens* complex [58]. The genome of *Cx. quinquefasciatus* with 18,883 protein-coding genes is 22% larger than that of *Ae. aegypti* and 52% larger than that of *An. gambiae* with multiple gene expansions for olfactory receptors and salivary gland genes [12]. Comparison of genomes from different mosquito species will enable assessment of whether specific functions are common to all mosquitoes or perhaps unique to individual species, genera or higher taxa. This information may help with development of future risk assessments in terms of understanding the characteristics of mosquito vectors and their potential adaptation to climatic variables.

Immature mosquito development and survival of adults are highly sensitive to environmental temperature. Several genes of Hsp families are upregulated in *Ae. aegypti* at 42°C and may be crucial in responding to stress induced by elevated temperature [24]. Eggs of *Ae. aegypti* resist desiccation surviving for several months under dry conditions. The serosal cuticle contributes to mosquito egg desiccation resistance. Formation of chitin may be a component step [59]. The *Ae. aegypti* chitin synthase A gene possesses two alternatively spliced variants, AaCHSIa and AaCHSIb, which are differentially expressed during *Ae. aegypti* embryonic development. Rezende et al. [59] demonstrated that at the moment of serosal cuticle formation, AaCHSIa is the sole variant specifically expressed. Goltsev et al. [60] performed whole-genome transcriptome assays with isolated serosa from *An. gambiae* embryos. They presented evidence that the serosal cells secrete a dedicated serosal cuticle, which protects the *An. gambiae* embryos from desiccation. Detailed temporal microarray assays of mosquito gene expression profiles revealed that the cuticular genes display biphasic expression during *An. gambiae* embryogenesis. The Hsps have also been shown to play a critical role in dehydration tolerance in three mosquito species, *Ae. aegypti*, *An. gambiae* and *Cx. pipiens* [61]. Knockdown expression of the *hsp70* gene resulted in females only being able to survive a 28% water loss instead of a 36% loss.

Adaptation of the livestock host

Livestock behavioural traits including tail-biting in pigs and feather-pecking by hens are affected by environmental stresses including density which may be in turn be affected by climate change due to requirements for shade, water and food [2]. Livestock density would affect the contact rates between individual livestock animals and aggressive behavioural traits would increase the risk of exposure of livestock to virus in an infected individual. This is of potential importance for those vector-borne viruses that may be transmitted by direct contact, e.g. African swine fever virus. Genomic approaches may increase our understanding of the genetic basis for the effects of stresses on livestock behaviour. Thus, Flisikowski et al. [62] have identified two sub-haplotypes of the dopamine D4 receptor that are associated with feather pecking behaviour in hens. The linked deformed epidermal autoregulatory factor 1 gene represents another candidate gene for feather pecking. Livestock behaviour and density could also affect the rate and nature of contacts of livestock animals with arthropod vectors.

Changing climatic conditions such as increasing temperature and drought will stress livestock, and breeds will be selected on the basis

of their ability to withstand these stresses. Thus some sheep breeds are more tolerant to heat with a temperate breed showing higher rectal temperature and respiration rate together with depressed serum thyroxine concentrations at 33.8°C compared to a tropical breed [63]. Different breeds also differ in their susceptibility to vector-borne pathogens. Indeed, the disease bluetongue was unknown until susceptible European breeds were introduced into BTV-enzootic parts of Africa, and in general "improved" European breeds are highly susceptible compared to African and Asian breeds [64]. Similarly, there is considerable variation in the susceptibility of the different breeds and genotypes of cattle, sheep and goats within the African continent to RVFV. Genomics approaches which can predict which breeds would be selected in response to changing climatic conditions could be used to assess the susceptibility of those breeds to different pathogens.

Potential Applications of Genomics in Risk Assessments for the Impact of Climate Change on Vector-Borne Diseases

In the future it may be possible to break down the classical risk assessment pathways for an arbovirus, for example, those presented for CCHFV in Gale et al. [31], and to build in the molecular interactions and individual biochemical processes at the levels of the three interfaces between the host, vector and virus. These could then be mapped back to specific loci on the genomes of the vector, the host and the virus. In this respect, future risk assessments may be looking for certain gene combinations and their expression profiles. Collectively, these technologies potentially offer a paradigm shift in the way that risk assessments for emerging viruses and their responses to climate change can be conducted.

Altered tissue or organ tropisms of the virus may facilitate novel transmission routes in new geographical regions following introduction. For example, tropism for placental tissue may increase the likelihood of transplacental transmission, while tropism for arthropod ovaries may increase the likelihood of transovarial transmission. In this respect, the potential for reassortment of RNA segments between different strains of BTV is important because the BTV-8 strain which caused outbreaks in north-western Europe between 2006 and 2009 has the capacity for transplacental (vertical transmission) in bovine and ovine hosts. This enabled the northern European 2006 strain of BTV-8 to overwinter in regions where its *Culicoides* vector is seasonally absent [65]. There exists a risk of reassortment between this BTV-8 strain and cocirculating strains of other serotypes which could result in the capacity for transplacental transmission spreading horizontally into strains against which no vaccine is presently available. Understanding the genetic basis for tissue tropism will facilitate prediction of new routes of transmission.

Changes in climatic variables, particularly temperature, directly affect the R_0 (basic reproduction ratio) not only through changes in the ratio of vectors to hosts and the biting and mortality rates of the vectors but also through changes in the replication rate of the virus and hence the EIP in the vector. Unlike in vertebrate host cells, there is a trade-off between a shorter EIP and a greater mortaility rate of the vector at elevated temperature. Thus, at 25°C only 5% of *Cx. tarsalis* survive for 8 or more days [54] while the median EIP of NY99 in that mosquito species is 10 days [4]. Some arbovirus strains replicate more efficiently in the vector at higher temperatures and the EIP was up to 4 days shorter for WN02 compared to NY99 in both *Cx. tarsalis* and *Cx. pipiens* at 27°C [66]. Comparison of RdRp genes from virus strains that are better adapted to replication at higher temperatures with those of temperature-sensitive phenotypes in conjunction with the three dimensional (3D) protein structure could shed light on how the polymerisation rate is affected by temperature and thus facilitate categorisation of virus strains according to their replication efficiency at different temperatures. Crystal structures of the RdRp catalytic domain have been published for DENV [67] and WNV [68]. Evidence against RdRp being a rate-limiting factor for the WNV EIP is that after intrathoracic inoculation there was no difference in EIP between the WN02 and NY99 strains in *Cx. pipiens*, suggesting that differences in their interactions with the mosquito midgut are important [66]. A shorter EIP at elevated temperature could reflect disruption of the cell junctions in the midgut epithelium which would allow leakage of the virus and hence more rapid infection of the salivary glands [5]. This however, would not necessarily explain the observed differences between WNV genotypes in transmission rates in *Cx. pipiens* at elevated temperature. Furthermore, the rate-limiting step in vector competence may differ between viruses. Thus, Cox et al. [20] have shown that the midgut binding potential in *Ae. aegypti* is the same for four genotypes of DENV and suggest that differences in vector competence are due to differences in viral replication and dissemination through the mosquito.

Future investigations on arbovirus RdRp should take into account the fidelity of replication at different temperatures. Indeed, Aggarwal et al. [69] observed that avian influenza A virus RdRp showed increased fidelity at lower temperatures (37°C compared to 42°C). Coffey et al. [70] describe a unique high fidelity variant of Chikungunya virus (CHIKV) with a single amino acid change in the NSP4 RdRp that increases replication fidelity and generates populations with reduced genetic diversity. In mosquitoes, CHIKV variant with high fidelity RdRp presents lower infection and dissemination titres than wild type. In newborn mice, high fidelity CHIKV produces truncated viraemias and lower viral loads in organs.

Where climate change (in combination with other factors such as increasing globalisation and changes in land use) affects the range, distribution and abundance of the pathogen, vector and host, new and unique combinations of host-pathogen-vector interactions will be tested. The rapid mutation rate of RNA viruses enables adaptation to dynamic environments through selection. For example, in CHIKV and VEEV single amino acid substitutions in the envelope glycoproteins affect the range of mosquito species which the virus is able to use as vector [71,72]. Such alterations would thus enable these viruses to adapt to changes in vector range. Recently some detail of the envelope glycoprotein heterodimers at 22 Å-resolution has been published for RVFV [73]. Knowledge of the 3D protein structure facilitates understanding the interactions of viral surface proteins and cellular receptors, although highly similar viral proteins within a single virus family can adapt to engage different receptors resulting in altered tropism and pathogenicity [74]. Although predictions on how single amino-acid substitutions within the envelope glycoproteins of the arbovirus affect vector specificity may be difficult, the currently available techniques of reverse genetics will enable experimental testing. Equally important for risk assessment is understanding the contraints on RNA virus evolution. Thus RNA viruses cannot find solutions to all major adaptive challenges and evolution of new modes of transmission, for example, is rare [9]. Furthermore, there are trade-offs to consider. Thus a single amino acid exchange in the NS4B RdRp protein of DENV while enhancing replication in mammalian cell lines resulted in decreased replication in mosquito cells [6].

Local adaptation between an arbovirus and its vector will undoubtedly be an important consideration in vector competence,

particularly where novel combinations of vector and pathogen occur. Thus, vector-driven selection may play an important role in shaping the genetic diversity of DENV [46] and specific vector genotype x virus genotype (G x G) interactions may promote adaptation of viral lineages to local mosquito genotypes in genetically diverse, natural populations. The results of Lambrechts et al. [46] challenge the general relevance of conclusions from laboratory systems that consist of a single combination of mosquito and DENV genotypes. In this respect, understanding the G x G interaction is of major importance for vector competence in risk assessment, and using genomics approaches in the future may enable reflection of the diversity of "real world" systems over experimental systems. Predicting vector competence may be complicated further by the fact that serine proteases in two mosquito species (*An. stephensi* and *An. gambiae*) may have changed roles in that the effect of serpin knockdown on the malaria parasite is different [75].

There is some genetic information on temperature, drought and desiccation resistance in mosquito eggs which may assist in assessing the outcome of the movement and expansion in range of mosquito vectors around the world through diapause and drought resistant eggs e.g. *Ae. albopictus* eggs in car tyres [1]. However, information on those genes which limit different midge or tick species to specific climatic ranges is generally lacking at present.

Acknowledgements

This work was supported by the EU Network of Excellence, EPIZONE (Contract No FOOD-CT-2006-016236). We thank our colleagues in EPIZONE Work Package 7.4 including Paul Phipps of Animal Health and Veterinary Laboratories Agency, UK for helpful discussion. Anthony Wilson is also funded by the Biotechnology and Biological Sciences Research Council (grant number BBS/B/00603).

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