Climate Changes and Emerging Wildlife-Borne Viruses in Norway:

Facts, Uncertainty and Precaution




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Climate Changes and Emerging Wildlife-Borne Viruses in Norway: Facts, Uncertainty and Precaution

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"The words of Dr. Gro Harlem Brundtland, former director of the World Health Organization (WHO), were indeed prophetic. In her speech at the United Nations Global Leadership Awards on April 19, 2001, she stated that in a modern world, bacteria and viruses travel almost as fast as money. With globalization, a single microbial sea washes over all humankind and there are no health sanctions. In actuality, that sea washes not over just all humankind, but also across all animal and environmental domains" (AMVA 2008).

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I. Preface

Globally spoken, some of the most burdening diseases of wildlife animals, livestock and human populations are caused by viruses with hosts and reservoirs in wildlife vertebrates like small rodents and bats. Many of these viruses are transmitted within and between vertebrate species by blood-sucking invertebrates such as mosquitoes, ticks and midges.

Until now, the occurrence and distribution of such viruses have, in a relative sense, been restricted to areas within the tropical, subtropical and temperate lower latitude regions of the planet. Through its impacts on competent virus hosts, reservoirs and vectors climate is a decisively important determinant for the distribution of a given virus. Suboptimal temperature is probably the most important single barrier to northwards spread of viruses that may have substantial influence on ecosystem as well as society health and resilience.

Through the ongoing global warming, and a diverse set of climate changes, the ecosystems and societies of northern Europe will most certainly be confronted with invading, potentially harmful, vertebrate- and invertebrate-vectored viruses. The human, livestock and wildlife populations in our part of the world will have no former evolutionary experience with, and may lack immunological protection against, the invading viruses.

The scenarios and prospects that are presented in this report call for risk assessment, governance and prevention according to a precautionary strategy. The approaches must be based on increased epi-disciplinary research efforts, including participants from all relevant scientific fields within the biological, medical and veterinary areas. Furthermore, the hosts, vectors and viruses do not respect any national or political borders. Accordingly, international cooperation within research, surveillance and monitoring will be totally essential for protection of biological diversity as well as ecosystem, animal and human health. There are huge knowledge gaps with regard to the effects of climate changes on local, regional and global virus-host-vector interactions and adaptations.

This report was made possible by a commission from the Norwegian Environment Agency (formerly the Directorate for Nature Management). It took substantially more time than stipulated to finalize the report, and I wish to thank our coworkers in the Agency for their support as well as their patience. I am grateful to my close friend and colleague Reidar Mehl for photos and valuable inputs. Likewise, my friend and colleague Thomas Bøhn has delivered substantial inputs and support. Finally, I gratefully acknowledge the contributions of Anne Myhr and Katrine Jaklin, which have resulted in the attractive design of the final report.

Contents

II. Abstract

Vector-borne viruses are threatening global biodiversity as well as ecosystem, wildlife, domestic animal and human health. The emergence and re-emergence of such viruses and their connected human and domestic animal diseases have been demonstrated nearly worldwide. Over 70% of emerging viruses are zoonotic in origin, i.e. viruses that originate in wildlife or domestic animals, and jump species barriers either directly or via arthropod vectors. In the case of viruses, further adaptation to a new species may increase the transmissibility and change their capability to cause disease. The emergence/re-emergence of such viruses is associated with complex factors, such as i) viral recombination, reassortment and mutation, leading to more virulent and adaptive strains; ii) urbanization and human activities creating more permissive environment for vector-host interaction; and iii) increased air travel and commerce. Climate is a major factor in determining the geographic and temporal distribution of virus vectors and host animals, the characteristics of vector and host life cycles, the consequent dispersal patterns of associated viruses, the evolution of the viruses; and the efficiency with which they are transmitted from vectors to vertebrate hosts. Although there are some different points of view with regard to the relative impacts of climate warming and other anthropogenic ecosystem changes, all *scientists emphasize the urgent need for greater understanding of the ecology of vector-borne viruses in order to understand and predict the effects of future climate changes and their impacts on the environment.*

In Norway, as well everywhere else, the present and future threats from vector-borne viruses must be mitigated by priority actions such as improving pre-emergence surveillance, monitoring and early response, establishing collaboration and communication inter-sectorally, and strengthening the prevention and control programmes along with improving biosafety aspects with regards to highly infectious nature of these vector-borne diseases. Evidence from research needs to be generated and priority areas for research defined.

Within the last twenty years several new vector-borne viruses emerged in Europe, and they may, assisted a.o. by climate changes, reach Northern Europe and Fennoscandia in a foreseeable future. In most cases the exact origin and route of the introduction remains unknown. Migratory birds are the suspected carriers of West Nile- and Usutu flaviviruses, while in the case of Bluetongue virus, the spread of serotype 8 to Western Europe, included Norway, still remains unclear. Besides the local transmission, certain insects may also be involved in the long distance spread of arboviruses, either by travelling on vehicles, ships and airplanes, or carried by the wind (e.g. midges).

Due to climatic changes, new arthropod species may adapt to the more moderate climate. They may overwinter and become residents in Europe, and also in Northern Europe and Norway. These arthropods may be competent vectors for emerging viruses. Some of these were not present in these regions before, and therefore the vertebrate host populations (including humans, domestic and wildlife animals) are highly susceptible for, and have no immunological protection against such

infections. The diagnostic and control measures (available tests, vaccines, treatment) for emerging viruses are to a much lesser extent available yet, compared to those for viruses that are already resident in Europe. The recent European Ckikungunya, Blue-tongue, West Nile and Usutu virus outbreaks are warning us that so-far exotic viruses with similar ecology, e.g. Dengue virus(es), Rift Valley fever virus, and a number of others, may also emerge in Europe. That may cause serious wildlife, human and animal disease outbreaks or "silent" aberrations in ecosystem balance and resilience capacity.

Knowledge about impacts of emerging vector-borne viruses on indigenous wildlife vertebrate/invertebrate vectors and reservoirs, or the food webs they are parts of, is extremely rudimentary and scarce all over the world. Furthermore, the distribution, ecology and impact of several resident, or indigenous, vector-borne viruses already circulating within ecosystems in Northern Europe, Fennoscandia and Norway, are not thoroughly investigated yet. Resident viruses may recombine with emerging relatives to create new viral progenies with unpredictable consequences for wildlife, domestic animal and human disease or fitness. Another field of universal knowledge gaps is connected to the fact that vector, host and reservoir animals may be infected with two or more vector-borne viruses at the same time. Whether, and to which extents, this may result in unpredictable interactive (e.g. synergistic) impacts on ecosystem, wildlife, domestic animal and human health conditions, in our part of the world, is completely unknown.

The time is already overripe for precautionary, science- and knowledge-based approaches related to emerging vector-borne viruses in our part of the world. The guidelines must be based on the definition of risk, in the sense that the consequences may be too dramatic to waste time on discussions of probabilities. It must also be realized that we are speaking about self-replicating organisms and problems. Once introduced and established within an area, they can not be exterminated without harming the ecosystems and their resilience even more. Anthropogenic ecosystem changes (e.g. various forms of ecosystem sequestration and endocrine disrupting as well as other chemical pollutants, i.e. EDCs, POPs) may act synergistically or additively with climate changes to increase the geographical distribution and change the biological characteristics and ecology of viruses.

Due to the advances in molecular biology, robust diagnostic tools became available for the rapid and sensitive detection of viruses. But one of the weaknesses of the present approaches is that they only detect what one asks for, i.e. you only find the viruses you already know. Hence, research for development of more "universal" laboratory detection methods must be integrated parts of precautionary research programs. Comprehensive, well-planned and –designed, surveillance and monitoring investigations are necessary for the early detection of the introduction and spread of exotic vector-borne viruses in northern Europe, and/or climate change-assisted abberations in the ecology and genetics of resident viruses, vectors and hosts/reservoirs. Besides the accurate identification of the vector, the host species, and the viruses, according to their ecological characteristics, early detection networks and effective control measures must be developed. These

activities require intensive collaboration of entomologists, virologists, and several other types of resource persons and authorities in, and between, the affected areas (e.g. medical, nature conservation, legislation, and agriculture). The right time is now; let's go to work!

III. Executive Summary

"The reason we want to draw attention to viruses is they're difficult to see, they have devastating effects, and we also don't think about them until it's too late." (Dr. William Karesh, as quoted by Dell'Amore, 2008).

"Vector-borne diseases can serve as 'the canary in the mine' as a first alert of changes due to climate" (Randolph, 2009).

"For real progress, the modeler as well as the epidemiologist must have mud on their boots (David Bradley 1982, quoted by Hudson et al., 2002).

Arthropod-borne (arboviruses) and vertebrate-borne (e.g. hanta- and lyssa-) viruses are major, global causes of diseases in humans and domestic animals. Hence, most scientific publications, as well as national and international research and risk management programs, are directly related to the significance and impacts of such viruses as human and domestic animal pathogens, the aims being to prevent and treat disease.

The main motivation and starting point for *this* report is, however*, i) the lack of knowledge related to the ecology of such viruses, ii) their direct effects on wildlife and hence iii) their indirect effects and impacts on ecosystem, domestic animal and human health. It cannot be strongly enough emphasized that research in relevant fields have, more often that not, been initiated first after viral epidemics in human populations have been discovered. Long-term, well-designed and -planned surveillance and monitoring programs are scarce. Really precautionary studies, trying to anticipate, protect and prevent by well-planned, long-term and continuous surveillance and monitoring programs have so far been nearly absent from scientific literature. Climate warming and xenobiotic as well as abiotic changes to the biosphere, the biomes and the ecosystems may have impacts on all the issues discussed in this report, directly or indirectly.*

At the present time a number of arboviruses and hantaviruses, with disease-causing potential for humans as well as for domestic and wildlife animals, are "on-the-move" from their original distribution areas in the tropic or lower temperate zones into areas of *increasingly higher altitudes and latitudes,* in the Old as well as the New World. This concerns, among others, mosquito-borne viruses like West Nile Virus (WNV) and Dengue viruses (DENV); tick-borne viruses like Tick-Borne Encephalitis virus (TBEV) and Crimean-Congo Hemorrhagic Fever virus (CCHFV); midge-borne viruses such as Bluetongue virus (BTV) and Schmallenberg virus (SBV); rodent-borne viruses like members of the *Hantaviridae* family; as well as bat-borne, rabies- and ebola-like viruses. A review of international scientific literature in relevant fields shows *unanimous support to the concept of climate change (global warming) as a strong driver for the invasions of arboviruses and vertebrate-borne viruses into areas of higher altitudes and latitudes*, although different authors may disagree on the relative impacts of global warming compared to other anthropogenic encroaches upon biomes and

ecosystems, e.g. chemical pollution in general and chemicals with *endocrine disrupting* biological activities in particular (EDCs, POPs) in particular.

The occurrence and geographical distribution of arboviruses are *governed by complex interactions between three main actors: the virus, the arthropod vector and the vertebrate host. These interactions are strongly influenced by the ecosystem conditions*. Under "stabilized" conditions the viruses are circulating between their natural vector and host species without causing overt disease/mortality in most cases, although recent studies have revealed that subtle, but ecologically important, changes in fitness, frcundity and behavior may be seen for some virus/vector/host combinations. Climate changes may disturb established relations and contribute to spread of resident, indigenous viruses out of their original distribution areas. This may happen through migrations of infected arthropods, wildlife mammals, birds or humans. Hence, new competent vector and host species may become involved, and immunologically naïve human, domestic animal and wildlife populations will become exposed and infected. For instance, when taking into account maximum climate change impact scenarios, both the short- (2030) and the longer-term changes are similar, suggesting that most of Europe, including Norway, might become favorable for establishment of exotic invertebrate and vertebrate species, including vectors and hosts for emrging as well as resident, native viruses. Such scenarios include the possibility of serious diseases in different vertebrate populations, both wildlife, domestic animal and human ones.

The reproduction rates, population sizes and distribution areas of competent small rodent and bat host species govern the occurrence and geographical distribution of many vertebrate-borne viruses. All these parameters may be strongly influenced by climate changes, sometimes in unpredictable ways, e.g. warmer winters in our part of the world may eliminate the protective snow covers, and hence make conditions harsher for small rodents. Such developments may diminish the chances for establishment of new viruses and also hamper the circulation of already indigenous and resident viruses. These trends may, however, be counteracted by other consequences of climate change.

In northwest Russian and Fennoscandian (including Norwegian) ecosystems a number of arboviruses and rodent-borne viruses are circulating within our wildlife populations, and there are most probably many more than we know about. Known, and partly characterized representatives within the families *Flaviviridae*, *Togaviridae*, *Bunyaviridae* and *Reoviridae* (mosquito-borne as well as tick-borne) are present. A number of rodent-borne members of the *Hantaviridae* family are circulating within populations of various small rodent species. Humans may become accidentally infected with hantaviruses by contact with small rodent secretes, excretes and droppings. Some of these infections give clinical symptoms, occasionally very serious ones.

In some areas of southern Europe bats are hosts and reservoirs for Rabies- or Ebola-like viruses within the family *Lyssaviridae* or *Filoviridae*, respectively. Bat species that are involved are also present in Norway, but no one has looked for the viruses yet. Climate change may lead to migration towards the north of new bat species.

In our part of the world small rodents are also carrying other viruses, e.g. *Poxviridae, Herpesviridae, Adenoviridae* and *Polyomaviridae,* with known or un-investigated potentials to jump species barriers and cause disease in new host species. Latent, genome-integrated and silent Endogenous *Retroviridae (ERVs)* in humans, domestic and wildlife animals may become activated, and cause disease as a result of climate change, in concert with polluting, endocrine disrupting chemicals (EDCs, POPs). It is conceivable that climate changes and new emergent infections in synergy with other anthropogenic ecosystem alterations may enhance the *probabilities for activation of persistent/latent infections with endemic viruses and initiate new dissemination and spread*.

Novel emergent arthropod- and vertebrate-borne *viruses* may arise through *mutations* enforced by changes to the ecosystem, induced a.o. by climate changes, and by genetic *recombinations or reassortments* between the genomes of invading and closely related indigenous and resident viruses. The biological characteristics, included pathogenicity, of such viruses are unpredictable. For some viruses it has been demonstrated that switching into new vectors or hosts may speed up these genome evolutionary processes.

In summary, viruses that have reservoirs and are circulating among wildlife animals cause many of the most burdening infectious diseases of human populations and their domesticated animals. Temperature barriers that prevent invasion of some species of mosquitoes and other arthropod vectors have so far protected Northern Europe and Fennoscandia. This situation may change drastically through the ongoing climate changes. However, by increasing our knowledge about viruses, vectors and host animals, as well as their occurrence and transmission routes within and out of the indigenous ecosystems, we might be able to break transmission chains or keep our activities out of "hot spot" locations. There is a void in knowledge about the ecological interactions for many important viruses. *In order to protect human, domestic animal, wildlife and ecosystem health, Norway should immediately initiate precautionary research, surveillance and monitoring related to emerging as well as indigenous, resident arthropod- and vertebrate-borne viruses.*

The time is now, and it is recommended that Norwegian authorities initiate the following concerted initiatives:

1. ***A multidisciplinary "task force"*** of resource persons with relevant competence, experience and interests for planning of present and future activities.

2. ***A national biobank for wildlife animal organisms and tissues***. This will be a crucial instrument for long-term monitoring of all sorts of environmental changes. It will also be a necessary source of baseline- and reference materials for future surveillance of wildlife infectious agents, as well as for a number of other purposes, e.g. release or escape of GMOs. The biobank may be established on the basis of already stored materials, frozen down from the 1960ies and onwards, from all over the Norwegian mainland. This valuable ecosystem repository is kept frozen by GenØk-Centre for Biosafety in Tromsø. Based on initial contacts it seems possible to establish a biobank that includes materials from terrestrial, marine and aquatic ecosystems in mainland and polar

Norway. The biobank should be based on aliquoted tissue blocs, homogenates and sections as well as purified DNA, RNA and protein fractions from the same samples. The biological materials should be stored for eternity, and samples should be available for all researchers upon application with relevant project description.

3. A competent, well-planned ***program for continuous surveillance and monitoring*** of putative vector-host-reservoir combinations within Norwegian ecosystems. The organization must be in constant close communication with related initiatives all over the world for general *scanning* surveillance approaches and with European partners in particular for targeted surveillance of specific organisms and viruses.

4. **A *continuous field-based collection program*** of relevant vector-host-reservoir organisms and tissues. The collections must take place at carefully selected locations and times of the year. The biological materials collected must, by molecular and immunological methods, be rapidly screened for a general set of viruses, and targeted methods must be employed in emergency situations, i.e. when "new" viruses have been detected in our neighboring countries. The collected materials should be continuously deposited in the newly established Biobank.

5. ***A new, holistic and multidisciplinary national research program*** targeted towards the understanding, anticipation, prevention and remediation of vector-borne and other zoonotic infectious agents in Norwegian ecosystems. The Ministry of Environment/Norwegian Environment Agency, on a biodiversity/ecosystem protection platform, should initiate such a program. However, it goes without saying that cooperation, coordination and communication with medical and veterinary research institutions and authorities all over the world, and particularly in Europe, will be important for the success of this initiative.

1. General Introduction

Climate Change as a driver for emerging arthropod and vertebrate-vectored viruses

"The ecology of climate change is receiving inadequate attention" (Dr. Jeff McNeely, IUCN chief scientist, quoted in Dell'Amore 2008).

1.1. Purpose and goals

With a special view to Norway and Europe, the main purpose and goals of this report is to:

- *Describe the complexity of vector-borne virus life cycles.*
- *Explore the influence of climate on these systems.*
- *Explore our capability to assess the potential impact of changes in climate on these systems.*
- *Place the issue of climate in the broader context of environmental change in general*
- *Outline the kinds of information that will be necessary for more accurate predictions of future climatic or environmental effects on vector-borne disease systems.*
- *Recommend research, surveillance and monitoring initiatives that may make the society able to act precautionary with respect to the described challenges.*

It cannot be strongly enough emphasized that research in relevant fields have more often that not been initiated first after viral epidemics in humans have been discovered. Really precautionary studies, trying to anticipate, prevent and protect against emerging viruses have so far been nearly absent from scientific literature. This is particularly true for investigations into impacts on ecosystem interactions, resilience and services.

1.2. Climate change and wildlife infectious agents

The incidence and spread of infectious diseases that were previously only seen in the tropics have considerably increased under the current climate change situation (Harvell et al., 2002). Of 14000 known infectious microorganisms and viruses, it has been shown that at least 600 are shared between animals and humans (Dell'Amore, 2008). In a relatively recent (Daszak et al., 2007) retrospective study of 335 emerging infectious episodes over a 64-year period (1940-2004), the role of wildlife as a source of emerging infections was strongly emphasized. An estimated 60% of emerging human pathogens are zoonotic. More than 70% of these pathogens have wildlife origins (Cutler et al, 2010). Such pathogens can unpredictably switch hosts by genetic *mutations*, *recombinations* and *reassortments* (see Chapter 6) that also induce altered pathogenic potential.

In the next decades, climate change may be a main driver for emergence of "new" infectious agents, and also for the spread of new and old infectious agents into new regions and territories. As certain

regions warm up, virus-carrying arthropods such as mosquitoes, ticks and midges may expand into new territories that are unprepared for their arrival. The same may hold true for virus-carrying vertebrates like small rodents, bats and birds.

In spite of the fact that infectious agents are integral parts of, and may dramatically change the ecosystems of this planet, we have very scanty and fragmented knowledge about the total occurrence as well as the ecology of wildlife infectious agents. The main reason for this, potentially dangerous, lack of knowledge is that research efforts have typically been focused towards viruses that may harm human populations or economically important domestic and wildlife animal species (Daszak et al, 2007; Cutler et al, 2010). However, infectious agents can cause rapid population declines or species extinctions without being directly pathogenic to human or domestic animals. Many pathogens of terrestrial and marine taxa are sensitive to temperature, rainfall and humidity, creating synergies that might affect biodiversity and also contribute to geographical spread of present, and creation of new, infectious diseases of wildlife, domestic animal and human populations. Infectious agents are strong biotic forces that may threaten biodiversity by catalyzing population declines and accelerating extinctions. Pathogens have been implicated in recent declines of vertebrates (e.g. Australian and Central American frogs, Hawaiian forest birds and African wild dogs) and invertebrates (e.g. Polynesian tree snail and marine limpets) as well as in threatened species such as lions, cranes, eagles and black-footed ferrets.

In the biosphere, biomes and ecosystems everything is connected and interacts with everything. Plant pathogens can cause problems not only for their immediate hosts but also for their associated fauna and ecological communities (reviewed in Drew Harvell et al., 2002). Since viruses and wildlife have evolved together over time, animal species have developed adaptations to cope with the viruses. Consequently, disease spikes in wildlife usually point to something “out of sync with nature” (Dell’Amore 2008).

Climate change is now accepted as a major environmental driver influencing vector-borne disease epidemiology. The Intergovernmental Panel on Climate Change (IPCC, 2001; IPCC, 2007) lists emergence of vector-borne diseases among the most likely consequences among the various effects of global warming. The sensitivity of vector-borne disease cycles to climate has resulted in the view that *vector-borne diseases can serve as ‘the canary in the mine’ as a first alert of changes due to climate* (Randolph, 2009).

Climate change, whether anthropogenic or not, may affect dissemination, prevalence, incidence, ecology and characteristics of already resident as well as invading and newly emerging virus species and strains. *Anthropogenic ecosystem changes (e.g. various forms of ecosystem sequestration and endocrine disrupting as well as other chemical pollutants, i.e. EDCs, POPs) may act synergistically or additively with climate changes to increase the geographical distribution and change the biological characteristics and ecology of viruses (Gould and Higgs, 2009; Tabachnick, 2010*). There is no disagreement in the literature about these general facts. Although there is some different points of

view with regard to the relative impacts of climate warming and other anthropogenic ecosystem changes, *everybody emphasizes the urgent need for greater understanding of the ecology of vector-borne viruses in order to understand and predict the effects of future changes in the environment* (reviewed by Tabachnick, 2010).

Developments of the human society have always had impacts on the ecology of infectious agents. The evolution of the domestic form of the mosquito *Aedes aegypti aegypti* (L.), for example, occurred after humans began storing water in containers. This type of water storage provided the niche for the evolution of this container-breeding mosquito and led to its urbanization and commensalism with humans, resulting in an increase in the level of transmission of both yellow fever virus (YFV) and dengue virus (DENV) (Tabachnick, 1991).

Climate change can impact the vector-borne disease epidemiology by influencing arthropod vectors, their life cycles and life histories. That may lead to changes in both vector and virus distribution and deviations in the ability of arthropods to transmit viruses. Climate can affect the way viruses interact with the arthropod vector as well as with the human or animal host.

Predicting and mitigating the effects of future climate change on the complex arthropod–virus–host ecological cycles require understanding of a variety of complex processes and interactions from the molecular to the population level. Although there has been substantial progress on many fronts, the challenges to effectively understand and mitigate the impact of potential changes in the environment on vector-borne viruses are formidable and at an early stage of development (review by Tabachnick, 2010).

1.3.Transmission and impacts of viruses with wildlife reservoirs and hosts

1.3.1.Transmission

Viruses with reservoirs/main hosts among wildlife animal species are either:

i. Spread and transmitted by arthropod vectors (biological transmission, see below). Such viruses are called arboviruses (arthropod-borne) and are found within a number of virus taxa, but most of them belong to virus families that have RNA genomes. RNA viruses have “proof-reading” systems of lower efficiency than DNA viruses, and hence they are more prone to develop and keep genome mutations (see chapter 6).

ii. Spread and transmitted by other routes and processes, i.e. directly between individuals, populations, species. Such viruses are found within all taxons, belonging to DNA as well as RNA genomic families. In the present context we collectively name them “vertebrate-vectored viruses”. In the present report rodent-vectored hantaviruses and bat-vectored Ebola-like and Rabies-like viruses are treated as special cases.

1.3.2. Hosts

Wildlife animals often harbor large numbers of persistent viruses, which can be the same viruses that can cause serious pathology in other, related species. The persistent infection seems to protect the animals from the acute phase of infection with the exogenous virus, but it can also provide a source of acute virus that can wipe out a population of related, sensitive animals. This scenario can allow invasion of new territory or can protect a resistant population from invasion by a sensitive population. In plants, invasive species can bring viruses with them that contribute to the process of invasion by weakening competing native species, as exemplified by the invasive annual grasses that are outcompeting native bunchgrass in California, uSA63. The process of invasion has not been well studied, and there may be many more examples that involve viruses (Roossinck, 2011).

1.3.3. Beneficial viruses?

In spite of the common perception of viruses as pathogens, many viruses are in fact beneficial to their hosts in various ways. There is significant evidence that they have played a major part in the evolution of life on earth. In some cases, viruses have been responsible for major evolutionary leaps, such as the retrovirus-based establishment of placental mammals. Some viruses, like the polydnaviruses of parasitoid wasps, for example, are even required for the survival of their hosts. Some provide a benefit only under certain environmental conditions; others have allowed the rapid adaptation of their hosts to extreme changes in the environment, which could be increasingly important in the future as we face changes to the earth's climate. It is likely that many more examples of mutualistic viruses will be discovered in the coming years, especially if researchers open their minds to the possibility that "viruses are not all bad" (Roossinck, 2011).

1.3.4. Reservoirs

Emerging viruses and other infectious agents threaten global biodiversity and the health of ecosystems and a myriad of organisms (Smith et al., 2006). Most zoonoses originate in wildlife and have, during the last decennia been increasing over time (Jones et al., 2008). However, the relative importance of different groups of wildlife hosts in the emergence of zoonoses and the processes driving such differences remain unclear (Luis et al., 2013).

Bats (Order Chiroptera) and *rodents* (Order Rodentia) are, beyond doubt, containing some of the important vector/host/reservoir species for zoonotic viruses (Luis et al., 2013). They share a number of characteristics that are hypothetically affecting their potentials in this context Luis et al., 2013). Both taxonomic orders are evolutionary ancient and diverse. Both include many species with peridomestic distribution, and species that commonly employ torpor or hibernation. In addition bats are indeed special, in that they host more zoonotic viruses and more total viruses per species than rodents, 61 vs 68. However, because there is approximately twice the number of rodent species as bat species, the overall number of zoonotic viruses identified in bats is lower than in rodents. Some specific traits that appear to promote viral richness across taxonomic orders have been identified (Luis, 2013). More zoonotic viruses are hosted by species whose distributions overlap with a greater

number of other species in the same taxonomic order (*sympatry*). Specifically in bats, there is evidence for increased presence of zoonotic viruses in species with smaller litters (one young), greater longevity and more litters per year. Given the importance of sympatry, future analyses should aim to determine the relative effects of *phylogeny* and sympatry more broadly in animal reservoirs of emerging zoonoses. Furthermore specific traits of zoonotic viruses may also be important in determining probability of spillover (species jumping). Both sympatry and viral traits may act together. The ability to replicate in the cytoplasm and bypass additional host-specific cell machinery may potentially allow viruses to more easily pass between sympatric species in the same taxonomic order. This might be compounded by increased rates of contact between different species. Luis (2013) points to this as a newly hypothesized mechanism to explain, at least in part, how bats host more zoonotic viruses per species. Interspecific transmission may be more prevalent in bats than in rodents (or other orders).

Interspecific transmission and spillover is one of the least studied aspects of disease ecology and should therefore be a focus of further studies. Processes enhancing virus transmission among bat species may be different from transmission from bats to humans. The mechanisms of interspecific transfer of pathogens, particularly to humans, remain poorly understood, but in some cases are complex and involve intermediate hosts. Gaining understanding of actual mechanisms of such pathogen transfer should be an active area of research in order to develop evidence-based policies to minimize risks, while conserving bats and the irreplaceable ecosystem services they provide.

In addition to host traits, *viral traits* affect spillover and emergence of zoonoses. RNA viruses are more likely to emerge than DNA viruses, and replication in the cytoplasm was the best predictor of cross-species transmission from livestock to humans (Pulliam and Dushoff, 2009). Additionally, there are some traits that may make bats and rodents more likely to host zoonotic viruses in particular and/or transmit them to other vertebrates. In evolutionary terms, bats and rodents are ancient mammals, and it has been hypothesized that viruses which evolved in bats may use highly conserved cellular receptors, thus enhancing their ability to transmit viruses to other mammals (Calisher et al., 2006). Consequently, we should also scrutinize some basic characteristics of viruses found in bats and rodents.

1.3.5. Co-infections with different vector-borne viruses

It will become obvious that many viruses share or overlap in geographical distribution as well as in use of vectors, reservoirs and hosts. This sets the stage for unpredictable effects, impacts and consequences for genuine vectors as well as for “victims” as humans and domestic animals. In spite of the implications of these facts, very little research has been devoted to relevant questions and hypotheses of importance. The research that has been performed has often given conflicting or confusing results (see review in Kuno and Chang, 2005 and references therein).

1.3.6. Do we know the viruses already circulating in Norway?

There are *huge gaps in our knowledge about microorganisms and viruses that are present in Norwegian ecosystems.* This statement is valid for vector-borne as well as non-vector-borne viruses, and this fact should become increasingly obvious during the accounts and discussions further on in this report.

1.3.7. Conclusion

The processes governing transmission and interspecific transfer of zoonotic viruses remain poorly understood. They are in many cases complex and involve intermediate hosts. Gaining understanding of actual processes of such virus transfer should be an active area of research in order to develop evidence-based policies to minimize risks, while conserving vector and host species and the irreplaceable ecosystem services they provide (Luis et al, 2013). Living in a rapidly changing world, we must be prepared in advance and prioritize resources to prevent or reduce the impacts of vector-borne and other wildlife-hosted viruses on ecosystem, animal and human health. *Unfortunately, we have a rudimentary understanding of the underlying processes that influence vectors, viruses, hosts, reservoirs and interactions between all these players. Our comprehension of vector-borne virus ecology and disease systems at all scales is fragmentary at the best. Consequently, forecasting the future of vector-borne viruses and diseases is fraught with uncertainty* (Tabachnick, 1998; Tabachnick, 2003).

1.3.8. General questions

Ideally, *before* an emerging virus has invaded a new area/location/ecosystem a number of crucial questions should have science-based answers, e.g.:

- May the invading virus engage in genetic recombination, or by other means achieve new genetic material? If so, will the hybrid offspring have changed their host preferences and virulence characteristics?
- May the invading virus or any hybrid or mutated offspring infect unexpected species?
- May the invading virus or any hybrid or mutated offspring integrate into the genomes of host cells?
- May other viruses that are present within the ecosystem influence infection with the invading virus or its offspring?
- May insects or migrating birds, or other vertebrates, function as vectors for the invading virus or its offspring, to disseminate viruses out of their initially invaded areas?
- For how long may the virus and its offspring survive outside host organisms under realistic environmental and climatic conditions?
- Are the virus and its offspring genetically stable over time?
- May the virus or its offspring establish long-lasting, clinically mute, persistent or latent infections in naturally accessible host organisms?

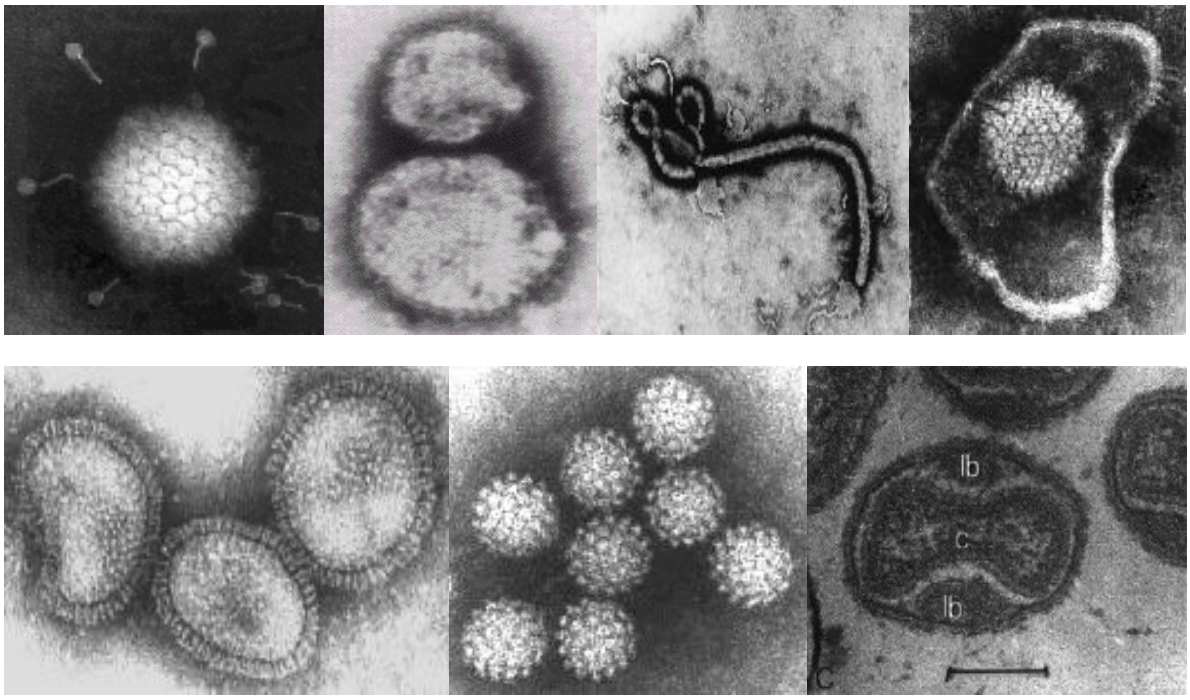
- May the virus or its offspring activate or aggravate naturally occurring latent or persistent indigenous virus infections?

Some of these questions deal with the biological and phenotypical characteristics of a supposed genetically stable emerging virus. But the situation becomes even more complex and unpredictable if the invading parental strain under certain conditions or circumstances is genetically unstable, giving rise to viral strains with altered characteristics.

It is obviously a very demanding task to find answers to these very complicated questions, but they may be sought by a combination of well-planned field, microcosm and mathematic model studies.

2. Viruses are not microorganisms or cells, they are viruses!

(Modified from Traavik, 1999; Myhr and Traavik, 2012)



Electron microscopy of representatives for some of the virus families mentioned in this report.
Upper row from left to right: Adenoviridae; Bunyaviridae; Filoviridae; Herpesviridae
Lower row from left to right: Orthomyxoviridae; Papovaviridae; Poxviridae

***Not "organisms"*:** Viruses are not "organisms", not even when you put "micro-" in front, and they are not "cells". Their genomes consist of *either* DNA or RNA. They are selfreplicating, intracellular parasites at the genetic level. The differences in genome strategies and life cycles between virus families are often more fundamental than between different mammalian or plant families. Although virus particles (virions) have diameters of tens to four hundred nanometers, "small size" is not included in the virus definition concept.

***Multiply or perish**:* Viruses multiply intra-cellularly in permissive host cells. One single virus particle (virion) infecting a permissive cell may give rise to millions of new particles during a short time (hours to days). These may then be transmitted to new hosts, of the same or different species, over varying periods of time.

Fully productive, persistent or latent – that's the question: In addition to fully productive infections, some virus/host cell combinations may result in persistent infection with virus shedding for extended periods, while others lead to latent infection with inactive viral DNA in a host chromosome-integrated or episomal state. Latent infections may be intermittently reactivated and accompanied by virus

shedding. Integration of viral DNA into the host cell genome may by itself have harmful consequences, irrespective of viral gene expression or replication.

Determinants of host- or cell-type preferences are often unknown: The host tropism, at the species-, organ- or cell type-level, is quite narrow for some viruses, while others have a much wider host-spectrum. For most viruses the molecular processes determining host-cell specificity are not known in detail. Restrictions may be present at various steps during a virus multiplication cycle, from the lack of cell membrane receptors to subtle incompatibilities with host cell enzymes necessary for viral nucleic acid transcription and replication. For a given host species, such restrictions may be relative and related to age, gender and environmental conditions.

***"Permissivity" is a relative term*:** For many virus/host cell combinations "permissivity" is a relative term, since it may be influenced to a considerable extent by the menu of genes expressed by the host cell, and by their exact levels of expression. In culture, the permissivity of a given host cell may be manipulated experimentally by activation of intracellular signal transmission pathways, i.e. by hormones, growth factors, cytokines etc. Such procedures may also enhance persistent or reactivate latent infections. At the intra- as well as at the inter-species level of host animals this is illustrated by a vast variation in susceptibility for a given virus strain. Such variation may be related to host gender, age, mating season, pregnancy, genetic differences, infection with other viruses or microorganisms, and environmental factors promoted by climate changes, season or pollution.

Infection without disease: It is important to be aware the distinction between viral infection and viral disease. An infected individual may shed virus and represent a transmission reservoir without showing clinical symptoms. Yet, other individuals within the same or other species may become clinically ill, or the viral infection may result in abortions, stillbirths, teratogenic or oncogenic effects. For persistent/latent infections, clinical symptoms may be present intermittently, only under special circumstances, or appear a long time after infection.

Even small genetic changes may give important biological effects: Different strains of the same viral species may have different virulence or pathogenicity, as well as host-cell or species tropism. Even genetic differences at the single point mutation level may result in virus strains with aberrant phenotypic characteristics (see also chapter 6).

Viruses show no respect for species barriers*:* The major sources of new human and domestic animal viral diseases are enzootic and epizootic viruses of animals. The opportunities for cross-species transfer of mammalian viruses have increased in recent years due to enhanced contact between humans and animal reservoirs. It is, however, difficult to predict when, where and how such events will take place, since the viral adaptations that are needed are multifactorial and stochastic. Recent examples of viruses that have crossed species barriers are HIV, hantaviruses, haemorrhagic fever viruses, arboviruses, avian influenza virus, SARS-associated coronavirus, Nipah and Hendra viruses, and monkeypox virus. The emergence of HIV exemplifies how multiple independent cross-species transmissions of simian viruses, that are not associated with disease in their natural hosts, eventually resulted in the establishment of two types of HIV in the human population. While adapting to its new

host the virus underwent a myriad of molecular changes. Aberrations in social behaviour of humans may well have offered opportunities for newly evolved HIV strains to become pandemic.

Most likely we know only a small fraction of the viruses infecting wild or even domesticated animals. The risks of such unrecognized viruses are highlighted by the emergence of SARS coronavirus (CoV), hantaviruses, Ebola and Marburg viruses, Nipah virus, Hendra virus, and human immunodeficiency virus type 1 (HIV-1) and HIV-2, all cross-species host switches of established enzootic viruses that were unknown before their emergences into humans (Parrish 2008 and references therein).

Crossing the species barrier from one animal species to another is most readily noticed when it is associated with overt pathology. In the past such events may have been overlooked as the underlying cause of the emergence of a new disease. When a virus translocates from wildlife host reservoirs, jumps a species barrier and causes a disease, that disease is called a *zoonosis*. If the transferred virus causes disease in humans, the disease may be labelled as an *anthropozoonosis.*

Human invasions with emerging viruses: When viruses that are innocuous in one population infect another, unprepared population or a new species, catastrophic epidemics or epizootics may be initiated. Human history is filled with examples of invasions of new territory. Recent estimates indicate that 90% of the native human population in the Americas died within 10 years of the European invasions. Although wars and massacres accounted for some of this, many native peoples were exterminated by viral infections, including smallpox, influenza and even the common cold (caused by rhinoviruses). The native populations had never been exposed to these viruses and had no immunity. A similar scenario with smallpox is thought to have decimated the Australian Aboriginal populations in the nineteenth century. In these cases the invading human population acted as virus vectors. In all of these examples, viruses carried by the invading populations benefited the invaders by clearing the new territory of its native inhabitants. However, the long-term effects on the human gene pool might have been less beneficial for the species as a whole (Roossinck, 2011).

The emergence of new viral infections often follows environmental, ecological and technological changes caused by human activities. Such activities may lead to an increased contact between humans and livestock on one hand, and wildlife animal hosts acting as reservoirs and vectors of zoonotic viruses on the other hand. Agricultural development, an increased exploitation of environmental resources, growth and increase in the mobility of the human population as well as trade and transportation of food and livestock, have been identified as important factors contributing to the introduction and spread of a number of new viruses in the human population.

Climate warming and xenobiotic as well as abiotic changes to the biosphere, the biomes and the ecosystems may have impacts on all the issues discussed in this chapter, directly or indirectly. This is one of the overriding working hypotheses behind this report.

3. Lifecycles of vector-borne viruses: participants and complexity

3.1.The ecological episystem

The vector-borne virus *episystem* encompasses all of the biological and environmental components and aspects of the entire vector-borne virus ecology system within specified geographical and/or temporal scales (Tabachnick, 2003). *The episystem includes the vectors, the hosts/reservoirs, the viruses, the biological controlling processes and all of the environmental factors that have an effect on viral spread and ecology within a defined spatio-temporal region.*

Episystems might occur at different levels of scale. For example, one might define the episystem for a specific virus at the local level of a village or town, which may be a different episystem, with different components and influences, than the same virus defined at the countrywide, continental wide or the global level. An episystem might be defined temporally if various controlling factors have different impacts over time. The West Nile virus (WNV) episystem in the northeast USA in 2000, for instance, may be different from the current episystem in the same region due to changes in vector populations, avian amplification host populations, human behavior and climate over the past decade.

A concept developed by Sutherst (2004) to emphasize the complexity of the interactions between some contributing factors is illustrated in Figure 1. The concept includes the direct and indirect influences of many factors on vector-borne disease. The disease cycle, represented by the vector–pathogen–host relationship, has multiple impacts that are interconnected and/or dependent on one another. Fig. 1 is a useful abstraction for visualizing vector-borne virus episystems.

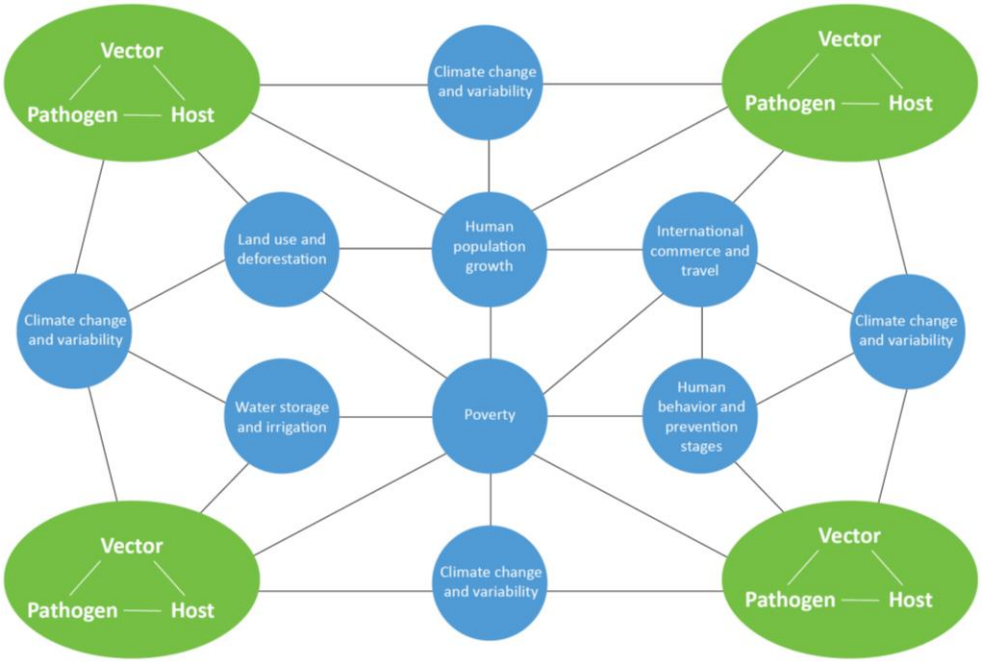


Fig. 1. The vector-borne virus episystem illustrating interactions between selected environmental factors with effects on the vector–pathogen/virus–host epidemiologic cycle [modified from Sutherst (Sutherst, 2004).

3.1.1. Virulence

The fitness cost to a host resulting from virus infection, is a dynamic trait fluctuating with the co-evolution of both host and virus as well as with their interactions with changing environments. Although newly emergent viruses are often more virulent, and many viruses have displayed decreased virulence over time, the avirulence hypothesis, the idea that viruses should always evolve away from virulent interactions with their hosts, has been largely disproven by epidemiological and experimental data demonstrating the persistence and/or evolution of highly virulent virus strains. Despite this, although vector-borne viruses are often associated with high virulence in vertebrate hosts, interactions between arthropod vectors and arthropod-borne viruses (arboviruses) have historically been characterized as benign. Although the term vector implies a lack of significant biological interaction between arthropods and the viruses they carry, it has become clear in recent years that such interactions are complex and are likely dominant forces shaping the evolution of arboviruses. The alternative to the avirulence hypothesis is the trade-off hypothesis, which proposes that virulence and transmission are coupled and that the extent of virulence at equilibrium is subsequently limited by the trade-off that maximizes virus transmissibility. Variability in modes of transmission, intrahost competition, and relationships between virulence and virus load for individual host-virus systems may argue against the broad applicability of this hypothesis to explain variations in virulence, the trade-off hypothesis nevertheless provides a useful framework by which to evaluate the capacity for virulence evolution in individual systems. The coupling of virulence and transmission has indeed been noted in many systems, yet to-date has not been evaluated for an arbovirus in an invertebrate host (for further inputs: see Ciota et al., 2013 and references therein).

3.1.2. Interactions between viruses circulating within the same episystems?

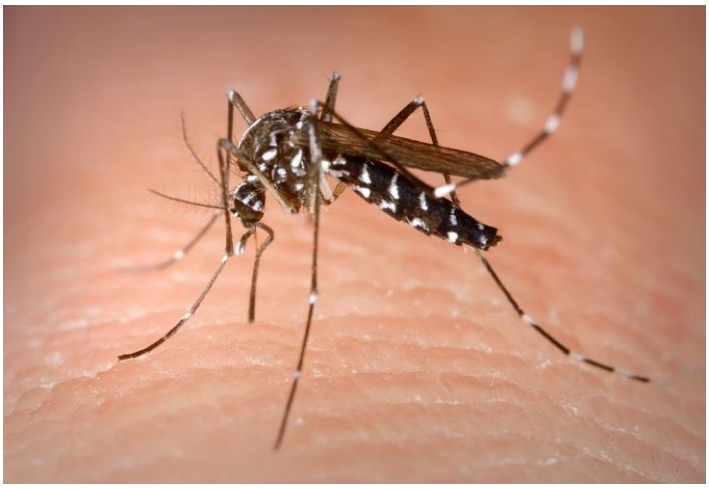
Issues of importance for this crucial question are treated under chapters 1.3.5 and 7.4.1.

3.2. Arthropod vectors[1]

3.2.1. Mosquitoes

In Europe the interest and awareness related to establishment and spread of invasive mosquitoes has grown during the last years. This has been due to the incursion of *Aedes albopictus* ("The Asian tiger" or "killer" mosquito) through the international trade in used tires and lucky bamboo, followed by onward spread within Europe through ground transport. More recently, four other non-European aedine mosquito species have been found in Europe (*A. aegypti*, *A. japonicus*, *A. atropalpus* and *A. koreicus*). In some cases populations have established locally and are spreading. Concerns have been raised about the involvement of these mosquito species in transmission cycles of pathogens of public

[1] For distribution maps of potentially important arthropod vector species in Europe: see the home page of ECDC, Home > Health Topics > Vectors > Vector maps



A female Aedes albopictus mosquito sucking human blood.
Photo: CDC/James Gathany, public domain licence

health importance, and these concerns were borne out following the outbreak of chikungunya fever in Italy in 2007, and subsequent autochthonous cases of dengue fever in France and Croatia in 2010. It is important to increase the current understanding of all exotic (five introduced invasive and one intercepted, *A. triseriatus*) *Aedes* species in Europe. The known import pathways, biotic and abiotic constraints for establishment, control strategies, and public health significance should be highlighted. The Europe-wide surveillance for invasive mosquitoes should be encouraged. By its very nature invasive mosquitoes are adaptable. Some species will in time become an established part of the European mosquito fauna and cause nuisance biting were they occur. We should be concerned about their virus vector status at any given time. *"If we learned any lesson from the last 20 years, it is that we should not be complacent"* (Medlock et al., 2012).

Mosquito-vectored viruses provide one of the earliest examples of viruses with a mutualistic role in their symbiotic partners. During feeding, mosquitoes must find their blood meal as rapidly as possible to prevent being killed by an annoyed host. *Aedes aegypti*, a mosquito vector of many parasites, was able to locate a host blood vessel more rapidly after feeding on hamsters infected with Rift Valley fever virus than after feeding on uninfected hamsters. The authors of that study speculated that the potential of the virus to disrupt haemostasis (that is, its ability to stop blood flow) could be the cause of this enhanced ability to find a blood vessel. Hence, Rift Valley fever virus seems to have a beneficial role in the life of the mosquito and thus enhances its own acquisition and transmission by the insect (Roossinck, 2011).

The important ecosystem services rendered by mosquitoes must be included in evaluations and priorities of combat strategies for mosquito-borne viruses and mosquito-related diseases (Fan, 2010).

3.2.2.Ticks

According to Obsomer et al. (2013), the incidence of tick-borne diseases is increasing in Europe. This follows an increase in the number of tick bites. This again is attributed to two factors: abundance of questing ticks and human exposure to ticks.

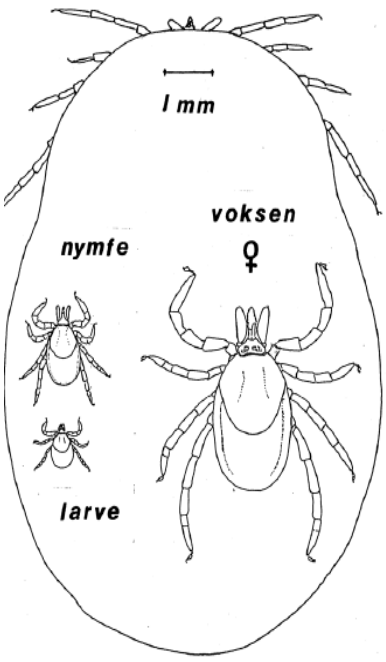
Knowing the local variations in the distribution of the species interacting in tick-borne diseases systems, including ticks, viruses and species influencing the presence and abundance of ticks and viruses, could provide new opportunities to estimate potential infection risks locally, identify local hot

Two adult *Ixodes ricinus*, one female and one male. Photo: Rolf Aasa/istockphoto.com

spots and develop targeted prevention, surveillance and control. Necessary information is lacking at national and sub national levels in many countries. The primary missing information concerns the presence and distribution of tick species. Efforts to characterise tick distribution on a European scale are limited by the information available at sub national level and only target major vectors such as *I. ricinus*. Other tick species less willingly biting humans sometimes harbour high virus prevalences and might contribute locally to additional virus lifecycles. The role of all tick species present should be investigated jointly per pathogen and their distribution clarified. The second missing information concerns the spatial distribution of hosts, predators and species influencing tick populations and virus prevalence in ticks. The presence and abundance of tick species varies locally according to many factors, including host availability.

Virus prevalence in ticks also varies locally according to availability of reservoirs, deadend hosts and vectors. The third set of missing information concerns viruses associated with ticks, e.g. their presence, reservoirs, vectors and distribution. Viruses detected by using classical PCR methods are those searched for only, while others might be present but go undetected. Because viruses are increasingly found in ticks, a more systematic approach is needed (Obsomer et al., 2013, and references therein).

In Sweden a northward expansion of the geographic distribution limit and an increased population density of *Ixodes ricinus* between the early 1980s and mid 1990s has taken place. This was followed by an examination of whether these events were related to climatic changes (Lindgren et al., 2000). The authors concluded that the relatively mild climate of the 1990s most probably was one of the primary reasons for the observed density and range increases.



Sketch that gives the relative sizes of the three metamorphosis stages of I. ricinus.

Effects of climate change on the distribution range and population density of *Ixodes ricinus* would be most easily recorded and documented close to its geographical distribution limits, and Norway embraces the northern distribution limit of the tick. In 1943 a distribution map based on collection of ticks from infested Norwegian cattle was published

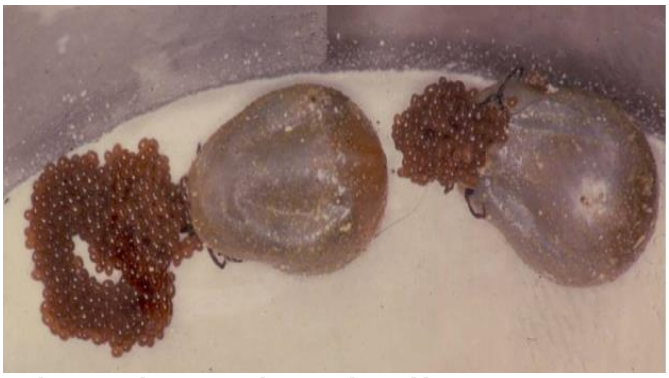
(Tambs-Lyche, 1943), setting the northernmost locations for *Ixodes ricinus* to Helgeland in Nordland County, at approximately 66° N. Forty years later a new overview of *Ixodes ricinus* distribution was published (Mehl, 1983), based on field-collected ticks from vegetation, birds and small mammals. The article arrived at a distribution map similar to the 1943 report. However, an analysis of multiple data sources published in 2012, demonstrated significant changes in the *Ixodes ricinus* ranges of distribution, with latitudinal and altitudinal shifts. The tick was now present up to an altitude of 583 metres above sea level, and in coastal areas up to approximately 69° N (Andreassen et al., 2012).

Fully engorged female I.ricinus
Photo: Reidar Mehl

Possible changes in the area inhabited by the ticks *Ixodes ricinus* and *Ixodes persulcatus,* the main transmitters of tick-borne encephalitis and Lyme disease in Russia, caused by temperature changes in 1976–2005 compared to 1946–1975 have been analyzed. It was shown that temperature changes could result in some areal expansion of these species. In the European part of Russia, *I. ricinus* expanded its areal boundaries to the east 100–300 km. *Ixodes persulcatus* expanded its areal in the Asian part of Russia. Its boundary moved to the north and northeast 100– 300 km.

Host changes during the life cycles of some tick species contribute to infection with viruses that can be transmitted to the next host. In many instances, viruses acquired by larval feedings are passed to the subsequent life stages of the individual (so-called, *trans-stadial transmission*). *Ixodes scapularis* is known to be able to feed on more than 100 host species in North America (at least 52 species of mammals, 60 species of birds, and 8 species of reptiles).

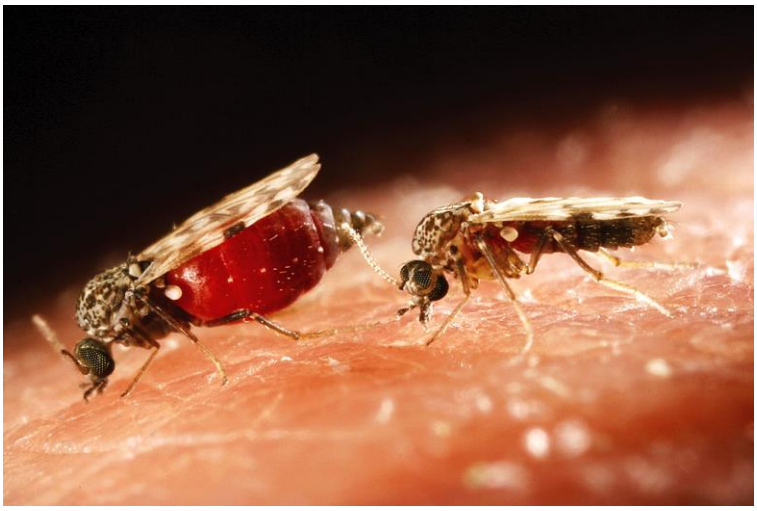
Ticks have relatively slow feeding processes, with firm attachment to their hosts. Hence, dispersal of the ticks are enhanced, as the host moves about in the environment. For example, *Ixodes scapularis* can travel over vast distances while feeding on birds. Some ticks are carried between continents in this manner. The slow feeding is associated with the need to produce new cuticle to accommodate the ever-increasing volume of blood. The saliva, metabolites, and excesses of fluids from the ticks are secreted back to the host. During this process viruses are transmitted to the host. Upon completion of feeding, the female can weigh 100 to 120 times its original weight. However, since so much water is secreted back to the host, the total volume of blood ingested may be two to three times the amount calculated from post-feeding weight.



Egglaying *Ixodes ricinus. Photo: Reidar Mehl.*

3.2.3. Midges

Members of the *Ceratopogonidae* family of biting midges can be severe nuisances; they are also the vectors of two important animal diseases in Europe: bluetongue virus and Schmallenberg virus. The Culicoides species are small, between one and five millimeters long. So far around 20 species have been identified in Norway, but it is assumed that approximately 40 species may be present (Mehl, 1996).



Culicoides after and before her meal (Photo: The Pierbright Institute, UK)

As regards human infections, there has only been a single isolation of Tahyna virus from *Culicoides* in Czechoslovakia (Halouzka et al, 1991). Therefore, the group has no apparent importance as vectors of human disease. On the other hand, hemoglobins of the midge family Chironomidae, potent human allergens, have been identified as causative allergens in asthmatic patients. A study in Sweden (Eriksson et al, 1989) concluded that Chironomidae might be allergens of clinical importance in asthma and rhinitis, that cross-allergy exists between chironomids and shrimp, and that cross-allergy might also occur among chironomids, crustaceans and molluscs.

3.2.4. Transovarial, transstadial and venereal virus transmission

Transovarial transmission occurs in certain arthropod vectors as they transmit viruses from parent to offspring by way of their eggs. *Transstadial* transmission occurs when a virus remains in the vector from one life stage to the next, and sometimes also to the third. If an emergent virus is able to be transmitted transovarially or transstadially by the invading or resident arthropod vectors, it will have a better chance of being firmly established in its new environment. Many mosquito-borne viruses can be transmitted from male to female during copulation, i.e. by *venereal* or horizontal transmission. These processes may, of course, enforce the influence on viral maintenance and overwintering in a given area (Hubalek 2008)

3.2.5. Climate change impacts on arthropods

Climate-sensitive, predictive models for risks of arbovirus emergence and spread in Europe have often led to the identification of numerous gaps in both the understanding and availability of relevant data. These gaps are mostly related to the biology of vectors and their interaction with hosts. According to Bayliss (2013), closing these knowledge gaps may allow the production of better models with more precise predictions. *Consequently, we need to enhance research on arthropod vectors that may be able to transmit arboviruses. Bayliss (2013) advocates training of a new generation of*

taxonomists, studies on the field biology of potential vectors, and increased coordination of vector surveillance and recording between countries facing similar threats.

The incidence of arthropods is particularly dependent on climatic factors because they have no internal control over their physiological temperatures, and the ambient temperature determines their reproduction rate, biting behaviour and survival. Their distribution may expand as the earth warms. Humidity and availability of water for breeding are important determinants of the distribution, longevity and behavior of arthropods. The incubation period of viruses inside vectors is temperature-dependent, and tends to become shorter at higher temperatures. Milder winters, warmer summers, wetter and earlier springs will most probably have, sometimes unpredictable, impacts on the density and distribution of many arthropods. Finally, human behaviour is likely to be affected by climate changes. This may alter our interaction with arthropods and the viruses they carry. These latter trends have allowed the range extensions into temperate latitudes of tropical vector species and viruses formerly constrained by winter severity.

Because arthropods are *poikilotherms*, transmission patterns typically are closely tied to warm temperatures that i) decrease the duration of immature development and thereby increase the rate of population growth and size, ii) decrease the duration of blood digestion and thereby increase the frequency of blood-feeding and host contact, and iii) decrease the duration of the incubation period of the virus within the arthropod, allowing transmission earlier in life by more individuals. Collectively these temperature-driven population parameters result in the seasonality of transmission patterns that typically peak in mid- to late summer. At temperate latitudes transmission subsides with the onset of cooler temperatures that arrest vector activity and often stimulate diapause. *Recent warming trends at northern latitudes have shortened this winter interlude and conversely lengthened the transmission season, precipitating outbreaks of tropical viruses at northern latitudes.* For example, unprecedented 10°C temperature anomalies in Saskatchewan, Canada, during 2003 and 2007 were accompanied by large epidemics of West Nile virus (Reisen, 2012). Saskatchewan is situated between 54**°** and 60**°** north, i.e. its northernmost part is at the same latitude as the southernmost part of Norway.

3.3. Vertebrate vectors and hosts

3.3.1. Birds

It will be recognized from various parts of this report that migrating birds may be utterly important as hosts and transporters of both new viruses and new virus arthropod vectors to new areas. During climate change, the suitable conditions for many bird species are expected to shift and perhaps improve. For European species, a recent modeling study has shown that a general northerly shift in the distribution of species is likely. For each bird species in Europe the recent distribution was modeled in terms of three climatic variables. That model was used to project the areas in which the climate is likely to be suitable for the targeted species under future climate scenarios. Overlaying such

projected ranges of all European species shows that the area of highest species richness is projected to shift northwards from southeast of the Baltic Sea into Fennoscandia, under the optimistic scenario of perfect dispersal. A marked increase in diversity in Arctic regions was contrasted by a decrease in southern and western parts of Europe. Such projected shifts pose significant challenges for conservation of species and "important bird areas" (Birdlife International, 2008), and also for surveillance and monitoring of invading vector-borne viruses and arthropod vectors.

Turdus merula (Common blackbird, Svarttrost)
Photo: Andrew Howe/istockphoto.com

3.3.2. Mammals

Wildlife mammals, especially rodents and bats, are hosts to an enormous number of viruses. We have very little knowledge about most of these viruses, which circulate readily within their specific ecosystem niches (Mackenzie and Jeggo, 2013). In general such viruses may be silent or asymptomatic in their natural hosts, but the balance may be tipped, and overt disease or behavioral abberations may appear due to climate and ecosystem changes. In some cases such viruses may cross species barriers and infect other wildlife species, humans and domestic animals.

Myodes (formerly Clethrionomys) glareolus (Bank vole, Klatremus) - Photo: Andrew Howe/istockphoto.com

Eptesicus nilssonii (Northern bat, Nordflaggermus) Photos: Wikimedia Commons

3.3.3. Reptiles and amphibians

Although mammals and birds appear to be natural vertebrate hosts for a number of viruses, it is often difficult to put their relative importance into perspective. Although put forward as potentially important reservoirs and maintenance hosts for many zoonotic viruses (Shortbridge and Oya, 1984), the knowledge and research in relevant fields to elucidate the roles of poikilothermice vertebrates are still meager and scanty.

There are some examples of roles for reptiles and amphibians in the natural history of arboviruses, but these are mostly of little relevance to conditions in our part of the world. Viremia levels of sufficient titer to infect mosquitoes were found after experimental infection of young alligators (*Alligator mississippiensis*). In Russia, the lake frog (*Rana ridibunda*) appears to be a competent reservoir for WNV

Non-mosquito-borne WNV transmission has been observed or strongly suspected among farmed alligators. Transmission through close contact has been confirmed for alligators in laboratory conditions but has yet to be documented in wild vertebrate populations (reviewed by Hayes et al., 2005). Some further examples are described in chapter 4.6.

3.4. Direct and indirect impacts of climate change on vector-borne viruses: relevant variables and effect parameters

Climate, i.e. temperature, precipitation, humidity, wind, etc. can influence various aspects of an arthropod vector's life cycle, including survival, arthropod population numbers, vector- virus-host interactions, virus multiplication, vector/host behavior and vector/host distribution.

Climate affects the range of viruses, while weather affects timing/intensity of transmission (Tabachnik 2010).

Comprehension of the climate influence on several current vector-borne virus episystems has provided knowledge about vector-borne disease epidemiology and has allowed greater ability to forecast vector-borne disease outbreaks. El Niño/Southern Oscillation (ENSO) and satellite imagery that included temperature and rainfall information was used successfully to predict a Rift Valley fever outbreak at the Horn of Africa. *Climate has influenced vector-borne diseases and in the future will continue to influence vector-borne disease at local, regional and continental scales.*

Climate has direct effects on the vector, virus and host, and on their interactions with one another. But climate also has direct impact on other environmental factors that in turn may directly influence vector-borne virus transmission cycles. *Poverty and human population size*, for instance, influence vector-borne virus cycles independent of climate. Climate in the form of rising temperature has been proposed to influence the surge of increased Dengue infections in the world in recent years. But

there is also good reason to believe that this may be due to the increases in the size and distribution of urban human populations, continuing poverty in many parts of the tropical world and an erosion of public health infrastructure in many regions (Gubler 2002; Gubler, 2008).

Summing up, climate changes may contribute to:

- Changing geographic ranges and reproduction levels, e.g. to higher altitudes and latitudes of:
    - Vectors
    - Host/reservoir species
- Changing arthropod vector blood sucking/biting habits (season, time of day, host preferences and availability)
- Changing (increasing or decreasing) prevalence and incidence of virus-carrying vectors and hosts/reservoirs
- Climate change may act synergistically or additively in concert with other anthropogenic ecosystem changes and sequestrations, e.g. immune system suppression (human, domestic animal, wildlife) due to endocrine disrupting chemicals (EDCs, POPs), et cetera
- Climate changes may activate persistent/latent infections with endemic viruses and initiate new dissemination, genetic and phenotypic alterations and implantations into new vectors, hosts and territories.

4. Arboviruses

4.1. Definitions

The term "arboviruses" comprises a large and heterogeneous group that only have in common that they are "arthropod-borne" to vertebrates. Consequently, the name is roughly describing the common ecological-epidemiological features of the members, saying nothing about their chemical, physical or biological properties. A definition that is generally accepted is that *"Arboviruses are viruses which are maintained in nature, principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by hematophagous arthropods"* (WHO Scientific group on arboviruses, 1967). The expression "biological transmission" is crucial in this context. It implies that after an infectious blood meal, a period of time will elapse before the arthropod is able to infect a new host by its bite (Casals, 1971). During this period the virus multiplies in the tissues of the arthropod (extrinsic incubation), which becomes able to transmit it when the amount of virus in the salivary glands has reached a certain level. *The extrinsic incubation period is defined as the period between feeding on infected blood and the appearance of virus in the saliva of the arthropod vector.*

4.2. Nomenclature and taxonomy

Arboviruses are mainly found within four families: *Togaviridae, Flaviviridae, Bunyaviridae* and *Reoviridae*. By 1996, 51 arboviruses had been reported from Europe and reviewed by Hubalek and Halouzka (1996). Many of these viruses are not known to cause human illness; some have only been isolated from arthropods, birds or animals, and their public or animal health significance is unknown. Others may cause significant human illness. *Arboviruses may be considered according to the four groups of arthropods that transmit them: mosquitoes, sandflies, biting midges and ticks* (WHO Europe 2004).

4.3. Lack of reliable disease and distribution data

There are an estimated 500 to 600 known arboviruses (arthropod-borne viruses) in the world, of which some 100 may give rise to disease in humans (ICTV, 2011). It must, however, be emphasized that these figures are very uncertain and preliminary. New arboviruses will continue to be detected, and "new" diseases in humans, domestic and wildlife animals, caused by "new and "old" arboviruses, will appear as time goes by. The difficulties in obtaining reliable data on the global distribution and disease burden were illustrated by a recently published, comprehensive review on dengue (Bhatt et al. 2013). Using innovative modeling techniques that took into account new evidence of risk factors, 8300 reports of dengue were evaluated. The researchers concluded that in 2010, 96 million people were seeking medical attention and missed school or work due to disease caused by dengue viruses. Furthermore, another 294 million were infected, but suffered milder or no symptoms. The totals are

almost 4 times higher than the previous assumptions of WHO. Commentators expect that future studies with even more complete data will show a still heavier burden of disease (Yuill, 2013). This tendency to under-report and –emphasize the distribution and burdens of arbovirus diseases may be the case for other viruses as well, and the situation may be even more under-estimated in the context of animal, wildlife as well as domestic, infections with the same viruses.

4.4. Natural life history and ecology

Arbovirus infection of a vector is established upon blood feeding of a susceptible female mosquito on a viremic vertebrate host. Arboviruses have a complex life cycle involving replication in both their invertebrate vectors, as well as their vertebrate hosts, where disease manifestations may or may not be present. This need to replicate efficiently in two completely disparate systems affects the viral properties and evolutionary patterns (Powers, 2009). Within the insect vector, arboviruses have a complex life cycle. It includes replication in the midgut, followed by systemic dissemination via the hemolymph followed by efficient replication in the salivary glands. Sometimes the virus may be transovarially and/or transstadially transmitted during the life cycle of the infected arthropod. This will make the virus more firmly established within the ecosystem. Transmission of an arbovirus to a naive vertebrate host during blood feeding requires high viral titers in the saliva. Anatomical and immunological barriers affect the ability of the virus to reach such titers and thus to accomplish successful transmission to a naive host. Despite efficient replication, arboviruses do not regularly cause overt pathology and are in many cases associated with minor fitness costs in the insect vector. This suggests that the insect immune system restricts virus infection to non-pathogenic levels (see chapter 7.5). Understanding the mechanisms of insect antiviral immunity may provide opportunities to restrict the spread of arboviruses (Merkling and van Rij, 2013).

The arbovirus definition means that a virus cannot be included among the arboviruses solely because of its isolation from an arthropod. The virus may have been accidentally present on the surface of the arthropod after contact with a host organism. The final proofs are lacking as long as the biological transmission cycles are not completely observed in nature or are experimentally reproduced (Casals 1971; Traavik, 1979). It must be realized and taken into consideration that arthropod vectors may spread some viruses that are not “true” arboviruses by modes included in the expression *“mechanic transmission”.*

There has been enormous progress in medical entomology since arthropods, more than 120 years ago, were first shown to transmit pathogens to humans. It is now accepted that vector-borne virus cycles are complex systems due to the requisite interactions between arthropod vectors, animal hosts and virus. These systems are under the influence of environmental factors that contribute, in complex ways, to variation in virus transmission (Tabachnick, 2010). Arbovirus maintenance in nature depends on host and vector coexistence in time and space. From an ecosystemic approach of transmission cycles, it is unlikely that the maintenance of a virus is restricted to determined vectors

and hosts, particularly when considering a multi-host-vector virus, like certain arboviruses. Therefore, the determining factors for the maintenance of an arbovirus in nature are the intertwined biological links that integrate a transmission network rather than a trans- mission cycle. Consequently, to better understand the activity pattern and transmission networks of arboviruses, it is fundamental to understand the species assembly of hosts and vectors, their interactions, and fluctuation through time and space (Diaz et al., 2013).

4.5. Determinants of occurrence, distribution and maintenance[2]

Considering the large number of arboviruses that have been isolated, it is not surprising that still relatively little is known about the transmission- and maintenance-competent vectors and vertebrate hosts under all possible conditions and climate zones. Each single arbovirus must be submitted to comprehensive laboratory investigations and rigorous, well-planned and -designed laboratory investigations in order to conceive its natural history. That is an exceedingly difficult task. There are now more than 500 recognized or candidate arboviruses, but information on many of them is still meager and scanty. Although mammals and birds appear to be natural hosts for a number of viruses, it is often difficult to place their importance into a total picture. Some species are often investigated first due to relative ease of collection and handling. For some viruses the attention has recently turned to poikilothermic vertebrates (reptiles and amphibians) because convincing information on arbovirus natural histories did not emerge from studies of birds and mammals. For a number of arboviruses that have been isolated from arhropods no vertebrate host has been found in nature, in spite of extensive virological and serological investigations.

Processes and actors behind long-term survival of arboviruses in the ecosystems are not well understood for most viruses. Arboviruses occur in virtually all regions of the earth, and are hence exposed to extremely different climatic conditions. The viruses probably circulate all year round in tropical regions. In temperate areas this is not conceivable since the activities of arthropods often ceases for a varying annual period.

Two main hypotheses have been advanced in order to explain the maintenance of an arbovirus when continuous arthropod-vectored transmission is interrupred: i) transovarial and interstadial transfer (see chapter 3.2.4), which can occur in mosquitoes and ticks, at least; and ii) overwintering in poikilothermc and homeothermic vertebrate hosts. The studies of poikilotherms have been largely responsible for the emergence of this field of virology (Shortbridge and Oya, 1984).

[2] Note of virus-ecological importance: Many arboviruses may persist, and overwinter, in eggs from infected females (hence these vectors are also reservoirs), next generation may transmit virus during first blood meal)

4.6. "New arboviruses will emerge at a place close to you"

The true magnitude of arboviral diseases and its associated human, economic and social costs are difficult to quantify, thus largely unknown (WHO 1985). In one recent study, the burden of Disability Adjusted Life-Years (DALYs) lost attributable to YFV, JEV, CHIKV, and RFV was estimated to fall between 300,000 and 5,000,000 (LaBeaud et al. 2011). DEN, considered as the most important human arbovirus, have increased in incidence by 30-fold in the last decade, with an estimated 50–100 million annual cases (WHO 2012).

The evolution and diversification of many arboviruses in the tropics resulted in more invasive and virulent strains (Weaver and Barrett 2004). There is no doubt that during the last 50 years or so patterns of emerging arbovirus range and disease have changed significantly. During the past decade a number of human- and animal-pathogenic arboviruses (see below for further discussions and examples) have emerged and caused epidemics and epizootics in North America, Europe and on the Arabian Peninsula. For example, unprecedented 10°C temperature anomalies in Saskatchewan, Canada, during 2003 and 2007 were accompanied by large epidemics of West Nile virus (Reisen 2012). The northernmost part of this province (approximately 60 N) is at the same latitude as the southern parts of Norway.

4.6.1. Climate

Climate is a major factor in determining:

- The geographic and temporal distribution of arthropods;
- Characteristics of arthropod and host/reservoir animal life cycles, as well as their geographic and temporal distribution
- Dispersal patterns of associated arboviruses
- The evolution of arboviruses
- The efficiency of arbovirus transmssion from arthropods to vertebrate hosts.

As stated by Gould and Higgs (2009): *"Thus, under the influence of increasing temperatures and rainfalls through warming of the oceans, and alteration of the natural cycles that stabilize climate, one is inevitably drawn to the conclusion that arboviruses will continue to emerge in new regions", and further "Undoubtedly, if the damage we have already done to our planet cannot be reversed, or at least if we cannot reduce harmful chemical emissions and prevent further damage, the emergence of either new or reemerging arthropod-borne virus (arbovirus) diseases in new areas of the world, such as southern and northern Europe, would be expected to continue to occur, perhaps with increasing frequency".*

4.6.1.1. Anthropogenic factors

Although arbovirus emergence may to varying degrees be attributable to the impact of climate change (Epstein et al., 2007; Chretien et al, 2007), a variety of other factors may contribute to different extents, for instance:

- Local levels of socio-economic development
- Increasing human travel
- Increasing domestic animal trade
- Commercial transportation
- Urbanization
- Deforestation
- Land reclamation
- Irrigation projects
- Human, animal and arthropod population density increase
- Political and military activities that lead to mass human and domestic animal evacuation (Smolinski et al., 2003).

And furthermore: the putative influence of *chemical pollution cocktails*, particularly when composed of *those with hormonal and/or immune system impacts,* should not be underestimated.

4.6.1.2. Virus impacts on their arthropod vectors

The traditional assumption is that vector-borne pathogens should evolve towards a benign relationship with their arthropod vectors. This has been challenged on theoretical grounds and empirical evidence. However, in the case of arboviruses (arthropod-borne viruses), although a number of investigators have reported experimental evidence for virus-induced vector mortality, others have failed to detect any significant impact. Whether this variation in the observed level of arbovirus virulence depends on biological traits or experimental design is unclear. A meta-analysis of studies across a range of mosquito–virus systems showed that, overall, arboviruses do reduce the survival of their mosquito vectors, but that the magnitude of the effect depends on the vector/virus taxonomic groups and the mode of virus transmission. Alphaviruses (see 4.6.1) were associated with highest virulence levels in mosquitoes. Horizontal transmission (intrathoracic inoculation or oral infection) was correlated with significant virus-induced mortality, whereas a lack of adverse effect was found for *Aedes* mosquitoes infected transovarially by bunyaviruses (see 4.6.1), a group of viruses characterized by high natural rates of vertical transmission in their enzootic vectors. Such findings are consistent with the general prediction that vertically transmitted pathogens should be less virulent than those transmitted horizontally. Varying degrees of virulence observed among vector–virus systems may reflect different selective pressures imposed on virus strains that are primarily transmitted horizontally and not vertically (Lambrechts and Scott, 2009). Different selective pressures might include climate change, different ecosystem conditions and genetic variation among virus strains as well as vector and host populations.

4.6.1.3. Virus impacts on vertebrate vectors and hosts

This topic, which is so potentially crucial in an evolutionary and ecosystem resilience context, is a definite "orphan in science". Under the headings of the specific virus families and species, some examples will be given of known impacts, and further examples will be found in some of the reviews referred to, e.g. Kuno and Chang, 2005. However, nearly all documented examples are related to serious disease or mortality observed either by chance under field conditions, or in experimental virus inoculation studies.

Viruses, ticks, muskrats and raccoon dogs: An illustrative tale of invasion.

The *muskrat*, *Ondatra zibethicus*, is one of the semi-aquatic rodents that have been introduced into many areas of the world. It is the largest species in the subfamily *Arvicolinae*, which includes 142 other species of rodents, mostly voles and lemmings. Muskrats are referred to as "rats" in a general sense because they are medium-sized rodents with an adaptable lifestyle and an omnivorous diet. They are not, however, so-called "true rats", that is, members of the genus *Rattus*. It is regarded as a most successful vertebrate invader. It was introduced into Finland in 1919 and the Kola Peninsula in 1931 for the purpose of fur hunting. From these areas it has spread "naturally" to Sweden and Norway. It seems quite natural that the muskrat, sooner or later, would spread into northern Norway, which shares borders with both the Kola region, ant the northernmost parts of Finland as well as Sweden. There were observations of muskrats in the river Alta area around 1960, and the first individual was captured alive in the Tana district in 1969. According to Danell (1996), a rapid muskrat population increase has taken place in Sør-Varanger since 1988.

The muskrat is a known host, and *I. persulcatus* a known vector, of a number of arboviruses that may give diseases in wildlife, domestic animals and humans, e.g. TBE (tick-borne encephalitis) and OHF (Omsk hemorrhagic fever). The spread of the muskrat falls together in time with the northwestwards spread of the taiga tick *Ixodes persulcatu*s. It is assumed that this migration is connected with the on-going climate changes, and *I. persulcatus* has already been detected in Finland. Jääskeläinen et al. (2006) isolated 11 Siberian subtype tickborne encephalitis virus (TBEV) strains from *Ixodes persulcatus* ticks collected in a TBEV-endemic focus in the Kokkola Archipelago, western Finland. Thus *I. persulcatus* and the Siberian TBEV were reported in a focus considerably northwest of their previously known range in Eastern Europe and Siberia. A further northwest move and establishment in Sweden and Norway would not be unexpected.

Muskrat (Ondatra zibethicus).
Photo: D. Gordon E. Robertson, Wikimedia Commons Licence

Raccoon dog (Nyctereutes procyonoides, "mårhund" på norsk*),* another successful mammalian invader, was quite recently (January 24, 2010) broadcasted in Norwegian media due to the first capture of a live individual. *Nyctereutes procyonoides* is native to Eastern Siberia, Northern China, North Vietnam, Korea, and Japan. Between 1927 and 1957, the fur-farming industry introduced from 4,000 to 9,000 raccoon dogs to the European and Asian U.S.S.R. Today, *N. procyonoides* is widespread throughout northern and western Europe in countries including Finland, Sweden, Norway, Poland, Romania, Czech Republic, Slovakia, Germany, France, Austria, and Hungary (Carr 2012).

In other parts of the world the raccoon dog is a known host and reservoir for a number of vector-borne and rodent-borne viruses.

4.7. Staying North or going North: Indiginous and emerging arboviruses in Europe

Table 1:

Details of selected arbovirus families

Virus family	Enveloped	Genome (sense)	Segmentation (number)	Example virus
Bunyaviridae	Yes	RNA SS[1] (-)	3	Rift Valley fever
Flaviviridae	Yes	RNA SS (+)	Nonsegmented	Dengue virus
Reoviridae	No	RNA DS[2]	10-12	Bluetounge virus
Rhabdoviridae	Yes	RNA SS (-)	Nonsegmented	Vesicular stomatitis virus
Togaviridae	Yes	RNA SS (+)	Nonsegmented	Chikungunya virus
Asfarviridae	Yes	DNA DS	Nonsegmented	African swine fever virus
Orthomyxoviridae	Yes	RNA SS (-)	8	Thogoto virus

[1]Single-standed

[2]Double-standed

From Johnson et al. 2012

4.7.1. Mosquito-borne viruses

The total number of mosquito-borne arboviruses on Earth is totally unknown. Most authors indicate that viruses thought to infect humans have been recovered from more than 150 species of mosquitoes, belonging to 14 different genera.

The number of mosquito-borne viruses known to occur in Europe at the moment seemingly stands at 11. These viruses belong to three families: *Togaviridae* (Sindbis, Chikungunya), *Flaviviridae* (West Nile, Usutu, Dengue, Bagaza), and *Bunyaviridae* (Batai, Ťahyňa, Snowshoe hare, Inkoo, Lednice). Several of them play a definite role in human or animal pathology (Sindbis, Chikungunya, Dengue, West Nile, Ťahyňa). Circulation of mosquito-borne arboviruses is strictly determined by the presence and/or import of particular competent virus vectors and their hosts. For emerging viruses it is impossible to predict whether competent vector-host combinations are present in any given threatened area. Ecological variables affect such viruses considerably. The main factors are population densities of mosquito vectors and their vertebrate hosts. These again are strongly influenced by climate factors like intense summer precipitations or floods, summer temperatures and drought, and presence of appropriate habitats, e.g., wetlands, small water pools, or intravillan sewage systems. Continuous, systematic surveillance- and monitoring-programs for mosquito-borne arboviruses, and the diseases they may cause in European wildlife, animal and human populations, is strongly recommended. Circulation of such viruses may often pass unnoticed or misdiagnosed, not only in free-living vertebrates but also in domestic animals and even in humans (Hubalek, 2008).

4.7.1.1. Resident or indigenous viruses

4.7.1.1.1. Togaviridae

Togaviridae is a family of viruses, including the following genera:

- Genus *Alphavirus*; type species: *Sindbis virus*, Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, Ross River virus, O'nyong'nyong virus, Chikungunya, Semliki Forest virus
- Genus *Rubivirus*; type species: *Rubella virus*

The *Togaviridae* family belongs to group IV of the Baltimore classification of viruses. The genome is linear, single-stranded, positive sense RNA that is 10,000–12,000 nucleotides long. The 5'-terminus carries a methylated nucleotide cap and the 3'-terminus has a polyadenylated tail, therefore resembling cellular mRNA. The virus is enveloped and forms spherical particles (65–70 nm diameter), the capsid within is icosahedral, constructed of 240 monomers, having a triangulation number of 4. The receptors for binding are unknown, however the tropism is varied and it is known that the glycoprotein spikes act as attachment proteins. After virus attachment and entry into the cell, gene expression and replication takes place within the cytoplasm. The vector for *Togaviridae* is primarily the mosquito, where replication of the virus occurs (Wikipedia, 2014).

Sindbis virus (SINV), *with subtypes Ockelbo, Pogosta, Karelian fever, Babanki and Kyzylagah viruses. SINV* is a member of the *Alphavirus* genus. It belongs to the American Western Equine Encephomyelitis (WEE) complex within that genus, and is closely related to the Whataroa virus from Australia.

SINV was originally isolated from Culex univittatus mosquitoes collected in Sindbis village in the Nile Delta of Egypt in 1952. The first European isolation was reported from a reed warbler (Acrocephalus scirpaceus) caught in Western Slovakia in 1971. Ockelbo and Karelian fever strains are identical to prototype SINV by polypeptide composition, but distinguishable by neutralization test. Certain antigenic and genetic differences have been described among other SINV strains isolated in different geographic areas. SINV is very widely distributed; it occurs in Africa, Eurasia and Australia. SINV strains have been detected in most parts of Europe, including Fennoscandia, with Norway, and northwest Russia (Hubalek, 2008 and references therein).

<u>*Arthropod vectors*</u>: Largely ornithophilic mosquitoes. In Europe, these are Culex pipiens, Culex torrentium (principal enzootic vector in Sweden), Culiseta morsitans, Coquillettidia richiardii, Ochlerotatus communis, Ochlerotatus excrucians, Aedes cinereus, and Anopheles hyrcanus. Laboratory transmission of SINV was documented also in Aedes albopictus (reviewed by Hubalek, 2008).

<u>*Vertebrate hosts*</u>: Wild passerine birds, e.g., *Turdidae, Fringillidae, Emberizidae, Corvus corone, Motacilla alba, Ardeola ralloides, Somateria mollissima, Anas platyrhynchos, Vanellus vanellus, Streptopelia turtur, Gallinago gallinago, Fulica atra, Acrocephalus scirpaceus, Sturnus vulgaris*,

occasionally rodents, and amphibians (*Rana ridibunda*). Migratory birds play an important role in the wide geographic distribution of the virus, including its probable introduction into Fennoscandia. Long-term persistence (53 days) of SINV in the central nervous system (CNS) of an experimentally inoculated pigeon has been observed (reviewed by Hubalek, 2008).

SINV or antibodies to SINV have been identified in wildlife in the following countries: Austria, Belarus, Bulgaria, Czech Republic, Estonia, Finland, Germany, Hungary, Italy, Moldova, *Norway*, Poland, Portugal, Romania, Russia, Serbia, Slovakia, Spain, Sweden, Ukraine, and also in the UK (ECDC,2007)

Disease: Virtually nothing is known about disease, fitness or fecundity effects for natural vectors and hosts, nor in domestic animals.

In humans the incubation period of SINV infection is often less than seven days. Maculopapular, and often itchy, exanthema over the trunk and limbs, mild fever, and joint symptoms, particularly in wrists, hips, knees, and ankles, are the hallmarks of acute SINV infection, sometimes accompanied by nausea, general malaise, headache, and muscle pain. Infectious and basic blood parameters are typically within normal range. In children, the clinical disease is usually mild, and can present without joint symptoms. Asymptomatic infections are not uncommon. Fatal infections have not been reported. In a considerable proportion of patients SINV infection leads to persistent joint manifestations that can continue for months or years, and in rare cases can even result in chronic arthritis. Outbreaks have been reported in Finland (Pogosta disease and Karelen fever) and Sweden (Ockelbo disease). In Finland, cases are reported every year, while larger outbreaks have occurred every seven years; during the 2002 outbreak in the highly endemic region of North Karelia, the incidence rate (counting serodiagnosed infections) was 81 cases/100 000 population. The proportion of subclinical and mild cases is probably high, but the available evidence is too limited to be able to quantify it (ECDC, 2007).

4.7.1.1.2. Bunyaviridae

Bunyaviridae is a family of negative-stranded, enveloped viruses with single-stranded, negative sense RNA genomes in 3 fragments: The small (S) fragment, encoding the nucleocapsid (N) protein; the medium (M) fragment, encoding two surface glycoproteins (Gn and Gc); and the large (L) fragment, encoding the viral RNA-dependent RNA polymerase. Though generally found in arthropods or rodents, certain viruses in this family occasionally infect humans. Some of them also infect plants.

There are currently about 330 viruses recognised in this family. The family Bunyaviridae contains the genera:

- Genus *Hantavirus*; type species: Hantaan virus
- Genus *Nairovirus*; type species: Dugbe virus
- Genus *Orthobunyavirus*; type species: Bunyamwera virus
- Genus *Phlebovirus*; type species: Rift Valley fever virus
- Genus *Tospovirus*; type species: Tomato spotted wilt virus

Bunyaviridae are vector-borne viruses. With the exception of Hantaviruses, transmission occurs via an arthropod vector (mosquitos, tick, or sandfly). Incidence of infection is closely linked to vector activity, for example, mosquito-borne viruses are more common in the summer (Wikipedia, 2014b).

4.7.1.1.2.1. Genus Orthobunyavirus

Inkoo (INK) and *Tahyna (TAH)* viruses are the European representatives of the California serogroup (CAL) within genus *Orthobunyavirus,* family *Bunyaviridae.* They are transmitted by a number of *Aedes* spp. mosquitoes (see Vapalahti et al., 1996 and references therein)

Inkoo virus (INK) was isolated from a pool of *Aedes communis* mosquitoes collected from Inkoo, Finland in 1964. The Finnish population has been shown to have a high seroprevalence against INK: in southern Finland about 20% and in Lapland 70-90 % of people has neutralizing antibodies to this virus (Brummer-Korvenkontio, 1969; Brummer-Korvenkontio et al. 1973, 1974). INK has also been isolated in Sweden, in the former USSR, and probably also in Norway (Traavik et al, 1978, 1985). The virus is transmitted by *Aedes communis* mosquitoes. This species feeds on large mammals, such as cows, reindeer and moose, which also show high INK antibody prevalence. Human infections occur during the summer, and are mainly mild or asymptomatic. However, cases of pneumonia, meningitis and encephalitis have been reported. The true incidence of clinical disease caused by INK remains unknown (Reviewed by Vapalahti et al., 1996).

Tahyna virus (TAH) was originally isolated in former Czechoslovakia in 1958 from a pool of *Aedes caspius* mosquitoes. High antibody prevalences have been demonstrated in Central Europe, with 30% seroprevalence, e.g. in Moravia, Czech Republic. The virus is *transmitted transovarially* in *Aedes vexans* and *Culiseta annulata* mosquitoes. Small mammals such as hares, rabbits and hedgehogs serve as amplification hosts. Human infection results in a febrile illness with respiratory and gastrointestinal symptoms, and occasionally also meningitis, but probably most infections are subclinical. The virus has also been isolated from human sera in Europe and antigenically *very* similar isolates, such as Lumbo virus (LUM), have been obtained from mosquitoes in Asia and Africa.

4.7.1.1.2.2. Reassortment of genome fragments

TAHV is a member of the California antigenic group, closely related to North American LaCrosse and Snowshoe Hare viruses. Genetic reassortment with all possible combinations of the three RNA segments has been demonstrated among them (Bishop et al. 1980), and the reassortants can appear during a mixed infection of a vector mosquito (Chandler et al. 1991)

Batai virus BATV) is an Orthobunyavirus that belongs to the Bunamwera group, It was originally isolated from *Culex gelidus* collected in Kuala Lumpur, Malaysia in 1955. Later on BATV has been detected in a number of Central European countries as well as Sweden and Finland. In Norway two Bunyamwera group viruses were isolated from *Anopheles claviger* collected at Trandum. These viruses are probably BATV strains, but that has not been finally demonstrated (Traavik et al, 1985, see below).

4.7.1.1.2.3. Viruses from mosquitoes in Norway (for reviews: see Traavik, 1979 and Hubalek, 2008)

California Encephalitis (CE) group-related strains (isolated from *Aedes spp*; Traavik et al. 1978, 1985). *Bunyamwera group* viruses (*Aedes spp*, Traavik et al., 1978, 1979a; 1985) *Flavivirus(es)?,* unknown, indicated by serological results in small rodent sera, *North of I. ricinus distribution area;* Traavik et al, 1985).

A total of 10 CE group viruses have been isolated from *Aedes spp*. mosquitoes collected in Norway. Three virus isolates were obtained from mosquitoes collected in 1975, while seven virus strains antigenically related to the California encephalitis (CE) virus group were isolated from Norwegian *Aedes* spp. mosquitoes collected in 1976. So far CE viruses have been isolated from five different *Aedes spp*. in Norway. The mosquito species and collection areas were:

- *Aedes sticticus; Øyern, Akershus*
- *Aedes diantaeus; Trandum, Akershus*
- *Aedes hexodontus; Masi, Finnmark*
- *Aedes punctor; Sjusjøen, Hedmark*
- *Aedes communis; Trandum, Akershus*
- *Aedes spp. (pool); Trysil, Hedmark*

Furthermore, two virus strains related to the Bunyamwera group were isolated from *Anopheles claviger* collected at Trandum in Akershus County. As earlier indicated these latter isolates may be BATV strains (Traavik et al., 1985; Hubalek, 2008).

Antibodies to CE viruses were demonstrated in 22 % of 1014 military recruits tested. Among 91 soldiers who were monitored by monthly blood samples during the mosquito season, sero-conversions were detected in 11 individuals. Specific IgM antibodies, indicating recent or ongoing infections, were found in seven of them. Disease symptoms in connection with the CE virus infections were not seen. The prevalence of CE antibodies in patients with CNS or respiratory infections was not higher than in control groups. Sero-conversions were not seen in any of these groups (Traavik et al., 1985). However, one of the earlier reported Norwegian CE strains caused a clinical CNS infection in the laboratory, probably by inhalation of infectious aerosol (Traavik et al., 1978).

Screening of sheep sera from six different areas in northern Norway indicated significantly different local degrees of CE virus activity. At Lyngseidet 75 % of the sheep had antibodies, while at Andøya the prevalence was only 7 %.

Based on prevalence of specific anti-CE antibodies in a very restricted number of individuals, passerine birds may be important CE virus hosts in Norway, while small rodents seem less important. Specific anti-CE IgM antibodies, indicating recent or on-going infection were detected in the sera of one of three hares and one of two squirrels (Traavik et al., 1985).

One of the CE isolates was made from *male A. diantaeus* mosquitoes collected early in the season (Traavik et al., 1978). This finding indicates some important virus-ecological traits: i) *A. diantaeus* seems to be a true vector of this virus; ii) The virus must have passed *transovarially*; and iii) This points to an *overwintering process for* CE viruses in Norway.

Due to lack of resources and methods, the Norwegian *Bunyaviridae* isolates were not species determined. Inkoo (INK) and Tahyna (TAH) viruses, are the known European representatives of the California serogroup (see 4.6.1.1.2.1).

4.7.1.1.2.4. Norwegian mosquitoes and their host animals

Descriptions have been given of the quantitative and qualitative composition of the mosquito fauna from seven biotopes where attempts were made to isolate mosquito-borne arboviruses. Observations concerning the phenology of larvae and imagines were also made. The mosquito species recorded in Norway have been tabulated and information concerning their host relations was presented (Mehl et al. 1983).

A Norwegian Aedes excrucuans mosquito has found her victim. Photo: Reidar Mehl

Aedes hexodontus (females) was the dominant species in an August collection from the sub-arctic birch forest in Northern-Norway. However, in June only *Aedes communis was found in the larval collections from the same location. This indicates that A. hexodontus* migrates down into the birch woods from the neighbouring treeless plateau. In the sub-alpine biotope in Southern Norway there was a progressional seasonal change in the dominant species from *Aedes impiger* in June to *Aedes hexodontus/punctor* and A. *communis* in the beginning of July, to *Aedes excrucians* in mid July. In two biotopes in the coniferous forests *A. communis* was the dominant species with *A. punctor*, *Aedes diantaeus* and *Anopheles claviger* as sub-dominant species. *Aedes cantans* dominated in a forest biotope on the southern coast. A mosquito fauna considered to be typical for inundation areas in Central Europe and previously unknown in Northern Europe was discovered in the delta region at the mouth of the river Glomma at Lake Øyeren, where *Aedes vexans*, *Aedes sticticus* and *Aedes russicus* were the dominating species. *Aedes dorsalis* dominated in a salt marsh biotope.

The host preferences have not been thoroughly investigated in Norway. But, as shown in the table below, reptiles and amphibian species are documented hosts for some of the mosquitoes found in Norway.

Table 4: A composite list of the mosquitos in Norway with information on their choise of hosts.

H = human, D = domestic animal, M = wild mammal, B = bird, R = reptile, A = amphibia. Symboles in paranthese indicate occasional hosts. Information from Natvig (1948), Carpenter & LaCasse (1955), Hopla (1966), Gutsevich et al. (1974), Service (1971), Zoltowski et al. (1978) and Utrio (1978, 1979).

	Norway		Finland	Germany		
	This	Natvig	Sweden	Britain	USSR	USA
Mosquito species	study	1948	Denmark	Poland	Siberia	Canada
Anopheles claviger (Meigen, 1804)	H	-	-	HDM	H	-
Anopheles maculipennis (Meigen, 1818 s.l.)	-	-	HD	-	HDB	-
Anopheles messeane (Falleroni, 1932)	-	-	H	HD	-	-
Anopheles maculipennis (Meigen, 1818 s.str.)	-	-	-	-	-	-
Culex pipiens (L., 1758)	(H)	(H)	B (HD)	B (HM)	(H) B	(H)
Culex territane (Walker, 1856)	-	-	-	RA	RA	A
Culex torrentium (Martini, 1925)	-	-	-	BM	-	-
Culiseta alaskaensis (Ludlow, 1906)	-	-	-	-	(H) M	HDMB
Culiseta annulata (Schrank, 1776)	-	HD	HD	HDMB	HMB	-
Culiseta bergrothi (Edwards, 1921)	H	D	H	-	DM	-
Culiseta subochrea (Edwards, 1921)	-	-	-	-	(H)	-
Culiseta fumipennis (Stephens, 1825)	-	-	-	-	-	-
Culiseta morsitans (Theobals, 1901)	-	-	-	HDMBR	HDBM	-
Aedes cantans (Meigen, 1818)	H	-	HD	HDMB	HM	-
Aedes caspius (Pallas, 1771)	-	-	HD	HD	HM	-
Aedes cataphylla (Dyar, 1916)	-	-	H	-	-	H
Aedes cinereus (Meigen, 1818)	H	H	HD	HDMB	H	H
Aedes communis (De Geer, 1776)	H	HB	H	HDB	HM	H
Aedes detritus (Haliday, 1833)	H	-	D	HDB	H	-
Aedes diantaeus (Howard, Dyar & Knab, 1912)	-	-	H	H	H	-
Aedes dorsalis (Meigen, 1830)	H	-	-	HDM	H	HD
Aedes excrucians (Walker, 1848 s.l.)	H	HB	H	HDB	H	H
Aedes geniculatus (Olivier, 1791)	-	-	H	HD	H	-
Aedes hexodontus (Dyar, 1916)	H	-	-	-	H	H
Aedes impiger (Walker, 1848)	H	-	-	-	HM	H
Aedes intrudens (Dyar, 1919)	-	HDB	H	-	H	H
Aedes leucomelas (Meigen, 1804)	-	-	H	-	H	-
Aedes nigrinus (Eckstein, 1918)	-	-	-	-	-	-
Aedes nigripes (Zetterstedt, 1837)	-	-	-	-	H	H
Aedes pionips (Dyar, 1922)	-	-	H	-	-	-
Aedes pullatus (Coquillett, 1904)	-	-	-	-	H	H
Aedes punctodes (Dyar, 1922)	-	-	-	-	-	H
Aedes punctor (Kirby, 1837)	H	HB	HD	HDB	HD	H
Aedes riparius (Dyar & Knap, 1907)	-	-	H	H	H	H
Aedes rossicus (Dolbeskin, Gorickaja & Mitrofanova, 1930)	-	-	-	H	H	-
Aedes sticticus (Meigen, 1838)	H	-	H	H	H	H
Aedes vexans (Meigen, 1818)	H	-	HD	HD	HDM	H

From Mehl et al., 1983, reproduced with permission.

4.7.1.1.3. Flavivirus(es) outside of the distribution area for Ixodes ricinus in Norway?

Small rodent (vole) sera were collected from three different locations in Norway. One of these was within the distribution area for *Ixodes ricinus*, and a tick-borne encephalitis (TBE) virus strain had been isolated from ticks collected there (Traavik & Mehl, 1977). The two other locations were outside the *I. ricinus* area, one in southern Norway, and the other at nearly 70° N. The sera were tested for anti-TBE antibodies by three different methods. All sera were also tested for antibodies to Uukuniemi (UUK) virus, and some positive TBE reactions were verified by separation of immunoglobulins and serum lipoproteins.

Animals containing TBE virus antibodies reacting in three different serological tests and animals with UUK antibodies were detected only from the location within the *I. ricinus* area. From the two locations outside the *I. ricinus* area we found animals, which had antibodies reacting with TBEV. Hence, circumstantial evidence and results indicate that flavivirus(es) related to, but not identical with TBE viruses are transmitted by other vectors than *I. ricinus* in parts of Norway. Flaviviruses transmitted by mosquitoes seems like a safe working hypothesis. However, it is also possible that TBEV cycles are kept up by alternative tick species. These findings were never followed up due to lack of resources.

4.7.1.2. Emerging viruses

4.7.1.2.1. Flaviviridae

The *Flaviviridae* family contains viruses that are primarily spread through arthropod vectors (mainly ticks and mosquitoes). The family gets its name from Yellow Fever virus, a type virus of Flaviviridae; flavus means yellow in Latin. (Yellow fever in turn was named because of its propensity to cause jaundice in victims.)

Flaviviridae have monopartite, linear, single-stranded RNA genomes of positive polarity, 9.6 to 12.3 kilobase in length. The 5'-termini of tgenus Flavivirus carry a methylated nucleotide cap, while other members of this family are uncapped and encode an internal ribosome entry site. Virus particles are enveloped and spherical, about 40–60 nm in diameter.

This family includes the following genera:

- Genus *Flavivirus* (type species Yellow fever virus, others include West Nile virus and Dengue Fever)—contains 67 identified human and animal viruses
- Genus *Hepacivirus* (type species Hepatitis C virus, also includes GB virus B)
- Genus *Pegivirus* (includes GB virus A, GB virus C, and GB virus D)
- Genus *Pestivirus* (type species bovine viral diarrhea virus, others include classical swine fever or hog cholera)—contains viruses infecting non-human mammals (Wikipedia 2014c).

West Nile Fever Virus **(WNV**, genus *Flavivirus*, family *Flaviviridae,* ss RNA genome; Vectors: High number of mosquito and tick species; Hosts: High number of bird, mammalian and reptilian species; disease in a number of species; no vaccine). NB! Among proven vectors 7 species have been *proven to be present in Norway* (Mehl, Traavik and Wiger, 1983): *Aedes cinereus, A. dorsalis, A. sticticus, A. vexans; Culex pipiens, C. territans; Culiseta morsitans.*

The virus: WNV is a member of the genus *Flavivirus*, in the family *Flaviviridae*. The virus is antigenically and genetically closely related to other flaviviruses in the Japanese encephalitis virus serological complex (de Madrid and Porterfield, 1974; Calisher et al., 1989), many of which cause human encephalitic infections in tropical and subtropical regions worldwide. On the basis of serological studies, virus isolation, and PCR-sequencing using samples obtained from healthy birds, horses, mosquitoes and ticks, there is now compelling evidence that WNV circulates widely and relatively harmlessly in Africa, Europe and many parts of Asia and Australasia among birds, horses, a range of other animal species and humans (Buckley et al., 2003; Gould et al., 2003; Buckley et al., 2006).

Distribution: In the Old World, WNV is most frequently associated with ornithophilic *Culex spp*. mosquitoes, which amplify the virus and transmit it to *resident and migratory birds*, thus facilitating the observed wide geographic dispersal of WNV. Detailed phylogenetic analyses of WNV strains originally identified two major clades of WNV, defined as lineages I and II. The lineage II viruses were primarily isolated in sylvatic African environments and were rarely associated with human epidemic outbreaks, whereas the lineage I viruses were mostly obtained during outbreaks of West Nile fever/encephalitis in Africa, southern Europe, the Russian landmass, India or Australia (Lanciotti et al., 2002). Subsequently, several new isolates of WNV from mosquitoes and/or ticks in the Volga region of Russia (Lvov et al., 2004) and in the Czech Republic (Bakonyi et al., 2005) have shown greater genetic diversity, *implying the possibility of further evolutionary divergence as these viruses have dispersed into more northerly climates.* The implications of the phylogenetic data, combined with the widespread serological evidence of WNV throughout Africa (Work et al., 1953, 1955), are that *this virus originated from ancestral African lineages less than 2000 years ago* (Zanotto et al., 1996*) and was dispersed out of Africa via migratory birds* (Gould 2002, 2003). This scenario is supported by several independent studies. Firstly, in the UK, healthy resident and migratory birds and sentinel chickens were shown to possess neutralising antibodies and viral RNA specific for WNV and Usutu virus (USUV) (Buckley et al., 2003; Gould et al., 2003; Buckley et al., 2006). Secondly, similar findings have been reported in many European and Asian countries, including Spain, France, Portugal, northern Italy, Poland and the Czech Republic (reviewed in Gould and Higgs, 2009). *From a virological perspective, this is not surprising,* as *Ockelbo virus*, a close relative of the African alphavirus, Sindbis virus (see 4.6.1.1.1), has been isolated from humans suffering with polyarthritis in Scandinavia (Lundstrom et al., 1993; Espmark et al., 1984) It seems most likely that Ockelbo virus was introduced into Scandinavia by *birds migrating from Africa.* Moreover, as cited above, *both WNV and USUV RNA sequences have been detected in mosquitoes collected in Portugal and Spain, i.e. regions of Europe directly beneath avian migratory flight paths to the UK and Scandinavia.* Recent evidence, based on sequencing and phylogenetic analysis, supports previous observations that both lineage II and lineage

I viruses are carried long distances by migratory birds (Mackenzie et al., 2002; Botha et al., 2008) *supporting the belief that these viruses could circulate at low levels in many species in northern Europe*.

The risk posed to the United Kingdom by West Nile virus (WNV) has previously been considered low, due to the absence or scarcity of the main *Culex* sp. bridge vectors. The mosquito *Culex modestus* is widespread in southern Europe, where it acts as the principal bridge vector of WNV. This species was not previously thought to be present in the United Kingdom. But recent findings strongly indicate that it has now established itself in parts of England. The addition of this species to the United Kingdom's mosquito fauna may increase the risk posed to the United Kingdom by WNV (Golding et al., 2012).

Currently there are no vaccines or antivirals with which to prevent and control WNV encephalitis in humans, although it is possible that individuals immunized against Japanese encephalitis virus, tick-borne encephalitis virus and yellow fever virus would be protected against the severest forms of infection by WNV as the result of immune cross-reactivity. However, this might also set the stage for disease caused by the phenomenon of ADE (antibody-dependent enhancement) of infection, see 7.5.2 (Cacel Tiredo 2003, Thomas et al, 2006).

WNV in North America: Lessons to be learned?

(Adapted from Gould and Higgs, 2009)

During the late summer of 1999, the discovery of unusually high numbers of dead birds (particularly corvids) and cases of human encephalitis in New York residents, heralded the first appearance of WNV in North America. Subsequent studies using nucleotide sequencing of the virus showed it to be closely related genetically to a strain of WNV from Israel (Isr98). The first isolation of the virus was from birds at the Bronx Zoo, and it has therefore been suggested that it could have been inadvertently introduced via imported birds on an incoming flight to New York Kennedy Airport, from Israel or Egypt. The weather in New York during the spring and summer of 1999 had been particularly warm and humid, conditions that favor intensive mosquito breeding and efficient arbovirus transmission. During the period between the commencement of the outbreak of West Nile fever/encephalitis and the onset of winter in 1999, when mosquito feeding activity stopped, hundreds of bird deaths were recorded in the metropolitan area of New York City, with 28 counties showing evidence of the presence of WNV in birds. Several cases of West Nile encephalitis were also identified in horses, and in total 69 human cases of meningoencephalitis were diagnosed, with seven fatalities. On the basis of sero-epidemiological evidence and a survey of individuals in the epicentre it was estimated that *thousands of asymptomatic or very mild viral infections occurred*, with less than 1% resulting in severe neurological disease (Mostashari et al, 1999).

The initial localised distribution of WNV in the New York area and the subsequent pattern of dispersal across North America during the ensuing years was remarkable, although, perhaps in the light of our knowledge of WNV in the Old World, not so surprising. By the end of the year 2000, WNV had been detected in birds in 136 counties, predominantly in those that surrounded the original 28 positive counties from 1999; but in addition the virus had clearly begun to disperse southwards on the eastern side of the US. Early in 2001, the virus was isolated in Florida and later in the year in the Midwest and north to the Great Lakes. Moreover, the first WNV-positive bird was identified in Ontario, Canada in August 2001. The virus continued to disperse westwards during 2002, and although the Rocky Mountains initially appeared to be a barrier to its dispersal, WNV was eventually isolated in birds in California during 2002. By the end of 2003, the virus had been identified in almost every mainland state of the US, and was beginning to be identified in Mexico and the Caribbean. The virus has since been identified as far south as Argentina.

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Following its introduction into North America, considerable resources were provided to study all aspects of the virus. It quickly became clear that WNV in North America had found a highly susceptible environment in which to amplify and disperse. In addition to avian species and humans, the virus has been shown to infect an extremely wide range of other mammals, and even reptilian species. Moreover, WNV has been isolated or demonstrated to be present in 62 different mosquito species (7 of these mosquito species have been demonstrated in Norway (Mehl et al. 1983). (http://www.cdc.gov/ncidod/dvbid/westnile/mosquitoSpecies.htm [accessed January 23, 2012]) (Higgs et al., 2004).

Also of major concern was the discovery that the virus can be transmitted to other humans via the blood and organs of apparently non-infected individuals. Furthermore, there is also circumstantial evidence for transmissi*on of the virus from mother to infant during breastfeeding*. Moreover, there is evidence based on laboratory investigations to suggest that WNV can be *transmitted non-viraemically between infected and non-infected mosquitoes*. If these two mechanisms of virus transmission do occur in the wild, it effectively overcomes the barrier of host susceptibility and thus increases the likelihood/efficiency of virus dispersal.

Overall, phylogenetic evidence supports the concept that WNV has been *introduced into North America and become established there only once*. Nevertheless, several studies have concluded that although WNV remains a relatively homogeneous virus population, with the most divergent strains containing *only a few nucleotide and/or amino acid substitutions,* a single WNV genotype that differs from the introduced strain has arisen since 1999 and has become dominant, largely displacing previously circulating strains throughout North America. Therefore, it appears to be undergoing *a process of adaptation to local transmission cycles*.

Interestingly, because the virus was frequently isolated both from sick and healthy birds it was widely assumed that *migratory birds were responsible for the observed dispersal patterns* that appeared to follow the recognized bird migratory routes. However, for some time it proved difficult to produce direct evidence that infectious migratory birds (i.e. birds that develop viraemia) are responsible for the observed pattern of WNV dispersal. This has now been evaluated by experimentally infecting birds in migratory disposition. *These birds display increased locomotor activity or restlessness, which can be recognised under captive conditions*. The results of this investigation support the concept that migrating passerine birds are probably the dispersal vehicles for WNV.

Another recent and interesting discovery resulting from the studies on WNV in North America also *relates to migratory birds*. It has been known for some time that *in the more northerly parts of North America the peak incidence of WNV infections in humans occurs in the late summer and early autumn period of the year*. Studies of the primary ornithophilic arthropod vectors of WNV in north east America, *Cx. pipiens* , and in California, *Cx. tarsalis* , suggest that *these mosquito species shift their feeding preferences from birds to mammals in the late summer, when the birds become less numerous as they begin to migrate south.*

This shift of feeding preference by Cx. pipiens may have a significant impact on WNV epidemiology in the northeast and north central parts of North America. A similar shift in feeding preference of *Cx. tarsalis* appears to have the same impact on WNV epidemic intensity in west and central North America. This can be explained as follows: the feeding preference for avian species in the early period of the summer intensifies epidemics of WNV infection among avian species, thus increasing the proportion of infected mosquitoes. The shift of feeding preference to mammals in the late summer then intensifies the epidemics in humans. These observations, at least in part, could also explain why WNV appears to have been more virulent for birds and causes a higher number of human infections in the New World than in the Old World. Other possible contributory factors to the *increased intensity* of epidemics in North America include: *(1) the lack of immunity in mammalian populations in North America before the introduction of WNV; (2) the fact that Cx. pipiens in the New World is a hybrid between European Cx. pipiens , a bird-biting mosquito, and Cx. molestus , a human-biting mosquito* (Fonseca et al, 2004*); and (3) the possibility that the strain of WNV introduced into North America is more virulent for American crows than for those circulating in the Old World (*Brault et al*.,* 2004).

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Since its introduction into North America in 1999, molecular epidemiologic studies have clearly demonstrated that *the virus has evolved to maximize its transmission potential within local transmission cycles.* This finding stimulated subsequent efforts to understand the details of the underlying evolutionary mechanisms that lead to population-level genetic and phenotypic changes in the virus. In brief, these studies demonstrated that WNV populations are genetically diverse within hosts, that genetic diversity may be shared between hosts, and that *mosquito infection drives genetic diversification of the virus population both through relaxation of purifying selection and through selection of rare genotypes resulting from RNA interference (RNAi; see 7.5.4). Thus, in the WNV transmission cycle, different host types differentially influence the virus population.* Whereas infection of mosquitoes leads to high levels of population variation and consequent adaptive plasticity, vertebrate infection maintains high fitness through strong purifying selection (reviewed by Ebel et al., 2011).

Although environmental conditions, in terms of local temperature and rainfall, are clearly very important in determining whether or not WNV is efficiently transmitted between vertebrates and mosquitoes, climate change, in terms of progressive increases of average temperature and rainfall, has not played an obvious role in the epidemic outbreaks of WNV seen in North America. The most important factors have been the *availability of competent vector species and the wide range and large numbers of susceptible species of migratory birds that have dispersed the virus throughout the Americas*. *Human activity,* in the form of animal transportation, farming practices, blood transfusion, organ transplantation, leisure activities, sanitation infrastructure, etc., have also contributed to local outbreaks and possibly, through air transport, to the original introduction of the virus from Israel/Egypt into the Western Hemisphere.

Usutu virus

(USUV, *Flaviviridae*, various mosquito spp, mostly in the *Culex pipiens* complex; disease in blackbirds - and others?)

During 2001, in Austria, the *Usutu virus (USUV*) was, for the first time, detected outside Africa. USUV is a rather unknown member of the *Flaviviridae* family of the Japanese encephalitis virus complex. It is closely related to the more common West Nile virus (WNV), dengue virus (DENV), Japanese encephalitis- virus (JEV) and yellow fever virus (YFV). USUV caused mass mortalities of birds, especially blackbirds (*Turdus merula*) and great grey owls (*Strix nebulosa*), in and around the capital city of Austria, Vienna. It was later confirmed that USUV overwintered in Austria. Although the initial number of dead blackbirds was very low in 2001 and 2002, an epidemic peak was observed during the extraordinary hot summer 2003. In the meantime, USUV was also observed in other Central European countries such as Switzerland, Hungary, and Northern Italy. The account of USUV in Europe has been reviewed recently (Rubel et al. 2008, Brugger and Rubel 2009).

USUV is circulating between arthropod vectors (mainly mosquitoes of the *Culex pipiens* complex) and avian amplification hosts. Infections of mammalian hosts or humans, as observed for the related West Nile virus (WNV), seem to be rare. However, USUV infection leads to a high mortality in birds, especially blackbirds (*Turdus merula*), and has similar dynamics with the WNV in North America, which, amongst others, caused mortality in American robins (*Turdus migratorius*).

Austrian researchers (Rubel et al. 2008) hypothesized that the transmission of USUV is determined by an interaction of developing proportion of the avian hosts immune status and climatic factors affecting the mosquito population. This process was implemented into a model that simulated the seasonal cycles of mosquito and bird populations as well as USUV cross-infections. Observed monthly climate data were specified for the temperature-dependent development rates of the mosquitoes as well as the temperature-dependent extrinsic-incubation period. The model reproduced the observed number of dead birds in Austria between 2001 and 2005, including the peaks in the relevant years. The high number of USUV cases in 2003 seemed to be a response to the early beginning of the extraordinary hot summer in that year. Extrapolation from the model suggested that only 0.2% of the blackbirds killed by USUV were detected by the Austrian USUV monitoring program (Chvala et al. 2007).

It should be noted that both blackbirds ("svarttrost") and great grey owls ("lappugle") are stationary in Norway.

Bagaza virus

(BAGV, Flaviviridae, various mosquito species, serious disease in some bird species)
In September 2010, an unusually high number of wild birds (partridges and pheasants) died in Cadiz, Andalusia, southwestern Spain. Reverse transcription PCR and virus isolation detected flavivirus infections. Complete nucleotide sequence analysis identified Bagaza virus (BAGV), a flavivirus with a known distribution that includes sub-Saharan Africa and India, as the causative agent. This virus was first isolated in Bagaza, Central African Republic, in 1966, from a pool of mixed-species female *Culex* spp. mosquitoes. BAGV has subsequently been found in mosquitoes in other countries in western Africa and in India, where serologic evidence suggests that this virus may infect humans, although its pathogenicity in humans is uncertain. BAGV has been shown to be synonymous with Israel turkey meningoencephalitis virus, a pathogen affecting poultry (turkeys) and reported only in Israel and South Africa (Aguero et al. 2011).

Sequence of BAGV from Spain was closely related to the 2 unique full-length BAGV sequences available in GenBank and showed greater similarity with the strain from Africa (94.1 percent nt identity) than with the strain from India (92.8 percent nt identity). Genetic relatedness between all 3 viruses was high (higher than 92 percent), which indicates that they belong to the same *Flavivirus* species (Bagaza virus). The tree based on the E region grouped Israel turkey meningoencephalitis virus and BAGV within the same cluster and showed that both viruses are closely related to Ntaya virus.

No signs of infection and no deaths were observed for other bird species. However, whether other bird species are susceptible to disease caused by BAGV should be determined because this virus is similar to Israel turkey meningoencephalitis virus, a relevant pathogen for turkeys. Also, other vertebrates could be at risk for infection with this virus. Thus, experimental studies on the pathogenicity of this virus in specific vertebrates should be conducted.

A study performed in the state of Kerala, India indicates the necessity of serious efforts to investigate the likely involvement of BAGV in sporadic human infections, and outbreaks in other vertebrates (Bondre et al. 2009). This can be achieved by developing BAGV-specific serological and molecular diagnostics for testing of human clinical specimens collected from the region. Additional studies addressing the potential of various mosquito species as vectors and birds as amplifying hosts, as well as sero-surveillance in domestic animals and the human population will add to our understanding of the epidemiology of arboviral diseases.

Dengue viruses

DENV**;** *Flaviviridae*, various, mainly *Aedes* spp, mosquitoes, high number of vertebrate species, serious disease in humans following multi virus type infections. Antibody dependent enhancement may be part of the pathogenesis for the most serious human infections (see 7.5.2).

Once rare, Dengue fever has developed into one of the world's major emerging infectious diseases, with recorded prevalence in more than 120 countries and an estimated >50 million clinical cases per year (LaBeaud et al., 2011) . Dengue viruses cause dengue fever (DF) and dengue hemorrhagic fever (DHF) in the tropics from Southeast and Southern Asia, the Caribbean, and many countries in South and Central America, and outbreaks are reported with increasing frequency globally (Jelinek, 2009). The four distinct dengue virus serotypes (DENV-1 to DENV-4) belong to the family *Flaviviridae*, closely related to JEV and WNV, and are among the most important vector-borne pathogens of humans, causing up to 100 million cases annually, and tens-of thousands of cases of the more severe and sometimes fatal *dengue hemorrhagic fever/shock syndrome (DHF/DSS syndromes), which is due to antibody dependent enhancement of infection* (see 7.5.2). Dengue viruses are mosquito-transmitted and circulate in both a sylvatic (enzootic) cycle involving non-human primates and various species of *Aedes* mosquitoes (such as *Ae. furcifer, Ae. luteocephalus and Ae. taylori*), and in a human (endemic) cycle principally vectored by *Ae aegypti* or *Ae. albopictus*. The only sylvatic DENV serotype that has been isolated in Africa is DENV-2. In contrast, sylvatic DENV-1, DENV-2 and DENV-4 have been isolated in Asia, although the last isolation of a sylvatic virus (of DENV-4) occurred in 1975, and the 'sylvatic' isolate of DENV-1 is not phylogenetically distinct from human lineages so that its origin is uncertain. Phylogenetic data suggests that sylvatic DENV are the ancestors of those viruses that now circulate endemically in human populations (reviewed by Cardosa et al. 2009).

DENVs that cause most human disease are not zoonoses, but exclusively utilize humans as reservoir and amplification hosts. Also unlike most arboviruses, they rely on transmission by mosquito vectors that live in close association with people; *Ae. aegypti* is the principal vector in most locations, with *Ae. albopictus* serving as a secondary vector in some locations.

The effects of global warming on DENV transmission are not as predictable as those of some other arboviruses, particularly for the sylvatic strains present in Asia. The biology of the mosquito vectors of these DENV strains is not well known, and their capacity for migration into temperate zones should be experimentally explored.

Because warming is expected to be most dramatic in temperate climates, the distributions of the 2 principal DENV vectors could expand. *Ae. aegypti* cannot tolerate cold winters and currently is limited to subtropical and tropical regions. Its northern and southern expansion could certainly be followed by extended DENV transmission into locations where human behavior and culture permits adequate exposure to this mosquito (e.g. water storage and the presence of artificial containers for larval development, and the lack of barriers to the movement of adult females into homes, such as window screening and air conditioning). However, in some subtropical regions, cultural practices limit exposure to this vector. One example is the southern U.S. where *Ae. aegypti*-borne DEN epidemics occurred beginning in the 18th century, but disappeared about a century ago, coincident with improved hygiene and the widespread use of window screening, and later, air-conditioning. In these locations, climate warming might have little or no effect on DENV. Although a less efficient vector, some strains of *Ae. albopictus* have a diapausing egg state and therefore greater tolerance for temperate winters. This species is less endophilic, so human exposure may be less dependent on home construction and probably occurs principally within the external, peridomestic environment (reviewed by Weaver and Reisen 2010).

Ae. albopictus has been detected in Northern Europe already, and will most probably spread further towards the north and east according to climate change based modelling (see EDCD 2009). Following the arrival and spread of *Aedes albopictus* in Europe, Rogers et al. (2014) created global risk maps based on remotely sensed satellite data from NASA's MODIS series. The risk maps showed that very few areas of rural Europe are suitable for dengue invasion and establishment, while several major cities appear to be at some degrees of risk. But this picture may, of course, change if the global warming process keeps developing.

Factors associated with emergence: Although most DENV strains have no vertebrate hosts other than humans, ancestral DENV are represented by strains that continue to circulate in the forests of West Africa and Southeast Asia. These DENV use nonhuman primates as reservoir/amplification hosts and canopy mosquitoes as enzootic vectors. Emergence into the evolutionarily and ecologically independent urban cycles occurred independently for each serotype an estimated hundreds of years ago. Experimental studies of vector competence and human models suggest that the emergence of DENV-2 required little or no adaptation to *Ae. aegypti* or humans, and epidemiologic and genetic analyses of human isolates indicate that sylvatic DENV-2 can cause typical DEN fever and some hemorrhagic manifestations (Cardosa et al., 2009). However, there is evidence that one particular genotype of DENV-2, termed "Asian," has evolved higher human virulence, in the context of immune enhancement, with limited antigenic cross-reactivity against previous DENV infections and greater infectivity for *Ae. aegypti* allowing it to displace the less fit "American" genotype from some parts of the Americas (reviewed by Weaver and Reisen 2009).

Togaviridae

Chikungunya virus

CHIKV, genus *Alphavirus* within the *Togaviridae*, ss RNA, a variety of *Aedes* spp, vector "victims", disease in a number of them. Has occurred in or nearby many of the forested areas of Africa, causing human Chikunguya fever. Later established as cause of febrile joint pains in humans. CHIKV circulate by *Aedes* mosquitoes among simian species.

"The silence of the virus": Neither vectors nor hosts display clinical evidence of infection. Following a virus-infected blood meal the virus is amplified in the vector and may be transferred to the eggs, which are then deposited in the forests. It is believed that, *in common with certain other arboviruses, CHIKV may survive for long periods of time in eggs.* If that were the case, then during rainy periods, these transovarially-infected eggs would hatch and subsequently produce adults able to immediately transmit virus to susceptible vertebrates (Gould and Higgs, 2009). There are no field or laboratory data to confirm this process of long-term virus survival, and alternative hypotheses have been proposed. One suggestion is that the virus may survive in wildlife species through constant transmission cycles moving in epizootic waves (Powers AM and Logue CH. 2007).

Genetic and phenotypic differences (selection of subtypes, mutations due to different vectors and hosts?): Spread of African CHIKV strains to a number of other, even distant geographic areas have been proven (Islands in the Indian Ocean, India, Thailand, Malaysia). However, *in 2007 an infected traveller from India arrived in Italy* and, within a few weeks, more than 200 indigenous cases of CHIKV-fever had been registered. Surprisingly, this virus strain ("Italian strain ITA07-RA1", GenBank accession no. EU244823) had the A226V mutation in the E1 envelope glycoprotein. Such strains had earlier only been isolated from *A. albopictus* vectors in India and La Réunion. Although *A. albopictus* is present in Italy, it is still an open question whether the virus mutation originated in India or Italy (Charell and Lamballerie, 2008). To test whether or not the A226V substitution had impact on the ability of CHIKV to infect different vector species, virus strains with and without the substitution were tested for their infection efficiency in *A. albopictus*. Using molecular methods and laboratory mosquito transmission experiments, it was conclusively demonstrated that the single amino acid substitution of alanine for valine in position 226 of the E1 glycoprotein directly influenced vector specificity by enhancing CHIKV multiplication and transmission in A. albopictus (Vazeille et al., 2007; Tsetsarkin et al., 2007). These data have general, important implications with respect to how viruses may establish a transmission cycle when introduced into a new area. More specifically, *A. albopictus* is now present along the Mediterranean areas of southern Europe (Spain, France, Italy). Quite recently *Aedes albopictus* was also demonstrated in the Netherlands (province of Noord Brabant) and in England.

In summary, *the success of CHIKV in invading* the Comoros Islands and subsequently dispersing to Mauritius, La Réunion Island and other nearby islands, and also India and Malaysia, *resulted from a combination of factors*. Firstly, increasing human mobility and commercial transportation of scrap car tyres and other water-retaining objects such as plants, both into and out of Africa and also between

and within the Islands, provided a mechanism for dispersal of *Ae . albopictus* . Secondly, the contribution of adaptive mutation of CHIKV to *Ae. albopictus* , resulting in increased transmission and amplification in this successful mosquito species. Thirdly, the presence of an *immunologically naïve human population*, including tourists, providing a high number of susceptible individuals, may have been important. Finally, the difficulties in rapidly implementing mosquito control measures and disseminating relevant information to local communities on the islands compounded the problems. It therefore seems reasonably safe to conclude that while climate change may have contributed to the epidemic outbreaks through a lack of socio-economic development, it is unlikely to have exerted a major influence on dispersal of the African virus to the Indian Ocean, India, Sri Lanka and Malaysia.

Currently, there are *no vaccines or antivirals* with which to control CHIKV epidemic outbreaks; thus, the only effective methods of avoiding infection are to reduce the number of potential breeding sites for *Ae . aegypti* and *Ae . albopictus* and to avoid exposure to infected mosquitoes.

Recently published long-term modeling maps indicate that Denmark, the southern parts of Sweden and Finland, *and also the Norwegian coast areas from the Oslofjord and at least up to Lofoten, will be suitable for Ae. albopictus establishment (ECDC, 2009).* Higher latitudes may be at risk if the global warming proceeds according to present prognoses.

Bunyaviridae

Rift Valley Fever Virus

RVFV, genus *Phlebovirus*, family *Bunyaviridae*, ss RNA genome in 3 fragments; Vectors: A number of *Aedes* spp, other mosquito species and *Phlebotomus* sandflies; Hosts: Vector "victims", disease in a number of them; Vaccine: Available.

<u>*Wide range of competent vectors*</u>*:* A wide range of mosquito species, including *Aede*s (*Neomelaniconion* and *Stegomyia*), *Culex* , *Mansonia* , *Anopheles* and *Eretmapodites* are capable of transmitting the virus, and also the sandfly *Phlebotomus duboscqi* (Turell and Perkins, 1990). While vector competence has not been determined for all of these species, most samples trapped during epizootics have tested positive for RVFV. Many other *Aedes* and *Culex* species have been implicated in disease transmission in different regions of Africa.

RVFV has been shown experimentally to replicate in a wide variety of mammalian species, but there is considerable variation in the response to infections in the environment. Sheep, cattle, goats and camels are most frequently associated with significant epizootics, primarily because they usually outnumber other potential hosts in the regions where disease is observed.

<u>*Mechanical transmission*</u> of the virus by *Culicoides* spp., and other insects such as the tsetse fly, none of which are permissive to the virus, has also been demonstrated (Hoch et al., 1985). This may be an important component of RVFV transmission and is largely attributable to the very high levels of virus found in the blood of sheep and cattle, combined with *the phenomenon of interrupted feeding.*

During this process, the insect may feed on more than one host within a few minutes, thus mechanically carrying infectious virus from one animal to another without replication of the virus between the feeding periods.

Immunologically "virginal" species and individuals: The first recorded outbreak occurred in Kenya in imported sheep, with very large numbers of abortions and many deaths in newborn lambs and older animals. No symptomatic disease was observed in indigenous animals kept nearby. This implies that the virus had *circulated relatively harmlessly for some time in Africa, among indigenous species, before its discovery in 1930* (Gould and Higgs, 2009).

Transovarial transmission: Climatic conditions are clearly an important driver of Rift Valley fever, because the primary vectors of virus transmission to animals and humans are *Aedes* spp. mosquitoes. Direct evidence that these mosquitoes can harbour the virus for long IEPs (inter-epizootic periods) was obtained by artificial flooding of the dambo formations in an epizootic area in the Central Highlands of Kenya. Millions of *Ae. mcintoshi* larvae hatched, and RVFV was isolated from the adult mosquitoes (including males), raised in the laboratory from the field-collected larvae. Thus, *transovarial transmission* of the virus provides a plausible explanation for the survival of the virus during the IEP and for its simultaneous emergence throughout epizootic areas, exhibiting similar environmental conditions (Gould and Higgs, 2009). Indeed, River Valley fever cases have occurred in areas separated by a thousand kilometres or more, virtually at the same time.

Remote sensing satellite imagery is now being used to study a variety of environmental parameters, such as cold cloud density and intensity of green vegetation, in order to evaluate their potential to predict the emergence patterns of mosquito vectors of RVFV (Linthicum et al., 1999). As knowledge and understanding of the information gained from these remote sensing methods increases, it is hoped that they can assist in the implementation of more effective vaccination and vector control programs before an epidemic and thus reduce the spread of RVFV.

In conclusion, climatic conditions have clearly been an important determinant of RVFV epidemiology in Africa and the Arabian Peninsula over a long period of time, and climate change could theoretically create conditions in southern/central European countries and the US that might enable introduced RVFV to become established in these regions. However, in addition to climate change, other factors, such as the movement of infected animals and/or competent mosquito vectors into non-RVFV regions, will determine whether or not the virus disperses beyond its current boundaries (Gould and Higgs, 2009).

4.7.2.Tick-borne viruses[3]

By 1972, some 68 different viruses had been recorded from more that 80 tick species, some 20 of which were believed to cause disease in man or domestic animals (reviewed by Hoogstraal, 1973). Since the publication of Hoogstraal's review, many other viruses have been isolated from ticks, although their role as causative agents of human or animal disease is often unknown or uncertain. Many areas of Europe remain poorly surveyed, and more viruses will certainly be found in further studies.

Tick-borne viruses belong to an ecological group of viruses characterized by their specific biological transmission via competent hematophagous hard (ixodid) or soft (argasid) ticks (*Ixodidae* and *Argasidae*, respectively) to endotherm (homeotherm, warm-blooded) vertebrates. Competent vectors are those arthropods that are able to imbibe the virus in the course of blood feeding on an infected donor vertebrate host, then support the multiplication of the virus in their organism and to finally deliver a sufficiently large inoculum to the recipient, uninfected vertebrate host. Usually certain minimum level of viremia ("infection threshold") in a donor vertebrate host is necessary for an efficient infection of particular arthropod vectors. Therefore, only those vertebrate species that produce at least moderate viremia have been regarded as competent, "true" or "amplifying" hosts of particular arboviruses. However, co-feeding ixodid ticks on a viremia-free host can sometimes also contribute to infection of noninfected ticks. Some viruses have been proven able to persist in tick eggs, and the individuals hatched from such eggs may be virus-carriers through their entire life cycle, i.e during metamorphosis from larvae to nymphs to imagos. Some tick-borne viruses are transmitted from larvae to nymphs and imagoes during metamorphosis (*transstadial transmission*, TST), from infected female to the offspring (*transovarial transmission*, TOT), and from male to female tick during copulation (*venereal* or *horizontal transmission*). These tick vector modes are extremely important ecologically: e.g., under conditions of TOT, the tick vector also plays the role of a long-term reservoir of the virus.

In addition to two "major" severe, occasionally re-emerging virus diseases transmitted by ixodid ticks in Europe, i.e. tick-borne encephalitis and Crimean-Congo hemorrhagic fever, there is a number of other, neglected tick-borne virus infections of vertebrates (see Box below). They are usually infrequent, although some of them are probably underdiagnosed, and other of these tiboviruses are nonpathogenic, or of low pathogenicity, for vertebrates.

[3] Very comprehesive and elucidating reviews have been published recently, e.g. Labuda and Nuttall, 2004; Gratz, 2006; Dobler, 2010; Hubalek and Rudolf, 2012

The number of tick-borne viruses ("tiboviruses") that have been detected in Europe stands at 27
(Adapted from Hubalek and Rudolf, 2012):

Flaviviruses: Tickborne encephalitis (TBEV), louping-ill (LIV), Tyuleniy (TYUV), and Meaban (MEAV)
Orthobunyaviruses: Bahig (BAHV) and Matruh (MTRV)
Phleboviruses: Grand Arbaud (GAV), Ponteves (PTVV), Uukuniemi (UUKV), Zaliv
Terpeniya (ZTV), and St. Abb's Head (SAHV)
Nairoviruses: Soldado (SOLV), Puffin Island (PIV), Avalon (AVAV), Clo
Mor (CMV), Crimean-Congo hemorrhagic fever (CCHFV);
Bunyaviruses: Bhanja (BHAV);
Coltiviruses: Eyach (EYAV);
Orbiviruses: Tribec (TRBV), Okhotskiy (OKHV), Cape Wrath (CWV), Mykines (MYKV), Tindholmur (TDMV), and Bauline BAUV);
Thogotoviruses: (Thogoto THOV, Dhori DHOV);
Asfivirus (African swine fever virus ASFV).

4.7.2.1. Resident and indigenous viruses

4.7.2.1.1. Family Flaviviridae

Tick-borne encephalitis virus (TBEV)

TBEV strains are currently divided into 3 closely related subtypes, i.e. Western-European (WE), Siberian (SIB) and Far Eastern (FE) (Heinz et al. 2000). FE TBEV is recognized as the most virulent pathogen with a 20–40% human case fatality rate. The SIB subtype is considered less virulent (7–8% case fatality rate) but chronic disease occurs more frequently (1–3%). WE strains are the least virulent with case fatality rates lower than 2%. *However, a range of clinical manifestations, from asymptomatic to encephalitic is observed for all TBEV subtypes (Gritsun et al. 2003a, 2003b) and the underlying basis for this has not yet been adequately explained.*

In addition to the three TBEV subtypes defined, the very *closely related Louping-ill virus* should be regarded as the fourth TBEV subtype.

Distribution: Conventionally, each TBEV subtype has been associated with distinct geographic ranges within the Old World region of the Northern hemisphere, hence the groupings Far East, Siberia and Western Europe (Heinz et al. 2000). However during recent decades the epidemiology of the TBFV appears to have been changing, with SIB TBEV becoming the dominant subtype apparently gradually replacing the WE or FE subtypes that previously appeared to monopolize many regions (reviewed by Khasnatikov et al. 2009) Moreover, the SIB subtype is being isolated more frequently from patients who develop the most severe forms of encephalitis, with the virus invading the entire brain in contrast with the more focal virus localization observed previously. Over a period of time, the most severe cases of TBE have been more frequently associated with the SIB strains than with the FE strains (Pogodina et al. 2004) indicating that this is not an artifact of increased surveillance.

Arthropod vectors: are ticks of the genus *Ixodes*: for TBEV WE *I. ricinus* (TST, TOT), the infection rate may attain 0.5 % to 3 % in natural foci and *Ixodes gi*bbosus (a vicariant, marginal vector in the

Mediterranean). Occasional vectors are other tick species such as *Ixodes hexagonus*, possibly *Ixodes arboricola.* Sporadically, metastriate tick species *Haemaphysalis inermis, Haemaphysalis concinna, Haemaphysalis punctata, Dermacentor marginatus, Dermacentor reticulatus* and *Hyalomma marginatum*. Main vector for TBEV FE ans SIB is *Ixodes persulcatus* (infection prevalence rates can reach frequently >2 %; TST, TOT; less often *Ixodes ovatus*, but also *Dermacentor silvarum, D. reticulatus, D. marginatus, H. concinna (TOT), Haemaphysalis longicornis, and Haemaphysalis japonica* (adapted from Hubalek and Rudolf, 2012).

<u>*Competent vertebrate hosts*</u>: Small forest mammals, especially rodents and insectivores (*Apodemus flavicollis, Apodemus sylvaticus, Myodes glareolus, Myodes rufocanus, Microtus agrestis, Sciurus vulgaris, Talpa europaea, Sorex araneus, Erinaceus concolor*); additional hosts may be (due to viremia) goat, sheep, rarely cattle. The role of some forest passerines and other birds as hosts of TBEV has not yet been fully elucidated; the virus was isolated occasionally from *Turdus pilaris, Turdus iliacus, other Turdus spp., Corvus monedula, Corvus corone, Pica pica, Sturnus vulgaris, Lanius collurio, Fringilla montifringilla, Fringilla coelebs, Loxia curvirostra, Carduelis flammea, Anthus trivialis, Motacilla alba, Motacilla flava, Emberiza spp., Jynx torquilla, Bonasa bonasia, Crex crex, Scolopax rusticola, Clangula hyemalis, Melanitta fusca, Anas querquedula, Fulica atra. A potential for TOT was demonstrated in some avian species* (*T. iliacus, T. pilaris, Turdus ruficollis, Turdus pallidus, Lanius cristatus, Emberiza fucata, Troglodytes troglodytes, Accipiter gentilis*) in Asian Russia by isolation of TBEV from their eggs. Experimental viremia has been demonstrated in many mammalian, avian, amphibian, and reptilian species: *Micromys minutus, Microtus arvalis, Microtus subterraneus, Myodes rufocanus, Myodes rutilus, Glis glis, Myotis myotis, Plecotus auritus, Barbastella barbastellus* cat*, Mustela nivalis, Mustela ermine*, *Coturnix coturnix, Anas platyrhynchos*, *Lacerta viridis, Lacerta agilis* and some other vertebrate species. Depending on route of inoculation, TBEV causes fatal disease in suckling and adult laboratory mouse, suckling rat, but not adult rat, newborn guinea pig, suckling hamster, rhesus monkey; lamb and kid. On the other hand, no mortality is produced by TBEV in adult forest rodents *Apodemus* and *Myodes* spp., adult rabbit. CEEV infection is usually subclinical in adult ruminants and pig; goats, sheep, and cows excrete virus in the milk. TBEV occasionally kills birds of some species, e.g., *C. flammea* (long-term viremia and the virus excretion in droppings up to 11 months was confirmed experimentally), *Passer domesticus*, and *F. atra* (adapted from Hubalek and Rudolf, 2012).

<u>*Human disease and epidemiology:*</u> Up to 14,000 human cases of tick-borne encephalitis (TBE) are recorded across Eurasia annually (Gritsun et al. 2003a, 2003b) TBE outbreaks are now registered in about 30 European countries, including Norway, with a recorded morbidity increase of about 400% during the past 30 years (Suss, 2008). TBEV is a member of the tick-borne flavivirus (TBFV) group that, together with mosquito-borne and “no-known vector” virus groups, comprise the genus Flavivirus within the family *Flaviviridae*. Human pathogens within the genus Flavivirus include Japanese encephalitis virus (JEV), Dengue virus (DENV) and Yellow fever virus (YFV) that cause annual epidemics of fever, encephalitis and hemorrhagic fever in the tropics and some sub-tropical regions (Grard et al. 2007, Gould et al. 2008)

In its natural habitat, TBEV is maintained by transmission between infected and non-infected ticks when they co-feed on small forest animals (Labuda et al. 1996, 1997). Humans are incidental hosts for ticks and may become infected by being fed on by an infected tick. The clinical manifestations caused by TBEV range from unapparent infections and fevers, with complete recovery of patients, to debilitating or fatal encephalitis. The proportion of fatal human infections varies widely in different regions and in different years. The factors that determine disease severity are poorly defined but *correlations between viral subtype and disease severity have been described*.

A vaccine produced from purified, inactivated TBEV is available. A mass vaccination campaign of Austrian population living in endemic foci led to a significant decline of TBE, and a similar 5-to-10 times decrease of TBE incidence has been reported in other European countries after frequent vaccination of population (review by Hubalek and Rudolf, 2012).

Louping ill virus (LIV)

LIV is very closely related to TBEV, and the two of them are nearly indistinguishable by most diagnostic methods. In some areas LIV goes under the name "Negishi virus".

LIV does not seem to occur outside Europe. Norway is the only country of the continental Europe where a typical LIV strain has been isolated. In Scotland "Louping ill" has long been recognized as a disease of sheep. LIV was first isolated from sheep brain in 1929. Hence it was the very first arbovirus to be isolated in Europe (reviewed by Hubalek and Rudolf, 2012).

Arthropod vector: Ixodes ricinus is the principal vector of LIV.

Competent vertebrate hosts: wood mouse (*A. sylvaticus*), common shrew (*S. araneus*), mountain hare (*Lepus timidus*), sheep, and red grouse (*Lagopus lagopus scoticus*). Dependent on inoculation route LIV infection is fatal to suckling rat, lamb, sometimes rhesus monkey. No symptoms are seen in adult *M. agrestis*, *Cervus elaphus* and *Capreolus capreolus*, although meningoencephalitis was demonstrated histologically in the deer. Furthermore, LIV has been isolated from a roe deer. LIV occasionally affects also cattle, pig (piglets), goat (kids), horse, dog and hare. Experimental infections in red grouse may give a mortality rate of 70–80 %, especially in juvenile birds. Some observations indicate that the grouse chicks die when they eat infected ticks. The typical course of LI in sheep is biphasic, first fever and weakness, followed by meningoencephalitis with cerebellar ataxia, generalized tremor, jumping (to "loup" means to leap in vernacular Scottish), vigorous kicking, salivation, clamping of jaws, progressing to paralysis, coma and death (lethality 40–60 %).

NB! Concurrent tick-borne fever (Anaplasma phagocytophilum infection) and external stress enhance the disease course (reviewed by Hubalek and Rudolf, 2012)

The symptoms of human LIV-infection are similar to those caused by TBEV. Nineteen naturally acquired human cases and 26 laboratory infections with LIV have been described in Great Britain between 1934 and 1990, including one fatal encephalitis in a butcher from northern Scotland. LIV transmission to man is obviously infrequent in the U.K. because the vector ticks only occasionally bite

people in endemic areas. It is primarily an occupational disease, affecting shepherds, crofters, veterinary personnel, forestry workers, butchers and laboratory personnel. However, human cases of LI with a milder symptomatology might remain underreported.

TBE vaccine is also protective aginst LIV. Control of LI is mainly based on vaccination of sheep; the inactivated LI vaccine is commercially available and in general use. Tick control by dipping the sheep with residual acaricides is also practicized. The methods of environmental control of ticks such as pasture rotation, cutting of burning grass and bush vegetation, and drainage are effective but economically less feasible (review by Hubalek and Rudolf, 2012).

4.7.2.1.2. Seabird-related tick-borne flaviviruses[4]

Tyuleniy virus (TYUV) was first isolated in 1969 from Ixodes uriae collected in nesting grounds of sea puffins (*Uria algae*) on Tyulenyi island, near Sakhalin in Asian Russia, and simultaneously off the western US coast

Principal vector is *I. uriae*, and both transovarial and transstadial transmission of TYUV has been demonstrated in this tick. Some *Aedes* spp. mosquitoes have been implicated as secondary or possibly mechanical vectors.

Vertebrate hosts are seabirds, particularly *U. aalge, Eudyptula minor*, and the suslik *Citellus undulatus.* Antibodies against TYUV have been demonstrated in a number of seagulls and other seabird species, as well as in some mammalian species. Antibodies were detected in a relatively high frequency among cattle in the North-European Russian taiga and tundra zones (Lvov et al., 1989).

Migratory seabirds are contributing to exchange of TYUV viruses between the northern and southern hemispheres (Lvov et al., 1989).

Meaban virus (MEAV) is related to TYUV. It was first isolated from argasid soft ticks (*Ornithodorus maritimus*) collected on islands outside Brittany in France (Chastel, 1988).

4.7.2.1.3. Family Bunyaviridae

4.7.2.1.3.1. Uukuniemi virus (UUKV)

UUKV is a member of the *Phlebovirus* genus, with a widespred distribution inside and outside Europe. The virus was first isolated from I. ricinus collected from cattle at Uukuniemi, southeast in Finland in 1959 (Oker-Blom et al., 1964). Identical or closely related virus strains from other locations received names like for instance Potepli virus and Sumakh virus.

[4] See more thorough review by Hubalek and Rudolf, 2012

Arthropod vectors: Transovarial and transstadial transmission have been demonstrated for the main vector *I. ricinus*. *I. persulcatus* is a less common vector. The virus has also occasionally been isolated from some mosquito species, but they are believed to be only mechanical vectors.

Vertebrate hosts: Forest rodents, like *Myodes glareolus* and *Apodemus flavicollis*, are important hosts, and so are birds, particularly ground-feeding passerines. Viremia and long-term persistence of the virus was demonstrated in experimentally infected birds of many species. Antibodies have also been detected in cows and reptiles. Experimental infections produce fatal meningoencephalitis with myositis in suckling mouse, but no symptoms have been observed in adult mouse or adult rat. UUKV was also pathogenic to suckling but not adult *M. arvalis, A. flavicollis or M. glareolus* and suckling rat.

Animal and human disease caused by UUKV has not been reported. Antibodies were detected infrequently (≤5 % persons examined) in a few areas, while only exceptionally at a higher frequency (e.g., 13–14 % in western Belarus and Hungary and much more often these serosurveys for UUKV were negative.

Distribution outside Europe: Azerbaijan, Asian Russia. Antibodies were detected in birds of Tunisia. Migratory birds play a role in the widespread distribution of UUKV; e.g., several strains of the virus have been isolated from immature *I. ricinus* collected on migratory passerines (Traavik 1979).

4.7.2.1.3.2. Seabird-related members of the Bunyaviridae family

The *Phlebovirus*, UUK-like, viruses Zaliv Terpeniya (ZTV) and St. Abb's Head (SAHV) and others were isolated from *Ixodes uriae* ticks collected in seabird colonies in France, UK, Ireland, Faroe Islands, Shetland Islands, Iceland, Norway and northwest Russia. Some identical or closely related virus strains have also been isolated from seabird colonies in Asian Russia, Canada and USA. In some cases such viruses have been linked to high chick mortality in seabird colonies (review by Hubalek and Rudolf, 2012).

Furthermore a number of virus strains within the genus *Nairovirus*, e.g. Soldado virus (SOLV), Puffin Island virus (PIV), Avalon virus (AVAV) and Clo Mar virus (CMV) have been isolated from ticks in seabird colonies of Europe, Asian Russia, Canada and USA.

4.7.2.1.3.3. *Orthobunyaviruses Bahig (BAHV) and Matruh (MTRV)*

These closely related viruses have ticks, and particularly *H. marginatus* as arthropod vectors and passerine birds as main vertebrate hosts. They were first isolated in North Africa, and have been isolated from ticks picked off northward migrating birds, indicating a possible means of dispersion to northern Europe if climate conditions allow (review by Hubalek and Rudolf, 2012).

Furthermore, some tick-borne *Bunyaviridae* that have not yet been assigned to any specific genus are now collectively named Bhanja group viruses (BHAV). The viruses have been isolated from a variety of ixodod ticks, particularly *Haemaphysalis punctata* and *Dermacentor marginatus*, in Portugal, Spain, Italy, Croatia, Bulgaria, Romania, as well as in a number of African and Asian countries. Migratory

birds may be important by transporting virus-carrying ticks over long intercontinental distances (Hubalek and Rudolf, 2012, and references therein).

4.7.2.1.4. Reoviridae

Viruses in the family *Reoviridae* have genomes consisting of segmented doublestranded RNA (dsRNA). The name "Reoviridae" is derived from respiratory enteric disease. Even though viruses in the Reoviridae family have more recently been identified with various diseases, the original name is still used. Reovirus infection occurs often in humans, but most cases are mild or subclinical. *Rotavirus*, however, can cause severe diarrhea and intestinal distress in children. The virus can be readily detected in feces, and may also be recovered from pharyngeal or nasal secretions, urine, cerebrospinal fluid, and blood. Despite the ease of finding Reovirus in clinical specimens, their role in human disease or treatment is still uncertain. Some viruses of this family infect plants. For example P*hytoreovirus* and *Oryzavirus*.

Reoviridae are non-enveloped and have an icosahedral capsid composed of an outer and inner protein shell. The genomes of viruses in Reoviridae contain 10-12 segments which are grouped into three categories corresponding to their size: L (large), M (medium) and S (small). Segments range from ~ 3.9 kbp – 1kbp and each segment encodes 1-3 proteins. *Reoviridae* proteins are denoted by the Greek character corresponding to the segment it was translated from (the L segment encodes for λ proteins, the M segment encodes for μ proteins and the S segment encodes for σ proteins).

Since these viruses have dsRNA genomes, replication occurs exclusively in the cytoplasm and the virus encodes several proteins which are needed for replication and conversion of the dsRNA genome into (+)-RNAs. The virus can enter the host cell via a receptor on the cell surface.

Reoviridae are organized into 2 subfamilies, counting a total of 15 genera. Members of this family have genomes composed of 10-13 dsRNA fragments. Hence, they have a great potential for genetic and biological evolution and variability through reassortment (see Chapter 6).

Eyach virus belongs to the *Colorado tick fever virus (CTFV)* group within genus *Coltivirus.* Such viruses have 12 ds RNA segments, while members of the Orbivirus genus have 10 segments. The virus was first isolated from I. ricinus ticks collected near Tübingen, Germany in 1972. It has been suggested that a CTFV strain was imported through US Army dogs carrying *Dermacentor* ticks to a military base after 2nd World War. Thereafter the virus should have evolved into Eyach virus under the selective pressure of the European ecosystem (Hubalek and Rudolf, 2012 and references therein). It might also, of course, have evolved by participating in a mixed infection and genome reassortment with another, still unknown virus relative, circulating within a common natural focus.

Arthropod vectors: *I. ricinus* and *I. ventalloi*. Small rodents and lagomorphs act as *vertebrate hosts*. The virus has so far been detected in Germany, France and Switzerland.

Tribec virus (TRBV) belongs to the *Orbivirus* genus, and is a member of the *Kemorovo group*. The first 28 strains of the virus were isolated in 1963 from *I. ricinus* collected in three different regions of Slovakia.

Arhropod vectors: *I. ricinus*, *I persulcatus* and *H. punctata*.

Vertebrate hosts: Small rodents, e.g. the voles *M. glareolus*, *M. subterraneus,* goat, European Starling (*S. vulgaris*) and chaffinch (*F. coelebs*). Disease in wildlife animals is unknown, but TRBV infection is fatal to suckling mice, suckling rats and suckling Syrian hamsters. The virus may cause an occasional febrile illness or aseptic meningitis in humans, but the disease is probably underdiagnosed, since specific antibodies have been detected in human populations in continental Europe.

Migratory birds have been implicated in the dispersal of Kemorovo group viruses over vast distances, i.e. a Siberian virus strain was isolated in Egypt from a southward migrating common redstart (*Phoenicurus phoenicurus)* (Hubalek and Rudolf, 2012 and references therein).

4.7.2.1.4.1. Seabird-related members of the Orbivirus genus

Okhotskiy virus (OKHV), Cape Wrath virus (CWV), Mykenes virus (MYKV), Tindholmur virus (TDMV) and *Bauline virus (BAUV)* have all been isolated from *I. uriae* ticks collected in seabird colonies in Ireland, UK, Faroe Islands, Iceland, Norway (Lofoten Islands), northwest Russia and Canada. Documented transatlantic flights of seapuffins from northwest Europe to New Foundland and vice versa contribute to the dissemination of viruses over wide geographical areas (Hubalek and Rudolf, 2012 and references therein)

4.7.2.1.5. Orthomyxoviridae

Orthomyxoviridae is a family of viruses with negative-sense single-stranded RNA genomes that includes *six genera*: *Influenzavirus A*, *Influenzavirus B*, *Influenzavirus C*, *Isavirus*, ***Thogotovirus*** and a recently discovered, still undescribed genus. The first three genera contain viruses that cause influenza in vertebrates, including birds (i.e. avian influenza), humans, and other mammals. Isaviruses infect salmon; *thogotoviruses infect vertebrates and invertebrates, such as mosquitoes and sea lice.* The virion is pleomorphic; the envelope can occur in spherical and filamentous forms. In general, the virus's morphology is spherical with particles 50 to 120 nm in diameter, or filamentous virions 20 nm in diameter and 200 to 300 (–3000) nm long.

The tick-borne orthomyxoviruses *Thogoto virus (THOV)* and *Dhori virus (DHOV)* have so far only been detected in Portugal, on Sicilia and in a number of African and South Asian countries. Although clearly an orthomyxovirus, THOV shares only 15-20% of its ssRNA genome sequence with *influenza viruses.*

The *THOV* genome is split into 6 and the *DHOV* genome into 7 ssRNA fragments. Hence the road for continuous evolution and divergent biological characteristics base on fragment reassortment is wide open for such viruses.

Known arthropod vectors: Hard ticks belonging to the *Boophilus*, *Rhipicephalus, Amblyomma and Hyalomma species.*

*Known vertebrate hosts***:** Cattle, camel, humans, bats, sheep, goat. Disease in sheep (abortion) and humans have been reported

4.7.2.1.6. Tick-borne arboviruses in Norway

Virus strains were isolated from *I. ricinus* and *I. uriae* during the Norwegian field based arbovirus studies in the 1970ies and 80ies (Traavik 1979). Attempts to isolate virus from engorged *I. trianguliceps* picked off trapped host animals were unsuccessful. A very restricted number of *A. vespertilionis* from bats and *H. marginatum* from passerine birds were also tested with negative results. The potentially interesting tick-species *I. hexagonus* was not investigated, mainly due to the practical difficulties connected with the collection of this strongly host-associated tick. In other countries *I. hexagonus* have been proven an effective vector. At the moment the "taiga tick" *Ixodes persulcatus* is invading Finland from southeast. It is a vector of a number of virus strains that are closely related to, but still different from the strains already circulating in Fennoscandia.

Collecting ticks for arbovirus isolations in Norway. Photo: Reidar Mehl

When will the tick species brought in each year by migrating birds (Hyalomma spp etc.) establish themselves, and overwinter in Norway? This is a very relevant question in the context of clima change and emerging tick-vectored viruses.

*4.7.2.1.6.1. Uukuniemi group viruses (*reviewed in *Traavik 1979)*

Three UUKV strains were isolated from field-collected ticks in Norway (Traavik et al., 1974; Traavik and Mehl, 1977). One strain was isolated from engorged I. ricinus nymphs picked off migrant, birds in the spring (SF El). Another isolate (By E50) was made from unengorged *I. ricinus* nymphs collected within an earlier established tick-borne encephalitis (TBE) virus focus (Traavik, T., 1973; Traavik et al., 1978) . These two strains seemed serologically identical, and also identical to the Finnish prototype strain S23. The third isolate (Ru E82) was made from unengorged *I. uriae* collected in a Common Puffin (*Fratercula arctica*) colony. Although clearly belonging to the UUK group of viruses, this strain demonstrated biological as well as antigenic differences to the other isolates.

Ticks and their host animals in Norway (adapted from Mehl, 1983)

Tick species; Hosts
Ixodes ricinus; Mammals, birds, reptiles
Ixodes lividus; Sand Martin
Ixodes caledonicus; Pigeons, Starling,
Ixodes arboricola; Birds
Ixodes frontalis; Passerine birds
Ixodes uriae; Seabirds in colonies
Ixodes hexagonus; Mammals
Ixodes trianguliceps; Small rodents and shrews
Hyalomma marginatum; Migrating birds, large mammals
Rhipicephalus sanguinea; Dogs, humans
Argas vespertilionis; Bats

Serological screening of human, bovine, small mammal and passerine bird sera for antibodies against UUKV strain By E50 indicated that UUK virus(es) are wide-spread in the Norwegian coastal areas where *I. ricinus* is distributed. Antibodies were demonstrated in human sera (5.0%); bovine sera (13.5%); small mammals, i.e. common shrews (*S. araneus*), bank voles (*C. glareolus*), field voles (*M. agrestis*), wood mice (*A. sylvaticus*); and passerine birds (12.5%).

A particularly interesting finding was the indications of mixed foci of UUKV and TBEV, based on both virus isolations and serological findings.

One reason for investigating *I. uriae* from the bird colonies on the island Runde as a potential virus vector, was the unusually high chick mortalities reported from these seabird colonies during the previous years. Alterations in the chemical milieu were reflected by the findings of pesticide residues in the birds. It might be worthwhile to consider whs of host animals to viruses and microorganisms.

4.7.2.1.6.2. Kemorovo group viruses

No virus strains belonging to this group have been isolated in Norway. However, restricted serological screenings performed in the 1970ies strongly indicated the presence of one or more such virus on the Norwegian mainland (Traavik, 1979). Antibodies to Tribec virus were detected in 7/186 human sera (3.8%), 4/84 bovine sera (4.8%) and 8/192 small rodent sera (4.2%).

4.7.2.1.6.3. Tick-borne encephalitis virus (TBEV)

Although thousands of ticks were collected during the years 1973-75, TBEV isolations were only successful from a small collection made in June 1976 (Traavik et al., 1978). Five virus strains were isolated from *I. ricinus* collected st three different locations in Sogn og Fjordane county. By the methods available by that time, the strains seemed mutually identical, and also identical to the Czech TBEV reference strain Hypr. These methods can, however, not exclude the possibility that one or more strain might actually belong to the LIV species, which is also present in the *I. ricinus* distribution areas.

Choice of locations for virus isolation attempts were based on prior serological screenings of cattle sera (Traavik, 1973), showing that animals with antibodies to TBEV could be found along the part of the Norwegian coastline investigated, from Ålesund in the north to Lista in the south. Antibodies in the sera of very young individuals indicated recent or on-going TBEV activity, and as earlier mentioned, some of these seropositive sera also contained anti-UUK antibodies.

Later on a TBEV antibody screening was performed with human sera from 341 individuals living at the western coast of Norway (Traavik, 1979). Among these unselected individuals, 20% had antibodies to TBEV. This is a high prevalence compared to supposed endemic areas in other European countries. It was noteworthy that some of the TBE-seropositive persons also had antibodies to other *I. ricinus*-vectored viruses. Two of them had antibodies to UUKV, and three had antibodies to Tribec virus. Furthermore, recent serological studies in wild cervids have demonstrated that TBEV and LIV (see 4.7.2.1.1) may co-circulate within the same biotopes in southern Norway (Ytrehus et al., 2013).

Newer studies in Norway have investigated the presence of TBEV in nymphs and related the detection data to climatic factors along the southern coast of the country. Virus specifc RNA was demonstrated in 7 different foci. The authors emphasized the possible importance of microclimatic conditions in relation to TBEV prevalence in ticks (Andreassen et al., 2012).

The first five human cases of human tick-borne encephalitis in Norway were reported from Tromöya, in Aust-Agder County from 1998-2001 (Ormaasen et al 2001; Skarpaas et al 2002). Serum specimens from 317 dogs in the same geographic area were collected. An enzyme immunoassay demonstrated antibody to human tick-borne encephalitis virus in 52 (16.4%) of the dogs, which supports the notion of an emerging disease (Csango et al. 2004)

Recently, field-based studies with the purpose of following the spread of *I. ricinus* towards higher altitudes and latitudes, in relation to recorded weather and climate change data have been initiated (Dr. Björn-Erik Kristiansen, personal communication, March 2012; further information can be downloaded at http://www.flåttinord.no).

4.7.2.1.6.4. Arboviruses in Norwegian seabird-colonies

In addition to the virus isolates earlier mentioned two identical strains of a coronvirus-like virus was obtained from *I. uriae* ticks collected in 1973. The virus received the name *Runde virus* after the island where the seabird colonies were located (Traavik et al., 1977, Traavik and Brunvold, 1978). The ecological circumstances of the isolations indicated a novel arbovirus circulating between *I. uriae* and its seabird hosts. Due to lack of resources these investigations have never been followed up.

4.7.2.2. Emerging viruses

4.7.2.2.1. Crimean-Congo Haemorrhagic Fever Virus

CCHFV, *Bunyaviridae*, ticks, disease in humans and a number of other species. *Crimean-Congo haemorrhagic fever (CCHF)* is one the most important and widespread diseases caused by tick-borne viruses. The causative agent, Crimean-Congo haemorrhagic fever virus does not cause disease in livestock, but vertebrates play a role in virus transmission as part of a tick-vertebrate-tick enzootic cycle. Although there is no evidence of clinical disease in animals, contact with viremic animals, tick bite, or crushing ticks taken from infected animals can lead to human infection. In humans, CCHF

virus causes a disease with four phases: incubation, prehaemorrhagic, haemorrhagic, and convalescence phases.

Geographical Distribution. The geographic distribution of CCHF virus is the widest amongst all tick-borne viruses. Currently CCHFV is endemic in Africa, Asia, Balkan countries, and Middle and Far East. During the last ten years, CCHFV has been rapidly introduced into new, previously nonendemic areas; especially into eastern and southeastern Europe including Greece and Turkey.

Vector. Hyalomma spp. are vectors and reservoirs for CCHFV, particularly *Hyalomma marginatum* . The geographical distribution of these ticks closely match the distribution of CCHFV and covers southern Europe, southern Russia extending to southern Asia, and most of Africa. The host range of these ticks varies from domestic animals (cattle, horse, sheep, and goats) for adults to small wild animals and birds for larvae and nymphs.

Given the continued emergence of CCHF clinical cases in Eurasia and focalized upsurges of *H. marginatum* populations in Europe, the potential of this vector species to be introduced into the United Kingdom was investigated. Immature forms of *H. marginatum* are frequent ectoparasites of passerine birds many of which migrate from Africa to the UK and also to Fennoscandia (R. Mehl, unpublished communication) each spring. Incoming birds were inspected for ticks during the spring migration in 2010 and 2011. A total of 68 ticks was collected from 971 birds (29 bird species), 21% (14) of the ticks were identified as *H. marginatum*. *Oenanthe oenanthe* (Northern wheatear) and *Sylvia communis* (Whitethroat) were infested by this tick in both years and with multiple ticks. Single specimens were also removed from *Acrocephalus schoenobaenus* (Sedge warbler) and *Phoenicurus phoenicurus* (Common redstart) in 2010. The cited study provides the first contemporary evidence for substantial importation of this tick species into the UK (Jameson et al 2012).

4.7.2.2.2. Omsk hemorrhagic fever virus

(OHFV, member of the virus family *Flaviviridae*). *Omsk hemorrhagic fever (OHF)* was described between 1945 and 1947 in Omsk, Russia from patients with hemorrhagic fever. The main host for OHFV is rodents, principally the water vole (Arvicola *terrestris),* but the virus also infects the *non-native muskrat (Ondatra zibethica).* OHFV is transmitted to the rodents from the bite of an infected tick (Dermacentor *reticulatus, Dermacentor marginatus, Ixodes persulcatus* are the major vectors). Humans usually get the disease from a tick bite.

I. persulcatus is migrating southwestwards from Siberia at the moment. It has been detected as a vector of arboviruses in Finland and may be expected to reach Norway in the future.

Humans are at additional risk of contacting OHF through their contact with muskrats. Muskrats, which are not native to the Omsk region, but were recently introduced to the area, like humans, fall ill and die when they are infected with the virus. Therefore, humans can contract OHF through contact with the blood, feces, or urine of an infected sick or dead muskrat. Experimental evidence shows that other rodents, i.e. narrow-skulled voles (*Microtus gregalis)* suffer similarly to muskrats; therefore,

contact with these animals may also cause disease in humans (CDC 2012).

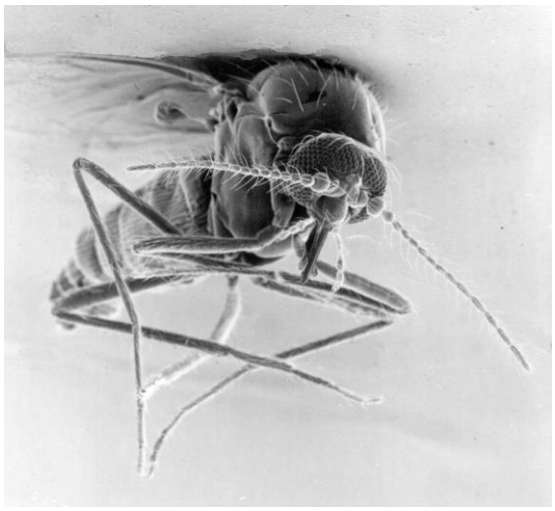
OHFV can be transmitted through the milk of infected goats or sheep and isolated from aquatic animals and water. This suggests that the virus is extremely stable in the environment.

4.7.3. Midge-borne viruses

4.7.3.1. Bluetongue virus

BTV, *Reoviridae*, genus *Orbivirus*, ds RNA in 10 fragments; *Culicoides* spp, vector victims, serious disease in domestic and wildlife ruminants, vaccine based on engineered recombinant protein(s) are under development.

This virus is *pathogenic for a range of domestic and wild ruminants*, and many aspects of its molecular biology and ecology has spurred studies by virologists, epidemiologists and veterinary scientists. Seasonal imports of the virus from Africa into more temperate latitudes, sometimes accompanied by disease, have occurred under favorable climatic conditions. The recent introduction of serotype BTV-8, and the establishment of a *transmission cycle that has resulted in its spread into northern Europe* including the UK (see below), is of significant economic importance. BTV is a member of the genus *Orbivirus* in the family *Reoviridae* but, unlike many other arboviruses, does not infect humans and therefore is not anthropozoonotic. There are 24 recognized serotypes of the virus, which contain between 10 and 12 segments of double-stranded RNA. Until recently BTV was considered to be almost exclusively a disease of some European breeds of sheep that, for commercial purposes, have been distributed widely in Africa, Asia and Australasia. In cattle and goats, clinical disease has been considered rare, and much milder than in sheep (Verwoerd and Erasmus, 1994). However, recent observations suggest that cattle frequently show disease symptoms resulting from infection by the BTV-8 serotype that is currently circulating in northern Europe (see below). *There is evidence that infected midges are carried on the wind for long distances* (Sellers 1980, 1981) *and it has been postulated that the major epidemics of bluetongue, in regions where disease occurs only sporadically, result from wind-borne carriage of infected Culicoides from distant endemic areas* (Gibbs, 1988).



A Culicoides obsoletus midge collected in Norway. Photo: Reidar Mehl

Different Culicoides species are main BTV vectors in different parts of the world. In South-East Asia and Australia it is *C. brevitarsis*, in North-America *C. variipennis sonorensis*, in South-America *C. insignis,* in Africa, the Mediteranians and South Europe it is *Culicoides imicola*. None of these midge

species have been detected in Middle- and North-Europe, and in connection with the BTV outbreaks in these areas during 2006-2008, it was found that a number of "novel" *Culicoides* species could act as BTV vectors, e.g. *Culicoides obsoletus*, *Culicoides scoticus, Culicoides dewulfi*, *Culicoides chiopterus og Culicoides pulicaris sensu stricto* og (Meiswinkel et al., 2008). All these five species are present in Norway (Hamnes, 2011)

Competent midges may be infected when biting viraemic vertebrates. The probability of infection depends in part on the genotype of the midge, the strain of virus, the level of viraemia and environmental factors (Mellor et al., 2000). The extrinsic incubation period (the period between feeding on infected blood and the appearance of virus in the saliva of the arthropod vector) is 1–2 weeks. Contrary to the BTV strains referred to above, the recent appearance of BTV-8 in northern Europe, including the UK, has unexpectedly been accompanied by the appearance of overt disease and mortality in cattle. Moreover, it is now recognized that healthy infected animals may remain ELISA- and RT-PCR-positive for at least 4 months (MacLachlan, 1994). This observation helps to explain how BTV-positive animals may be detected in mid-winter in the UK when midge transmission activity is presumed to be minimal. Symptoms of BTV infection in sheep are variable but typically include fever. Facial edema results in swelling and soreness of the lips and nose with muco-purulent discharge, which is exacerbated by champing to produce frothy saliva. The term *`bluetongue' is derived from the cyanosis of the tongue that is observed in some cases.* Erosion of the coronal band above the hooves and musculoskeletal damage cause pain and lameness induce the sheep to adopt a disease-typical posture.

BTV circulates widely throughout tropical and subtropical regions, but until relatively recently the disease had been observed only infrequently in some areas of southern Europe. However, during the past decade, six strains of BTV are known to have spread across 12 European countries, and significantly the virus has gradually dispersed further north in central and Western Europe. This dispersal has probably been driven by the northward expansion of the range of *Cu. imicola*, the main BTV vector, and by climate change, which has probably contributed to increased persistence during winter. Consequently, the subsequent risk of transmission over larger geographical regions (Purse, 2005) and an extended period of time have increased. To the north of the *Cu. imicola* range, other species (*Cu. obsoletus, Cu. pulicaris, Cu. chiopterus and Cu. dewulfi*) with distributions extending across central and northwestern Europe (Mellor and Wittman, 2002) were probably involved in the appearance of BTV-8 in Belgium, France, Luxembourg, Germany and the Netherlands in August 2006, and subsequently in the UK in September 2007. In Denmark and Fennoscandia BTV cases appeared during 2008-2009, but Norway was declared BTV-free in early 2011 (Sviland and Kjeang, 2011). The presence of multiple vectors of BTV-8 appears to apply to large parts of northern Europe and has almost certainly contributed to the dramatic spread of this arbovirus across this area. In addition to the impact of climate change on vector range expansion and the northerly establishment of BTV-8, the commercial transportation of asymptomatic infectious ruminants and the wind-borne dispersal of infected midges are believed to be highly significant contributory factors to the rapid dispersal of the virus.

Understanding the described sequence of events may aid predictions of the emergence of other midge-borne pathogens, such as the more devastating Schmallenberg virus, another animal pathogen in the genus *Orbivirus* that may be transmitted by several of the same vectors as BTV.

Another important observation has appeared as the result of the invasion of BTV into northern Europe. Conventional opinion has previously considered it extremely unlikely that BTV could be transmitted *vertically to newborn offspring* of infected animals. New evidence suggests that this virus may be transmitted across the bovine placenta to infect the fetus, causing an unusually high rate of malformed, stillborn and weak calves born on holdings with a known history of BTV infection (Gould and Higgs, 2009). This observation has not been confirmed through systematic investigation. Nevertheless, whether or not this represents an acquired new characteristic of BTV-8 clearly needs close attention. Transplacental infection has also been associated with attenuated BTV vaccine viruses. In further support of these reports, the recent unpublished finding of imported heifers in Northern Ireland, leading to the suspicion that *newborn calves infected in utero can act as virus reservoirs for the Culicoides vector*, is another worrying development that needs immediate investigation (Gould and Higgs, 2009).

Methods for controlling BTV include reducing exposure of the animals to the competent midges, the use of insecticides to repel the midges from biting the animals, and the use of vaccines. While the strategies of reducing exposure and using insect repellents might reduce the levels of BTV transmission, clearly these measures cannot be expected to eradicate BTV from northern Europe.

Vaccination is associated with several practical difficulties. Firstly, there are 24 serotypes of BTV, and while there is some antigenic cross-reactivity between different serotypes, the preparation of a single live attenuated virus multivalent vaccine to protect against all 24 is impractical, partly because different serotypes may outcompete each other in the vaccine, partly because at the moment only BTV-8 is circulating in northwestern Europe and partly because of the costs and time involved in producing a multivalent vaccine. Moreover, the use of live attenuated vaccines presents a low but potential risk of *reversion to virulence*, or in some circumstances the possibility of *reassortment of the RNA gene segments between different serotypes of BTV.* However, for reasons beyond the control of the manufacturers, the production of a vaccine in time to prevent the reemergence of BTV-8 in northern Europe during 2008 is proving to be seriously problematic. It will be interesting to see whether or not BTV-8 is brought under control in the UK and northern Europe during 2008. Non-infectious vaccines based on engineered recombinant proteins are also under development, but in addition to the requirement for multiple dosing, these vaccines are likely to be expensive and therefore not favoured by farmers.

4.7.3.2. ***Schmallenberg virus***

(*SBV*, *Bunyaviridae*, *Orthobunyavirus,* newly emerging (2011), no clinical disease in adult ruminants, but disease, stillbirth and malformations in fetuses; *Culicoides vectors (Some of them are present in Norway).*

In summer and autumn of 2011, farmers and veterinarians in North Rhine-Westphalia, Germany, and in the Netherlands reported to the animal health services, local diagnostic laboratories, and national research institutes an unidentified disease in dairy cattle. Affected animals went through a short period of unclear clinical signs, including fever, decreased milk production, and diarrhea. All classical endemic and emerging viruses could be excluded as the causative agent. To identify the cause of the disease, blood samples from affected cattle were analyzed (Hoffmann et al. 2012).

By metagenomic analyses of blood specimens from infected dairy cows, a number of sequences that, beyond any doubt, belonged to S, M and L fragments of a bunyavirus were generated. Sequence comparisons strongly indicated close relationship to Shamonda viruses within the *genus Orthobunyavirus.*

Schmallenberg virus: where did it come from?

SBV has spread across Europe infecting most domestic ruminant species. Transmission is thought to occur through midge vectors such as *Culicoides spp*., and infection is mostly sub-clinical in adult animals, occasionally causing clinical signs (such as a drop in milk yield, pyrexia, anorexia, and diarrhoea), which correlate with the viraemia and persist between days 2 and 5 post-infection. Losses in SBV-infected herds are associated with infection of susceptible pregnant animals resulting in abortion, stillbirth and, most frequently, congenital musculoskeletal and neural malformations observed in new-born animals leading to their death shortly after birth

The susceptibility of wild red (*Cervus elaphus*) and roe (*Capreolus capreolus*) deer to SBV infection has been confirmed on only one occasion in Belgium in the autumn of 2011 (Linden et al., 2012). Antibodies were found in 43% of animals hunted between October and December 2011 in an area approximately 250 km from the city of Schmallenberg, where the virus was first identified.

It was recently reported from Poland that Schmallenberg virus (SBV) RNA was detected in the serum of an elk (Alces alces) calf captured on the outskirts of Białowie_za National Park (BNP) in December 2012, and shortly afterwards the calf died of acute bronchopneumonia. Furthermore, serum samples from 169 wildlife ruminants, including bison, red and fallow deer, originating from eight locations situated in four Polish Provinces, were tested for the presence of SBV-specific antibodies between

Deer (Capreolus elaphus)
Photo: taviphoto/istockphoto.com

Elk (Alces alces) Photo: Hagerty Ryan, Creative Commons Licence

2011 and 2013. Although no antibodies were found in samples collected up to July 2012, positive samples subsequently appeared between November 2012 and January 2013 in all of the sampled regions. The introduction of SBV infection to the European bison (Bison bonasus) population of BNP between July and November 2012 was also confirmed (Larska et al., 2013).

In Norway SBV seems to be widespread all over the Southern part of the country. The virus was probably introduced by midges in late summer 2012. Viruset ble trolig introdusert med sviknott til landet i løpet av sensommeren 2012. Serological investigations indicate that the virus arrived through Sweden or Denmark. A number of important virus-ecological and –epizootological questions are lacking answers, e.g. i). Will SBV be able to overwinter in midges in Norway? ii). Will SBV become endemic in Norwegian wildlife and domestic ruminants? Iii). If ”yes”: what will the consequences be for national husbandry? (Tønnesen and Jonassen, 2013).

What is not known is the source of Schmallenberg virus. Where did it come from?

“European virologists, epidemiologists, veterinarians, physicians and others have served as arbovirologists for decades. Excellent work was done, diseases described, viruses isolated, antibody detected, reagents made and distributed, meetings held and students educated.

Most of the early work focused on human diseases and the details of mosquito and tick life-cycles and biology. When bluetongue began to be considered as a disease of economic importance, culicoids were added to the list of insects to study.

By now, millions of culicoids must have been collected, ground and tested for viruses. So why only now has Schmallenberg virus appeared? Was it in Europe before now? Is it a recent reassortant of Akabane virus (not previously known in Europe) and another (what?) virus? Has it been introduced only recently? How? Intentionally?

Perhaps the methods used to detect viruses in culicoids have been specific for bluetongue viruses, in which case only bluetongue viruses would have been detected. Are there pools of culicoids stored in freezers in Europe, pools that contain Akabane virus or Akabane-like viruses but which have not been properly and rigorously tested? Are only state-run laboratories involved in these studies, rather than universities, whose findings usually are more transparent?

In the bedlam that usually follows the discovery of a new and important pathogen, retrospective studies usually await a time when the epidemiologic situation settles down and investigators have time to put their feet on their desks and think. That time cannot come soon enough. If this was anything like an intentional (and successful) introduction, there will be another and another after that.Use of the word "intentional" is only paranoia if it is not the case.

It appears to me that the excellent groups working to study and prevent this virus from spreading, while well intentioned, have not been formed into a pan-European, cohesive organization with someone in charge. OIE and WHO might count cases but they are not suited to the sort of work that needs doing, such as answering the questions "How long has Schmallenberg virus been in Europe?", “If so why was it not detected earlier” and "Where did it come from?"

(End of quotation from Charles H. Calisher, Ph.D. Professor Emeritus, Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, Fort Collins, CO 80523-1690. College of Veterinary Medicine and Biomedical Sciences Colorado State University, USA <calisher@cybersafe.net>).

4.7.4. Sandfly-borne viruses and others

Sandfly Fever Virus (SFV)

The sandfly-transmitted viruses are all within the genus Phlebovirus within the *Bunyaviridae* family. Globally, some 45 viruses are associated with sandflies. Some Phleboviruses are transmitted by mosquitoes, e.g. Rift Valley fever, whereas others are transmitted by ticks. The sandfly-transmitted fever viruses identified in Europe include Arbia virus, Corfou virus, Naples virus, Radi virus, Sicilian virus and Toscana virus. Arbia virus has been isolated from sandflies in Italy and Corfou virus from *Phlebotomus major* on Corfou Island, Greece. Neither of these viruses appears to be of public health importance.

4.7.5. Can climate changes explain the processes behind "arboviruses going north"?

(Adapted from Gould and Higgs, 2009)

The answer is different for each virus. Firstly, as an arthropod is a critical component of any transmission cycle, viruses are inevitably dependent on specific climatic conditions for their geographic range. *Nevertheless, each virus has emerged and become established in new areas primarily as the result of: (1) human travel and/or invasion by foreign species (e.g. CHIKV); (2) climatic conditions and/or commercial transportation of animals (e.g. RVFV, BTV); (3) natural patterns of bird migration (e.g. WNV)*. In the case of CHIKV, a single mutation in the viral genome that facilitated adaptation to the mosquito species *Ae. albopictus* has played a major role in its emergence. The transportation and mass storage of scrap car tyres, as recently illustrated in the Netherlands and UK, and plants as primary methods by which this mosquito species has dispersed globally in the tropics and subtropics have been cited. If global climate change continues according to the predictions of some experts, *Ae. albopictus* and *Ae. aegypti* will certainly disperse beyond their current geographic boundaries, and we could expect to see more cases of epidemic outbreaks typified by the incursion of CHIKV into northern Italy. One cannot ignore the possibility of outbreaks of other arboviral diseases for which these species are the primary vector, namely *Dengue virus and Yellow fever virus*. In the case of RVFV, climate has always been the major factor for the onset of new outbreaks, due to emerging competent mosquitoes in flooded areas. Human activities, including irrigation projects, the movement of herded animals and importation of animals to feed large numbers of humans, have almost certainly contributed significantly to RVFV epidemics.

Climate change may play a greater role if the specific environmental conditions required for the development and maintenance of appropriate competent vector species become established in regions beyond the Arabian Peninsula. Epidemics of WNV encephalitis in Europe have always correlated with warm and humid summers; thus, once again climate is an important factor. However, the presence of large numbers of susceptible migratory birds, the availability of competent vectors and human commercial and leisure activities have been major factors in the emergence of WNV in Europe as a human epidemic virus. As this virus already circulates in northern Europe, via migratory birds, the induced low levels of immunity might be expected to reduce disease severity in northern Europe. The impact of climate change may be to move the disease further north by increasing virus

transmission efficiency (increased vector population densities and vector—vertebrate encounters, and shorter extrinsic incubation period), but *new vaccines and antivirals that are being developed may provide the means by which this virus can be controlled*.

Finally, *BTV is a proven example of a virus that has moved into and become established in northern Europe, partly as the result of climate change*. Nevertheless, the exportation of animals across Europe and other factors such as wind-borne midges have clearly contributed to the northerly dispersal of BTV.

5. Vertebrate-borne viruses in Europe

5.1. Rodent-borne viruses in Europe, Fennoscandia and Norway

Most members of the *Hantaviridae* are emerging, rodent-borne viruses. They cause two significant human diseases, *haemorrhagic fever with renal syndrome* in Asia and Europe, including Fennoscandia and Norway, and *Hantavirus cardiopulmonary syndrome* in the Americas. Very recently, several novel hantaviruses with unknown pathogenic potential have been identified in Africa and in a variety of insectivores (shrews and a mole). There is very limited information available on the possible impact of climate change on hantaviruses, but it may reasonably be concluded that climate change will influence hantaviruses through impacts on the hantavirus reservoir host populations. We can anticipate changes in the size and frequency of hantavirus outbreaks, the spectrum of hantavirus species and geographical distribution (mediated by changes in population densities), and species composition and geographical distribution of their reservoir hosts.

The early effects of global warming have already been observed in different geographical areas of Europe. Elevated average temperatures in West-Central Europe have been associated with more frequent Puumala Hantavirus outbreaks, through high seed production (mast year) and high bank vole densities. On the other hand, warm winters in Scandinavia have led to a decline in vole populations as a result of the missing protective snow cover. Additional effects can be caused by increased intensity and frequency of extreme climatic events, or by changes in human behavior leading to higher risk of human virus exposure. Regardless of the extent of climate change, it is difficult to predict the impact on hantavirus survival, emergence and epidemiology. Nevertheless, hantaviruses will undoubtedly remain a significant public health threat for several decades to come (Klempa, 2009).

Although discovered more than 30 years ago, hantaviruses are still considered to be “emerging viruses” because of their increasing significance as human pathogens. These zoonotic viruses cause two human clinical syndromes but are not known to cause disease in their rodent reservoir hosts. Haemorrhagic fever with renal syndrome (HFRS) is a significant medical problem in Asia and Europe, whereas Hantavirus cardiopulmonary syndrome (HCPS) is responsible for significant morbidity and mortality in both North and South America. Both diseases are acute febrile infections that are usually acquired through the inhalation of aerosols or dust particles contaminated with virus-containing rodent excreta, which is often encountered in rodent-infested buildings, such as log cabins or farm buildings, when they are being cleaned. The initial symptoms of infection are very similar and include an abrupt onset of high fever, malaise, myalgia, back and abdominal pain, and other influenza-like symptoms. HFRS is mainly characterized by renal failure and haemorrhages varying from small petechiae to severe internal bleeding and the disseminated intravascular coagulation syndrome. On the other hand, pneumonia and cardiovascular dysfunction are characteristic of HCPS (Khaiboullina et al. 2005).

Approximately 150 000 HFRS cases are estimated to occur worldwide annually. More than 90% of them are reported from China, the far-eastern parts of Russia, and Korea where the most severe cases, with case fatality rates reaching 15%, are recorded (Kariwa et al 2007). The type species of the genus Hantavirus (family *Bunyaviridae*), is Hantaan virus (HTNV). Although HFRS has been recognized since the 1930s, the causative virus eluded discovery until 1976 when it was finally isolated from its rodent reservoir, the striped field mouse, *Apodemus agrarius*, trapped near the Hantaan river in South Korea (Lee et al. 1978). Seoul virus (SEOV), as it became known, is the only hantavirus known to be distributed worldwide because of the global dispersal of its natural host, rats (*Rattus rattus*, *Rattus norwegicus*). The most commonly recognized European hantavirus is Puumala virus (PUUV), which causes a mild form of HFRS, usually called Nephropathia epidemica (NE) and is transmitted to humans by the reservoir host, bank voles (*Myodes glareolus*). On the other hand, severe cases of HFRS are also reported in Europe, mostly in the Balkan region. These cases are caused by Dobrava–Belgrade virus (DOBV) (Kruger et al. 2001).

In South America, Andes virus (ANDV) is the most important causative agent of HCPS, with case fatality rates occasionally reaching 50%. ANDV is so far the only hantavirus with reported human-to-human transmission (Padula et al. 1998).

European hantaviruses: Five hantaviruses are known to circulate among rodents in Europe, and at least two among insectivores. Four (Dobrava, Saaremaa, Seoul, and Puumala [PUUV] viruses) are clearly associated with hemorrhagic fever with renal syndrome (HFRS). PUUV is the most common etiological agent of HFRS in Europe. It is carried by the bank vole (*Myodes glareolus,* earlier named *Clethrionomys glareolus*). This is one of the most widespread and abundant mammalian species in Europe. This host–virus system is also the most studied one among hantaviruses in Europe. However, HFRS incidence varies throughout the continent. The spatial as well as temporal variation in the occurrence of HFRS is linked to geographic differences in the population dynamics of the reservoir rodents in different biomes of Europe. Rodent abundance may follow mast-seeding events in many parts of temperate Europe. But in northern Europe multiannual cycles in population density exist. This is the result of the interaction between rodent populations and specialist predator populations in a delayed density dependent manner. The spatial distribution of hantaviruses further depends on parameters such as forest patch size and connectivity of the most suitable rodent habitats, and the conditions for the survival of the virus outside the host, as well as historical distribution patterns (phylogeographies) of hosts and viruses. In multi-annually fluctuating populations of rodents, with population increases of great amplitude, one should expect a simultaneous build-up of recently hantavirus-infected and -shedding rodents. The increasing number of infectious, virus-shedding rodents leads to a rapid transmission of hantavirus across the rodent population, and to humans. PUUV is the only European hantavirus for which there is a reasonable, yet still far from complete, ecological continental-wide understanding (Olsson et al 2010).

Hantaviruses in Norway: In Norway the strategies and approaches used for field-based arbovirus-studies in the 1970ies were used for hantavirus-studies in the 1980ies and poxvirus-studies in the 1990ies. The efforts resulted in a number of articles published through the 1980ies (Lähdevirta et al.;

Traavik et al., 1983; Traavik et al, 1984; Sommer et al., 1985; Sommer et al., 1988). Nephropathia epidemica (NE) antigen was detected by IFAT (indirect fluorescent antibody technique) in the lungs of 14 of 97 bank voles (*Clethrionomys glareolus*) collected in three endemic areas. The distribution of antigen positive voles within an endemic location was scattered. Anti-hantavirus antibodies were detected in 12 of 14 NE antigen positive bank voles and in 15 of 83 that were antigen negative. NE antigen positive voles exhibited higher antibody titres. Antibodies to hantavirus were demonstrated in sera from *C. rutilus* and *C. rufocanus* collected more than 200 km north of the distribution area for *C. glareolus*. It appears likely that these vole species can serve as virus vectors for NE cases occurring north of the bank vole area. NE antibodies seemed to diminish with time after infection in some NE patients, while for others such antibodies were detected up to 12 years after the disease. Antibodies to KHF were detected in eight of 106 healthy forestry workers with no clinical history of NE. No serological cross-reactions were detected between NE/KHF antigens and representative *Bunyaviridae* present in Norway. *NE/KHF-like viruses appear widespread in Norway, both within and outside of the distribution area of the bank vole.*

Small rodents were collected live in two different locations within a Nephropathia epidemica (NE) endemic area, and tested for both antiviral serum antibodies and viral antigens in lung sections. In one location, only *Apodemus sylvaticus* (woodmice) were found in the traps, in the other, both *A. sylvaticus* and *Clethrionomys glareolus* (bank voles) were collected. Among the woodmice from the former location the prevalence of NE virus markers was significantly lower than for either woodmice or bank voles from the other location, and no NE antigen-positive animals were found. The woodmice co-existing with bank voles had a lower prevalence of NE antigen and antibodies than the bank voles, and fewer woodmice had both antibodies and antigen. The results emphasize the important role of bank voles as a major NE virus reservoir and probable source of human infections.

In Norway, NE-like disease has been reported since 1946 and about 50 cases are diagnosed annually; however, the causative agent has not been characterized. A virus originating from bank voles (Clethrionomys *glareolus)* trapped near the town of Eidsvoll (Akershus County) was isolated and passaged in laboratory-bred bank voles. The bank vole strain was identified as a PUU virus by serological typing and by sequence analysis of the S and M gene segments. For comparison, complete or partial S sequences were determined for wild-type PUU strains from five locations in Sweden. Two locations were inhabited by the southern variant of bank vole present in Fennoscandia, and three by the northern variant. Phylogenetic analysis showed that Norwegian PUU strains are clustered together with Swedish strains from the first group forming a well- supported sublineage within the PUU genotype, distinct from other sublineages from northern Sweden, Finland, Russia and France. The results are consistent with the view of a complex evolutionary history of PUU strains in post-glacial Fennoscandia. Analyses of the current collection of nucleotide sequences suggest that PUU is the most variable genotype of the known hantaviruses (Lundkvist et al., 1998).

5.2. Bat-borne viruses

Myotis daubentonii (Vannflaggermus) collected in Norway. Photo: Reidar Mehl

5.2.1. Ebola-like

Lloviu virus

LLOV, *Filoviridae,* novel Ebola-like virus isolated from *bats* in Spain (Negredo et al. 2011).

The family *Filoviridae* is the taxonomic home of several related viruses that form filamentous virions. The family is classified within group V in the Baltimore system, having a single-stranded, negative polarity RNA genome. Two members of the family that are commonly known are Ebola virus and Marburg virus. Both viruses, and some of their lesser known relatives, cause severe disease in humans and nonhuman primates in the form of viral hemorrhagic fevers. All accepted members of the family (all ebolaviruses and marburgviruses) are Select Agents, World Health Organization Risk Group 4 Pathogens (requiring Biosafety Level 4-equivalent containment), National Institutes of Health/National Institute of Allergy and Infectious Diseases Category A Priority Pathogens, Centers for Disease Control and Prevention Category A Bioterrorism Agents, and listed as Biological Agents for Export Control by the Australia Group. It is expected that "cuevaviruses", proposed to be additional members of the family, will be classified in a similar way in the near future.

The family *Filoviridae* contains some of the most lethal of primate pathogens. Family members have only been reported as natural infections in sub-Saharan Africa and the Philippines, where bat infections with the Ebolaviruses and Marburgviruses do not appear to be associated with disease. Bats naturally or experimentally infected with such viruses are healthy and shed virus in their feces for up to 3 weeks (cited by Negredo et al. 2011).

In 2002, colonies of Schreiber's bats (*Miniopterus schreibersii*), sustained massive die-offs in caves in France, Spain and Portugal. *M. schreibersii*, family *Vespertilionidae,* comprises at least four geographically discrete lineages distributed in Oceania, southern Europe, southern Africa, and Southeast Asia. A distinct new filovirus was identified in dead insectivorous bats. It was provisionally named Lloviu virus, after the site of detection, Cueva del Lloviu, in Spain (Negredo et al. 2011). *This is the first documentation of an ebola-like virus in Europe. The novel virus was detected and characterized under the research program ''Rabies surveillance in Spain'', demonstrating how ecosystem surveillance programs can reveal totally unknown viruses circulating in the ecosystems.*

Recently, the discovery of genome-integrated filovirus elements has led to the proposal that filoviruses have co-evolved with mammals over millions of years (Belyi et al 2010, Taylor et al 2010). Phylogenetic analyses of LLOV indicate a common ancestor of all filoviruses at least 150,000 years ago.

There are various interesting questions related to this "new" filovirus, for instance: i) The LLOV was detected "by chance" through a surveillance program targeted on rabies. May systematic, targeted surveillance programs reveal filoviruses in other regions and other bat species?; ii) May climate changes lead to migrations of South-European bat species into Fennoscandia? iii) May climate change, ecosystem pollutants and new emergent virus infections lead to activations of endogenous filoviruses?

In the autumn some Norwegian bat species seem to migrate southwards on the European continent. It is known that some bat species may fly more than 1000 km to reach their overwintering habitats. The discovery of a novel filovirus in a distinct geographical niche suggests that the diversity and distribution of filoviruses should be studied further, also in Fennoscandia and Norway.

5.2.2. Rabies-like

Family Rhabdoviridae contains viruses that have non-segmented negative sense RNA genomes. There are more than 200 rhabdoviruses known, and this is probably still an underestimate of the total. The main characteristics of the member viruses are: (i) the viruses infecting vertebrates and invertebrates are bullet-shaped, and the viruses infecting plants are usually bacilliform; (ii) the viruses have particle lengths varying from 130 to 380 nm and widths varying from 60 to 95 nm; The family is split into 5 genera.

The Lyssavirus genus of the family Rhabdoviridae consists of five serotypes: classical rabies virus (serotype 1), Lagos bat virus (LBV) (serotype 2), Mokola virus (serotype 3), Duvenhage virus (DUVV) (serotype 4), and European bat virus (EBV) (serotype 5). The viruses within the genus share serologic relationships, but the sero- types and stable species-associated variants within serotypes can be distinguished by the reactivity profiles of monoclonal antibodies (Mab) directed against nucleoprotein and glycoprotein antigens. Analysis of the nucleotide sequence of the nucleoprotein gene has also shown genetic clusters along the same lines as serologic analysis, except that serotype 5, EBV, has been separated into two genotypes, EBV1 and EBV2 (Bourhy et al. 1992).

European bat lyssaviruses (EBLVs) were first identified in Europe in 1954 and over 850 cases have subsequently been recorded between 1977 and 2008. Most reports have involved EBLV-1, and 95% of these were linked with serotines, especially *Eptesicus serotinus.* By contrast, EBLV-2 in bats has been reported only 20 times to date, in Daubenton's bats (*Myotis daubentonii*) and pond bats (*Myotis dasycneme*). Eight of these records are in *M. daubentonii* in the UK (Harris et al. 2009)

There have been three confirmed cases of EBLV in humans: one EBLV-1 case in Russia in 1985 and EBLV-2 cases in Finland in 1985 and Scotland in 2002. European bat lyssavirus type 1 is believed to have been responsible for an earlier case of bat-associated rabies in Ukraine in 1977, although the virus was not characterized. In all four cases, there was no record of prophylactic immunization against rabies. Spillover of EBLV-1 into sheep has occurred on two separate occasions in Denmark, in 1998 and 2002, and into a stone marten in Germany. EBLV-1 neutralizing antibodies also were

detected in a domestic cat in Denmark. In 2007, the first case of rabies to infect a domestic animal in France since the country was declared free of the disease in 2001 was diagnosed in a cat, and was believed to be an EBLV-1 of bat origin (reviewed by Harris 2009).

In recent years, confirmed (virus-positive) EBLV bat cases in Europe have increased in direct association with in-creased *scanning (passive) and targeted surveillance,* giving rise to renewed concern regarding the possibility of bats translocating the virus perhaps even across the English Channel to the UK. Scanning (passive) surveillance (submission of dead bats) was initiated in the UK in 1987, with tested submissions of over 7,800 UK resident bats, and 12 bats (of six species) of European origin (Harris et al 2009).

The main reservoir for EBLV-1 in mainland Europe, *E. serotinus,* is also present in Norway, and its close relative *E. nilsonii* is the most common Norwegian bat species. The established EBLV-2 reservoir *M. daubentonii* is also present in Norway.

A recent report from Norway (Hansen et al. 2007) concluded: "Even though no rabies cases have been recorded on the mainland of Norway or in Sweden during the last hundred years, rabies in bats can exist undetected, as until recently was the case in the UK. A small number of Norwegian bats have been examined for rabies with negative results. In Sweden more bats have been analysed, but the virus hasn't been detected here either. Further, little information exists on the migration patterns of the bat species found in Norway. In continental Europe several species migrate in a Northeast to Southwest direction. Some species share migration routes as well as resting and mating areas, enabling a possible transmission of rabies virus between species and individuals. The formation of colonies may in some cases enhance the spread of virus (due to the gregarious behaviour of bats and high density of individuals). Some studies have shown that bats may shed virus without any signs of clinical disease. This can cause difficulties in detecting EBLV. The expected climatic changes for the next hundred years may density of individuals). Some studies have shown that bats may shed virus without any signs of clinical disease. This can cause difficulties in detecting EBLV. The expected climatic changes for the next hundred years may change the northern distribution limits of certain bat species. Southern bat species may thus to a greater extent inhabit northern regions, with the possibility of bringing rabies virus to Norway and other regions."

5.3. Other viruses with wildlife reservoirs

Non-vectored viruses with reservoirs in wildlife animals

Besides the vector-borne viruses we have discussed so far, wildlife animals are carrying an unknown number of viruses belonging to various families, e.g. *Poxviridae*, *Herpetoviridae*, *Adenoviridae*, *Papovaviridae* and *Retroviridae*. Jumping across species borders has been proven for some wildlife viruses, but the whole area has been very "stepmotherly" investigated. According to extrapolations from other viruses, and plausible hypotheses, many of these viruses have in common that they may become activated and spread when the infected animals are exposed to ecosystem and climate

changes, and also by EDCs and POPs. Another point of interest is that such viruses will share hosts with the emerging vector-borne viruses already discussed. *The consequences of mixed infection of the same individual with a number of different viruses, and often also other pathogens, concomitantly or during a short time are in most cases virtually unknown.*

Common hosts: Moreover, a post-glacial re-colonization hypothesis has been used to explain the distribution of different Puumala *Hantavirus* (PUU) genotypes in Norway and Sweden, and it is interesting that both PUU and orthopoxviruses have bank voles as reservoir (Horling et al., 1996; Lundkvist et al., 1998; Sandvik et al., 1998).

Whether, how and to which extent viruses with reservoirs in wildlife animals are being influenced by climate change, and whether effects of different human-made environmental impacts in the same ecosystems will add synergistically or additively to such influence is a very important issue for environmental risk assessment and management in general.

6. Emergence of new viral species and subspecies: evolutionary pressure and genetic modifications

6.1. Prerequisites for exchange of genetic material

The exchange of genetic material between viruses can occur in nature during cellular co-infections by two or more virus lineages either by *recombination or reassortment*. Such genetic exchange is presumably an underlying reason for the existence of segmented viral genomes that allows unique or novel versions of distinct mutations to be combined, while undesirable changes are removed from the gene pool (Pringle, 1996). The creation of chimeric nucleic acid molecules derived from the genome of each parental donor, termed *recombination*, is one mechanism for this type of exchange. *Reassortment* is the exchange of complete genome segments in multisegmented viruses, and is another mechanism for genetic exchange

6.2. Recombinations

Inter-strain and inter-species between related, naturally occurring viruses. This may happen within all families irrespective of genome type.

The family *Flaviviridae* includes important human pathogens, such as dengue (DEN) virus, yellow fever (YF) virus and hepatitis C virus, many of which have emerged or re-emerged in recent years. Until recently, flavivirus evolution was thought to proceed in a clonal manner, with diversity generated mainly through the accumulation of mutational changes. However, this assumption has now been shown to be invalid, with homologous recombination demonstrated in all three genera of the *Flaviviridae*. Since recombination has important implications for the study of virus evolution, a survey of recombination in the viruses of the genus Flavivirus was carried out. Using envelope gene sequence data and a combination of graphical and phylogenetic analyses, hitherto unreported recombinations in Japanese encephalitis virus and St Louis encephalitis virus was detected, as well as further recombinants in DEN virus. However, no evidence for recombination was found in West Nile or YF viruses, nor in the tick-borne flavivirus group. It has been proposed that the difference between the mosquito- and tick-borne viruses can be accounted for by their differing modes of transmission, whilst the variation among the mosquito-borne flaviviruses reflects both the ecology of the particular host and vector species and also bias in the sampling process (Twiddy and Holmes 2003).

6.3. Reassortment of genomic RNA fragments: (Bunyaviridae, Orthomyxoviridae, Reoviridae)

The genomes of viruses within the *Bunyaviridae* consists of three fragments: the small (S) fragment, encoding the nucleocapsid (N) protein; the medium (M) fragment, encoding two surface glycoproteins (Gn and Gc); and the large (L) fragment, encoding the viral RNA-dependent RNA polymerase. Reassortment of genomic RNAs has been reported within the vertebrate, plant or arthropod host for viruses of all genera in the *Bunyaviridae*, including orthobunyaviruses (Webster et al. 2011) It was established for hantavirus PUUV in Finland that exchange and reassortment of RNA fragments happen quite frequently within a bank vole (*Myodes glareolus*) population. The fragments were often further changed by accumulated point mutations (Razzauti et al 2008). For orthobunyaviruses within the Akabane group, evolution of new viruses with reasserted genomes have been detected in a number of instances (Yanase et al. 2010) Reassortment also seems to be the process responsible for the novel emerging orthobunyavirus Schmallenberg virus (Hoffmann et al. 2012)

As in the case of other viruses that have segmented genomes, the occurrence of genetic reassortment has been reported among orthobunyaviruses in nature. Ngari virus (NRIV), which has caused severe human illness in East Africa, was generated as a result of reassortment between the Bunyamwera and Batai viruses (BUNV and BATV) (Yanase et al., 2006). The Jatobal and Tinaroo viruses (JATV and TINV) are probably reassortants containing a part of the RNA segments from OROV and AKAV, respectively.

Genetic reassortment might have contributed to the evolution of the genus Orthobunyavirus, as revealed by phylogenetic analyses. Previous studies have indicated that reassortants generated in nature and in the laboratory exhibited changes not only in terms of antigenicity but also virulence (Briese et al., 2006). *To determine the effects of genetic reassortment on evolution and on virulence to mammalian hosts, genetic characterization of field-isolated viruses should be conducted especially for orthobunyaviruses, because of their public health and veterinary importance*

6.4. Mutations (point, insertion, deletion)

The possible impacts of genomic mutations for TBE, WNV, CHIKV and hantaviruses have already been discussed. Mutations take place, under natural conditions, in all virus families, but more frequently for viruses with RNA than DNA genomes. The reason is that the fidelity of “proof-reading enzymes” is lower for the former.

All genome changes may lead to new host and vector preferences and host cell tropisms. There are a number of illustrating examples in the literature (e.g. Khasnatinov et al. 2009; van Slyke et al. 2012; Razzauti et al. 2008; Tsetsarkin et al. 2007; Bennett et al., 2007; Vapalahti et al., 1996)

A general truth to remember: RNA viruses may shift hosts across Kingdoms

"ABSTRACT
Emerging and reemerging diseases that result from pathogen host shifts are a threat to the health of humans and their domesticates. RNA viruses have extremely high mutation rates and thus represent a significant source of these infectious diseases. In the present study, we showed that a plant-pathogenic RNA virus, tobacco ringspot virus (TRSV), could replicate and produce virions in honeybees, *Apis mellifera*, resulting in infections that were found throughout the entire body. Additionally, we showed that TRSV-infected individuals were continually present in some monitored colonies. While intracellular life cycle, species-level genetic variation, and pathogenesis of the virus in honeybee hosts remain to be determined, the increasing prevalence of TRSV in conjunction with other bee viruses from spring toward winter in infected colonies was associated with gradual decline of host populations and winter colony collapse, suggesting the negative impact of the virus on colony survival. Furthermore, we showed that TRSV was also found in ectoparasitic *Varroa* mites that feed on bee hemo-lymph, but in those instances the virus was restricted to the gastric cecum of *Varroa* mites, suggesting that *Varroa* mites may facilitate the spread of TRSV in bees but do not experience systemic invasion. Finally, our phylogenetic analysis revealed that TRSV isolates from bees, bee pollen, and *Varroa* mites clustered together, forming a monophyletic clade. The tree topology indicated that the TRSVs from arthropod hosts shared a common ancestor with those from plant hosts and subsequently evolved as a distinct lineage after transkingdom host alteration. This study represents a unique example of viruses with host ranges spanning both the plant and animal kingdoms.

IMPORTANCE
Pathogen host shifts represent a major source of new infectious diseases. Here we provide evidence that a pollenborne plant virus, tobacco ringspot virus (TRSV), also replicates in honeybees and that the virus systemically invades and replicates in different body parts. In addition, the virus was detected inside the body of parasitic *Varroa* mites, which consume bee hemolymph, suggesting that *Varroa* mites may play a role in facilitating the spread of the virus in bee colonies. This study represents the first evidence that honeybees exposed to virus-contaminated pollen could also be infected and raises awareness of potential risks of new viral disease emergence due to host shift events. About 5% of known plant viruses are pollen transmitted, and these are potential sources of future host-jumping viruses. The findings from this study showcase the need for increased surveillance for potential host-jumping events as an integrated part of insect pollinator management programs."

Quoted from Lian et al., 2014

7. Prediction, precaution and prevention

7.1. Can we reliably predict impacts of climate and ecosystem changes on indigenous and emerging viruses?

”Predicting the emergence of infectious diseases has been touted as one of the most important goals of biomedical science, with an array of funding schemes and research projects. However, evolutionary biology generally has a dim view of prediction, and there is a danger that erroneous predictions will mean a misuse of resources and undermine public confidence. Herein, I outline what can be realistically predicted about viral evolution and emergence; argue that any success in predicting what may emerge is likely to be limited, but that forecasting how viruses might evolve and spread following emergence is more tractable. *I also emphasize that a properly grounded research program in disease prediction must involve a synthesis of ecological and genetic perspectives.”* (Holmes, 2013).

The process of emergence is, to some extent, synonymous with the cross-species transmission of viruses to new hosts. Predicting what might emerge is essentially equivalent to predicting which viruses are better able to jump species boundaries and spread in new hosts. Our conception of viruses emerging in the future can be improved. It is obviously important to continue metagenomic surveys of the viruses that circulate in potential reservoir species, although these will be costly if many animals need to be surveyed. As many metagenomic studies are opportunistic, they might be better focused by collating the global species range of likely reservoir species, and dissecting their virus load in those parts of their home range that most often overlap with humans or which are most prone to human disturbance. However, it is important to recall that *identifying a virus through its genome sequence is not the same as isolating a virus, and that its exact biological properties cannot easily be determined from sequence data alone.* More generally, it is critical to recall that cross-species transmission and emergence represents an intricate balance between ‘genetics’, defined as the processes and determinants by which a virus is able to productively infect the cells of a new host species and spread to multiple individuals within that

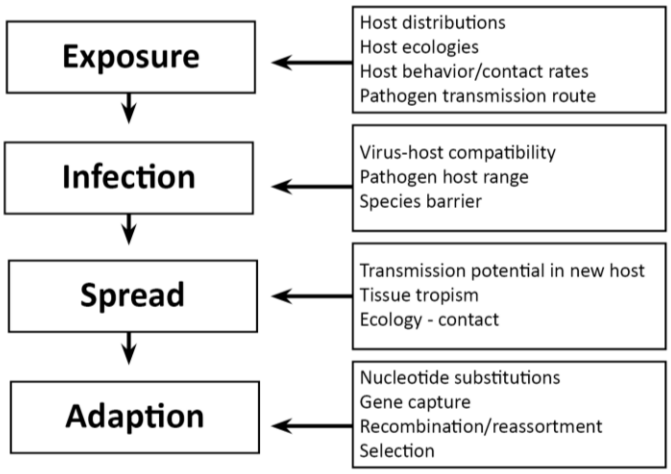


FIG 3. The steps involved in the emergence of host-switching viruses, showing the host and viral processes that can be involved in the transfer and adaption process (based on data from refrence 149).

From Parrish et al. 2008

species, and 'ecology', representing the likelihood that animals are exposed to a specific virus and that there are sufficient connections to enable the virus to maintain its spread at the epidemiological scale. Only when all these conditions are satisfied will an epidemic occur. Finally, more attention should be devoted to revealing the common evolutionary and epidemiological patterns exhibited by those viruses that have successfully jumped species boundaries. For example, a comprehensive survey of the phylodynamic patterns exhibited by currently circulating viruses will do much to help us understand how a new virus will evolve and spread once it has emerged. Specifically, it should be possible to compile a cross virus data base of the parameters that correlate most with successful emergence, such as how rapidly each virus evolves, its mode of transmission, its major host or vector species, its cell receptors of choice, key aspects of phenotype such as virulence and antigenicity, its population growth rate, its phylogeography, and whether it has jumped species boundaries in the past. Although such data will not enable us to predict future emergence with any certainty, they may allow broad-scale conclusions as to which groups of viruses are most likely to emerge in humans, which animal species in which geographical locations need to be surveyed most intensively, and how evolution will proceed following a host jump (adapted from Holmes, 2013 and references therein).

7.1.1. No answers to important questions

Three stages of viral emergence leading to successful host switching can be identified: (i) initial single infection of a new host with no onward transmission (spillovers into "dead-end" hosts), (ii) spillovers that go on to cause local chains of transmission in the new host population before epidemic fade-out (outbreaks), and (iii) epidemic or sustained endemic host-to-host disease transmission in the new host population. Variables that affect successful disease emergence influence each of these stages, including the type and intensity of contacts between the reservoir (donor) host or its viruses and the new (recipient) host, host barriers to infection at the level of the organism and cell, viral factors that allow efficient infections in the new host, and determinants of efficient virus spread within the new host population.

When emergent vector-borne viruses, or their known vectors/hosts, approach a new area, there is a high level of uncertainty with regard to the consequences for ecosystem, wildlife, human and domestic animal health. This uncertainty is, among others, related to lack of answers to questions like:

- Are any indigenous vector species competent to initiate and keep up transmission cycles for the emergent, invading virus?
- May local vertebrate species function as reservoirs or intermediate hosts for the emergent, invading virus?
- May the emergent, invading virus be more virulent for indigenous arthropod and vertebrate species?
- May genetic changes and adaptations of indigenous viruses give new viruses that are efficiently transmitted by the emergent, invading vector species?

7.1.2. The "vector competence" challenge

Gauging the direct influence of the environment on vectors, viruses, hosts and vector-borne disease episystems is a difficult challenge. Predicting the consequences of future environments and future climate changes on current episystems or the potential for the development of new episystems is much more difficult. Tabachnick explored the issues and challenges to predicting emerging vector-borne virus transmission in different regions of the world and the consequences associated with the purposeful introduction of modified vectors to prevent virus transmission (Tabachnick, 1998; Tabachnick, 2003). The difficulty in making successful predictions about virus transmission due to potential environmental changes is due to the paucity of available information on the processes controlling and influencing specific components of the complex vector–virus–host cycle. This is illustrated by the lack of information about mechanisms controlling *vector competence* for specific viruses. Vector competence is the susceptibility of the vector to infection with the pathogen and the ability of the infected vector to transmit the virus to a host during blood feeding. Vector competence is a key component in the vector–virus cycle. There are many examples of both genetic and environmental change causing *variation in vector competence between vector species, populations and between individual vectors* (Beerntsen et al., 2000; Tabachnick, 1994). *However, the complexity of genetic and environmental effects on vector competence has hardly been explored*. The specific genes influencing vector competence in nature are virtually unknown. The arrays of vector competence phenotypes produced by various genotypes in different environments, the norm of reaction of the genotype, have yet to be thoroughly characterized (Tabachnick, 2003).

During their lifecycle, mosquitoes are exposed to a variety of microbes, some of which are needed for their successful development into adulthood. *Symbiotic microbiomes* are beneficial to their insect hosts in many ways, including dietary supplementation, tolerance to environmental perturbations and maintenance and/or enhancement of host immune system homeostasis *However, recent studies have suggested that the adult mosquito's midgut microflora is critical in influencing the transmission of human pathogens* (Weiss and Aksoy, 2011).

It has been shown that a mosquito's microbiota influences Dengue virus (DENV) infection of the mosquito, which in turn activates its antibacterial responses. The reciprocal interactions between the mosquito's midgut microbiota and dengue virus infection were assessed (Ramirez et al., 2012). These interactions are, to a large extent, mediated by the mosquito's innate immune system. A marked decrease in susceptibility to DENV infection was observed when mosquitoes harbored certain field-derived bacterial isolates in their midgut. The results suggested that the mosquito's microbiota elicits a basal immune activity that seems to act against DENV infection. Conversely, the elicitation of the mosquito immune response by DENV infection itself influenced the microbial load of the mosquito midgut.

The complexity of the environmental effects on a vector-borne virus is illustrated by *Culex pipiens quinquefasciatus* (Say) competence for WNV. *Culex p. quinquefasciatus* infection with WNV increases with temperature. However, the influence of temperature changes due to the age of the adult, due to the virus dose or viremia, and the effect of these factors on the effect of temperature were non-

linear (Richards et al., 2007). The effects on vector infection also differed between two strains of the species, demonstrating that *different genotypes respond completely differently to complex environments* (Richards et al., 2007). *Culex p. quinquefasciatus* vector competence for WNV was also different from its vector competence for the related St Louis encephalitis virus (SLEV). *These studies illustrate that norms of reaction under different environments were not linear, and one could not predict vector competence under different environmental conditions* (Richards et al., 2007; Richards et al., 2009).

There are no examples of specific *genes that control vector competence* in natural populations for any vector– virus system. Nor is there information about environmental influences on a specific controlling genotype, the norms of reaction, under interacting arrays of environmental factors. There are complex environmental effects on vector genotypes that are not fully known. *We do not yet understand the genetic systems controlling vector competence, the full array of environmental factors influencing genotypes, nor how environmental factors interact with one another within any vectorborne disease episystem on a local, regional or higher level*. Therefore, it is not surprising that we have little ability to predict the future behavior of episystems under changing environmental conditions. There is much to be learned by exploring these issues with current vector-borne virus episystems.

7.1.3. Virus impacts on vectors and hosts

According to Kallio et al. (2007) the effect of parasite infection on the host population is one of the major questions in infectious disease ecology. In wildlife, a parasite's impact on its host population may also affect the parasite's own persistence, and furthermore, the infection risk to other species, including humans. Endemic parasites tend to persist for long times in host populations with rather stable prevalence. They do not usually induce severe pathogenicity or obvious decreases in survival or reproduction of their hosts. Yet, they may induce deleterious effects, and thus, decrease the fitness of the hosts. These effects may be difficult to separate from other factors that influence fitness of wildlife populations.

For population-level regulation, the parasite must influence the host reproduction or survival in a densitydependent manner. Although host regulation by parasitism is best demonstrated by experimental studies, evidence for parasites regulating their hosts is still rare. All information on the influence of parasitism on host fitness, both at individual and population levels, is valuable in evaluating the role of parasites in host population dynamics (Kallio et al., 2007 and references therein).

The bank vole (*Myodes*, ealier *Clethrionomys*, *glareolus*) is the host of Puumula virus (PUUV, genus *Hantavirus*, *Bunyaviridae*). Hantavirus infection in the rodent host is chronic, i.e., the immune response of the host does not clear the infection and virus replication is persistent. Consequently, the host may be infectious for the duration of life, and transmission of hantavirus is horizontal. Despite some evidence of cellular-level effects, hantavirus infections have been thought to be asymptomatic in their rodent hosts due to the long coevolution between them. No clinical illness, increased

mortality, or reduced fecundity caused by hantaviruses has been reported in rodent hosts (Kallio et al., 2007 and references therein).

Since hantaviruses have coevolved with their hosts, they are generally thought to have little or no effect on host survival or reproduction. Kallio and coworkers (2007) challenged this concept by investigating the effect of PUUV infection on overwintering survivals on bank voles. *The authors demonstrated that PUUV-infected voles had a significantly lower survival probability than uninfected voles*. The conclusion was that PUUV had a significant negative impact on host survival, and that endemic viruses in general deserve more attention in studies of host population dynamics (Kallio et al., 2007). Furthermore, it has been shown for some other hantaviruse that infected host rodents had slower weight gains than uninfected rodents (reviewed in Johnson et al., 2010),

7.2. Probabilities and Risks

7.2.1. Invoking the Precautionary principle

The establishment of biological transmission has always been determined by the possibilities for encounters among the three essential actors, the virus, the vector, and the vertebrate. Infinite numbers of new encounters with new partners occur daily worldwide, but nearly all are purely accidental or abortive, allowing only extremely small numbers of the contacts to meet all required conditions in time and space to establish biological transmission. Furthermore, among the very small number of encounters that gave a virus the chance, sooner or later most will become extinct when the required conditions are disrupted irrevocably (Kuno and Chang 2005).

However, for a number of vector- and rodent-borne viruses the on-going climate changes may increase the probabilities of new successful encounters with competent vectors, hosts and reservoirs.

In this connection it is once more prudent to remind about the inherent nature of viruses: One successful encounter may be enough to establish a given virus within a new location. The billions of new virus particles resulting from this encounter may pave the way for an emerging virus infection with dire consequences for ecosystems, wild life animals, domestic animals and/or humans.

Furthermore, because the concept of “risk” is frequently confused with “probability”, it seems warranted to repeat *the definition of risk:* Risk is defined as the *probability* that a given event shall happen *multiplied with the consequences* it will have if it happens. It is evident that applied to the global emerging and reemerging patterns of vector- and rodent-borne viruses we neither have enough knowledge about the probabilities nor the consequences.

7.3.Surveillance and monitoring programs



All photos: Reidar Mehl

7.3.1. A Norwegian Environmental Surveillance and Monitoring Program?

There are a number of international programs in operation. They are of high quality in relation to their explicit purposes and goals, which are directly related to prevention and control of human and/or domestic animal disease, and hence they deal only with "the known unknowns" and not with "the unknown unknowns". The technical report on invasive mosquito surveillance by European Centre for Disease Prevention and Control (ECDC) is a good, high quality example of this (ECDC, 2012).

7.3.1.1. Earlier strategies for detection and montoring of vector-borne viruses in Norway

Investigations performed during the 1970ies and 1980ies strongly indicated that a number of tick-, mosquito- and rodent-borne arboviruses might be circulating in Norwegian ecosystems (Traavik, 1979). No unequivocal proof of such viruses was at hand when those studies were initiated. The ambitions were to elucidate some features of the occurrence, characteristics and significance of arboviruses in Norway.

The scope and aims of the Norwegian arbovirus project were to (reviewed by Traavik, 1979):

- Carry out serological screenings of wildlife and domestic animals supposed to be hosts for potential arbovirus vectors, mainly ticks and mosquitoes. This should give rough indications of the extent and distribution of virus foci in nature.
- Use serological "maps" to spot biotopes with high virus activities and thereby increase the chances of isolating virus (es) from vector-collections.
- Carry out vector-collections within selected biotopes all over Norway.
- Identify and characterize prospective Norwegian virus isolates and compare, at least some of them, to known members of actual virus taxons.
- Use our local virus strains in studies aimed at evaluating the virus/vector/host interrelationships and the possible significance to human, domestic animal and wildlife health.
- Specifically study the ability of Norwegian virus isolates to establish persistent infections in cell cultures and/or laboratory animals.

- Study the effects of arbovirus mixed co-infections and sequential infections on laboratory animals.

All the intended goals were by no means reached, but it might be worthwhile to take elements of this scheme for field-based studies of arboviruses in Norway into consideration during the future surveillance- and monitoring-based research.

Mice study
Photo: Reidar Mehl

7.3.2. A professional, multi-disciplinary "task force" for surveillance and research on emerging vector- and rodent- borne viruses?

It is highly recommended that a group consisting of resource persons with complementary competence and interests are being established as soon as possible.

7.3.3. Rapid detection methods for surveillance of emerging arboviruses

A key aspect in preparing for the emergence of arthropod-borne diseases is the establishment of tests capable of detecting them. The available repertoire has recently been discussed in an excellent review by Johnson et al. (2012). During development of such tests a number of fundamental issues must be addressed, including key features such as sensitivity of the assay and its specificity for the target virus. The latter issue is tricky for arboviruses since a number of different closely related strains and species may be circulating within the same ecosystems. The assay must also be validated to provide assurance of its reliability, or at least give an indication of what might be missed. The assay under development needs to compete with existing methods in terms of cost and speed of delivery. Some tests may be applied to virus surveillance, in which case the test needs to be amenable to cost-effective delivery of high volumes of samples. This in turn can complement serological surveys for particular viruses or be applied to sampling arthropod vectors in order to provide early warning of potential virus invasion.

For some methods, the cost of individual tests is prohibitive for application to large numbers of samples. Molecular detection techniques have been very competitive as evidenced by the numerous tests developed in recent decades. Many of those assays may offer conclusive results considerably faster than more traditional detection methods such as virus isolation and plaque-reduction neutralization tests. Genetic variability of viruses is an inherent challenge in the use of molecular detection techniques with primer-mismatch being a constant problem. This has to some extent been overcome by the wealth of sequence data now available on many of the emergent arboviruses discussed in this report. Given that most vector-borne viruses have small genomes, it is not unrealistic to develop databases with extensive genetic information using rapid and large-scale sequencing

options. Once developed, this database would be a fundamental tool in the development of molecular-based, rapid diagnostic tests, including real-time PCR, microsphere/liquid-bead detection and chip-based microarrays.

In addition, because other etiological agents cause many arboviral-like illnesses, shotgun sequencing and metagenomic analysis of a diverse array of pathogens will also aid in revealing occurrence and disease diagnosis for vector-borne agents (Powers, 2009).

7.3.4. Inherent problems with viral ecosystem surveillance

One of the problems with virus ecosystem surveillance is that you very often are trying to find something you don't know is there until you have looked for it, and then you often find something you did not look for at all. It can be very difficult to convince politicians, competent and responsible authorities and the public that the considerable investments are really necessary. You do not, however, need a lot of insight or imagination to realize that the money spent on surveillance programs will be well worth compared to the expenses running, in terms of human and animal suffering, ecosystem imbalance and loss of biodiversity, and economic losses, if some of the worst case scenarios connected to emerging viruses should become reality. And it is just a matter of time before the first examples of serious ecosystem, animal or human disease caused by emerging viruses take place. The climate changes proceed. The northwards migrations of potential vectors and hosts, and hence also the viruses, are *en route*. Sooner or later the emerging viruses will find additional local vectors, hosts and reservoirs already infected with related viruses. This opens up for wild life co-infections with two or more related viruses. By genomic mutations, recombinations and/or reassortments such incidences may lead to generations of changed, e.g. chimeric, viruses with unpredictable biological characteristics in a short while, or after a number of incidences over a longer period of time.

7.3.5. Warning systems

It might be a good idea to establish a network of local Norwegian professional and *pro bono* working organizations and individuals to report observations of aberrant wildlife behavior and unexpected mortality.

"An "early warning system" based on an international wildlife-monitoring network may be the only effective defense, said William Karesh, a report co-author and vice president of Global Health Programs at the New York-based Wildlife Conservation Society. Observing wildlife could yield crucial signals of potential outbreaks. "Without the presence of wildlife, we would be clueless about what's going on in the environment," Karesh told a briefing at the International Union for Conservation of Nature (IUCN) World Conservation Congress in Barcelona." *(*Quoted from Dell'Amore 2008*).*

On-the-ground monitoring has already been shown to work, according to the Wildlife Conservation Society's Karesh (Dell'Amore 2008). In Brazil forest communities that spot primates sick with yellow fever report back to their health agencies, which in turn start vaccinating for the mosquito-borne

illness.

In the Republic of the Congo a group of local hunters has been trained to pinpoint symptoms of Ebola hemorrhagic fever in animals. The strategy has led to three years without a single human case in that region, said Karesh.

The Global Avian Influenza Network for Surveillance also draws on indigenous knowledge through a system of people in 34 countries, who monitor wild bird populations for signs of sickness. Of course, other unnatural forces are contributing to the spread of disease, experts added. For instance, the illegal wildlife trade, especially robust in Asia, is bringing people and animals into closer quarters, according to the Wildlife Conservation Society (Dell'Amore 2008). The 2002 outbreak of severe acute respiratory syndrome (SARS) was traced to civets. The cat-size mammal, prized for its meat, had ended up in wildlife markets in China.

7.3.6. Collection of biological materials: A biobank for research, surveillance and monitoring of emerging and re-emerging viruses in our ecosystems

A biobank of this kind would also be valuable for studies and surveillance of other infectious agents, released/escaped GMOs and chemical pollutants. The bank could initially be based on the collections that Reidar Mehl et al. carried out, all over the country, from the 1960s until the present time, of:

- Potential vectors – *Ixodes* spp ticks, *Aedes/Anopheles/Culex/Culiseta* spp mosquitoes, *Culicoides* spp, *Phlebotomus* spp etc.
- Carcasses and blood samples from potential host/reservoir wildlife animals – Nine different species of small rodents and shrews; rats, hares, bats, squirrels, passerine birds and others.

The materials have been kept frozen down ever since collection.

New collections must be carried out during a *Scientific Study and Surveillance Program (SSSP)* established by public funding. The Norwegian Agency for the Environment might take initiatives for funding and practical execution of field collection expeditions.

7.4. Precautionary science and research

As pointed out earlier the different virus families have their specific life cycles and host-specificities. Hence it is impossible to make risk assessment schemes that are valid for all potential virus vectors. Risk assessment must be performed on a case-by-case, step-by-step basis, taking into account the characteristics of the ecosystem into which the virus invades, and the ability of the virus to engage in trans-boundary movements.

The most evident risk issues related to invading viruses are the questions of (i) whether infectious cycles can be substantiated over prolonged periods of time, and (ii) whether recombinations or reassortments with naturally occurring relatives can take place.

7.4.1.Some holistic research questions lacking good answers.

As indirectly demonstrated in this report, *co-infections between different viruses* carried by the same vector and/or host/reservoir will most certainly occur. As seen elsewhere, and according to the studies from Norway in the 1970ies and 1980ies, such situations might occur through:

i) Combinations of different mosquito-borne viruses;
ii) Combinations of mosquito-borne and tick-borne viruses; and by
iii) Combinations of both mosquito- and tick-borne viruses with rodent-borne viruses.

Will the effects of such mixed infections be different from single virus infections in terms of:

- *Levels of multiplication for the individual viruses?* This question can only be answered by well-designed and executed field- and laboratory- based studies.
- *Transmissibility of the individual viruses?*
- *Effects on vector, host or reservoir health and fecundity?*

There are few published studies dealing with suchquestions. In experiments performed in Norway, approximately 12-day-old mice were infected intracerebrally with tick-borne encephalitis (TBEV) virus (strain Hypr), Unkuniemi virus (UUK, strain By E50) and Kemorovo (Tribec virus), as single virus inoculations, coinfections with two or three viruses, and sequential infections with two or three viruses at 24-hour intervals. The effect of mixed infections on mortality, morbidity and average survival time was recorded. The main findings were that:

1) Some mixed infections with TBE and UUK viruses reduced the mortality and acute morbidity significantly as compared to single infections with each virus. The average survival times were lengthened.
2) Mixed infections with TBE and Tribec did not affect the 100% mortality of TBEV.
3) Mixed infections with UUK and Tribec seemed to result in a cumulative effect of the two viruses.
4) With triple co-infections (TBEV + Tribec + UUKV simultaneously), the mortality and acute morbidity rates were reduced significantly as compared to TBEV single infections.
5) Some of the mixed infections tended to result in persistent disease among the survivors (Traavik 1978).

Are vectors/hosts/reservoirs affected by the viruses they carry and transmit? There are published studies related to WNV-infected birds. *These birds display increased locomotor activity or restlessness, which can be recognized under captive conditions*.

Synergy between climate change and different anthropogenic environmental changes (e.g. climate warming + endocrine-disrupting POPs + radiation + ecosystem sequestrations): May it affect the course and expression of virus infections?

Climate change is likely to pose additional stress to individuals, and, because different endocrine systems are important for enabling animals to respond adequately to environmental stress, EDCs may interfere with adaptation to enhanced stress situations. Thus, when taking into consideration the

long-range transport of novel EDCs into the northern Palearctic ecosystems, the combination of EDCs, climate change and new emergent virus infections may be a worst-case scenario for mammals and seabirds in these parts of the world. However, knowledge of the responses of animals, and humans, to multiple natural and anthropogenic stressors is at the present time not sufficient for investigators to forecast the combined effects of these three stressors. *Clearly there is a need for more focus on the interacting effects of multiple stressors (natural or anthropogenic) on wildlife (Munro Jensen 2006), as well as on humans and domestic animals. Dioxins, PCBs, asbestos, benzene, flame retardants, certain pesticides, and other chemicals are known to be immuno-toxicants. Hence, vertebrate populations already under influence of chemical pollutions may cope differently with novel, emergent virus infections when they are also exposed to the ecosystem effects of climate changes (Centers for Disease Control 2010).*

It is difficult to find literature references concerning how EDCs and other pollutants affect the permissiveness for virus infections. This is curious, because the types of cellular functions that are usually affected by EDCs, such as the composition and permeability of cell membranes, including potential virus receptors, the synthesis of nucleic acids and proteins, etc., can very well be envisaged to have an impact on the possibility for horizontal and vertical transfer of viruses. The extent to which xenobiotics affect living cells and organisms depends upon the specific physico-chemical conditions, such as the type of soil, the temperature, the water content and the pH, factors which, in turn, may be affected by other types of contamination, local emissions, etc. (Traavik 1999).

7.4.2. Research approaches and goals

- Collect baseline data and host/vector/reservoir biobank materials for surveillance and detection of invading vector/host/species and emerging viruses over time.
- Use of experimental and indigenous animals as “sentinels” for emerging viruses
- Sampling along the routes and “transit sites” of migratory birds.
- Improvements of molecular and serological tests for rapid detection and diagnosis.
- Using optimal methods and parameters for registration and documentation of weather conditions and climatic changes
- Identify, separate and re-integrate the effects of multiple climate variables.
- Identify, separate and re-integrate other, potentially contributing, anthropogenic ecosystem changes (e.g. ecosystem sequestration and endocrine disrupting pollutions, POPs).
- Using population and mathematic models to explain virus dynamics and predict epizootological changes.
- Develop ability to forecast and monitor invasions of new potential virus hosts and vectors
- Develop ability to forecast invasions and disease outbreaks (based on field studies and collections, lab experiments, mathematical modeling, remote sensing)
- Evaluate the effects of climate on virus evolution (mutations, reassortments, recombinations, selection of virus subpopulations may happen rapidly) and adaptation to new hosts and vectors.

- Evaluate whether climate warming plus other anthropogenic ecosystem effects may activate persistent or latent endogenous viruses in vector/host/reservoir animals.
- Evaluate whether climate change plus other anthropogenic effects may make vector/host/reservoir species more permissive to clinical infections with "novel", invading viruses

7.5. Prohibit and interrupt

7.5.1. Vaccine Development and Use

Immunization of human and domestic animal populations is one of the first remedies a modern society is considering when epidemics or epizootics are emerging. That will also be the case for emerging arbovirus and hantavirus infections. It must, however, be remembered that when epidemics and epizootics are discovered, the viruses have already been established in the fauna of the local ecosystems (Traavik 1999). Hence, a precautionary regional and national strategy must always *be grounded in surveillance-based risk assessment and management.*

Bait-administered vaccination of potential vectors, hosts and reservoirs may be one of the strategic measures to be taken. Vaccines that are intended for such use must be resistant to rapid environmental degradation. This calls for the use of recombinant vaccines based on virus vectors, e.g. pox- or adenoviruses, or naked DNA constructs (Myhr and Traavik 2012, Nalca et al. 2003, Mota et al 2005). Recombinant vaccines for release into the open ecosystems carry their own environmental risks, *for instance in terms of new viruses emerging as the result of recombinations between the vaccine vector and wild type virus relatives circulating within the ecosystems* (Traavik 1999; Okeke et al 2011; Myhr and Traavik 2012)

Arboviruses and rodent-borne viruses cause significant human illness ranging from mild, asymptomatic infection to fatal encephalitis or hemorrhagic fever. The most significant arboviruses causing human illness belong to genera in three viral families, *Togaviridae, Flaviviridae,* and *Bunyaviridae*. These viruses represent a significant public health threat to many parts of the world, and, as evidenced by the recent introduction of the West Nile virus (WNV) to the Western Hemisphere, they can no longer be considered specific to any one country or region of the world. Like most viral diseases, there are no specific therapies for the arboviral encephalitides; therefore, effective vaccines remain the front line of defense for these diseases (Nalca et al. 2003). With this in mind, the development of new, more effective vaccines and the appropriate animal models in which to test them become paramount. In fact, for many important arboviruses (e.g. California serogroup viruses) there are currently no approved vaccines available for human use. For others, such as the alphaviruses, human vaccines are available only as "investigational new drugs", and thus are not in widespread use. On the other hand, safe and effective vaccines against tick-borne encephalitis virus (TBEV) and Japanese encephalitis virus (JEV) have been in use for decades. New challenges in vaccine development have been met with new technologies in vaccine research. Many of the newer vaccines are now being developed by recombinant DNA technology (Traavik, 1999b; Nalca et al. 2003, Mota et

al 2005). For example, chimeric virus vaccines have been developed using infectious clone technology for some arboviruses including, WNV, JEV, and TBEV. Poxvirus vectors like MVA (Modified Vaccinia Virus Ankara) have been employed for a number of arboviruses (Myhr and Traavik, 2007, 2012). Other successful approaches have involved the use of naked DNA encoding and subsequently expressing the desired protective epitopes. Naked DNA vaccines have been used for, among others, TBEV, JEV and WNV. The development of less expensive, more authentic animal models to evaluate new vaccines against arboviral diseases will become increasingly important as these new approaches in vaccine research are realized.

There are, however, some disturbing experiences related to vaccination campaigns against other zoonotic diseases caused by single stranded RNA viruses with fragmented genomes. Recent studies have shown that Influenza A H5N1 is mutating faster in countries that have been implementing large-scale vaccinations of poultry. The genetic changes accrued by the viruses rendered the vaccinations ineffective and increased the risk that the viruses might jump to other host species (Cattoli et al., 2011).

7.5.2. Antibody dependent enhancement?

In general, virus-specific antibodies are considered antiviral and play an important role in the control of virus infections in a number of ways. However, in some instances, the presence of specific antibodies can be beneficial to the virus. This activity is known as antibody-dependent enhancement (ADE) of virus infection. The ADE of virus infection is a phenomenon in which virus-specific antibodies enhance the entry of virus, and in some cases the replication of virus, into monocytes/macrophages and granulocytic cells through interaction with Fc and/or complement receptors. This phenomenon has been reported *in vitro* and *in vivo* for viruses representing numerous families and genera, including vector-borne members, of public health and veterinary importance. These viruses share some common features such as preferential replication in macrophages, ability to establish persistence, and antigenic diversity. For some viruses, ADE of infection has become a great concern to disease control by vaccination. Consequently, numerous approaches have been made to the development of vaccines with minimum or no risk for ADE. Identification of viral epitopes associated with ADE or neutralization is important for this purpose. In addition, clear understanding of the cellular events after virus entry through ADE has become crucial for developing efficient intervention. However, the mechanisms of ADE still remain to be better understood (Cacel Tiredo 2003, Thomas et al, 2006).

Dengue Haemorrhagic Fever (DHF), the most serious clinical manifestation of dengue virus (DENV) infection is due to ADE. There are 4 different DENV virus types. When an individual is initially infected with one type, antibodies able to neutralize this serotype are produced. These antibodies will bind to, but not neutralize, the invading virus if the same individual is later on infected with another virus serotype. This sets the stage for ADE and DHF. In October 2013 it was announced at the International Conference on Dengue and Dengue Haemorrhagic Fever in Bangkok that after years of work developing a vaccine predicated upon the need to address four distinct strains of dengue virus,

scientists have identified a fifth. Researchers screening DENV samples came across one collected in 2007 during an outbreak in Malaysia's Sarawak state that seemed different from known DENV strains. Sequencing it, they discovered it to be phylogenetically distinct; further experimentation showed that the monkey antibodies produced against it differed significantly from those produced against the four original serotypes. This discovery will complicate - and delay - an already difficult and complex research undertaking (Yuill, 2013).

In a study from Norway (Traavik 1979) it was demonstrated that antisera from various animal species containing antibodies to a tick-transmitted virus were not able to neutralize virus infection in newborn mice, the outcome of which was an acute, fatal CNS disease. There was, however, one noticeable exception. Mixtures of virus and hyperimmune mouse serum or ascitic fluid inoculated intracerebrally into newborn mice resulted in a persistent infection and a chronic disease that had previously only been recognized in 2 to 3-week-old virus-inoculated mice. A serum pool from persistently infected mice had the same effect, though this was less pronounced. The addition of unheated guinea pig serum to the virus-hyperimmune serum mixtures reinforced the tendency to persistence and chronic disease, and unheated guinea pig serum alone modified the infection in the same way. The results suggested an immunological basis for the virus persistence and chronic disease in suckling mice.

7.5.3. Creating incompetent vector populations

None of the vaccine strategies mentioned so far can be used to make potential arthropod vectors the "dead end street" for arbovirus propagation and transmission. Hence there are at the moment massive efforts to create *genetically modified mosquitoes* that are unable to transmit some of most feared arboviruses, e.g. the Dengue viruses and Japanes B encephalitis viruses. For this purpose genetically modified mosquitoes produced by transgenic modification techniques are already in highly controversial field trials (Reeves et al., 2012; see Box below). A new strategy has appeared through the demonstration of RNAi (interference) pathways as a natural regulator of virus infections in arthropods. The RNAi pathway acts as a gatekeeper to the incoming virus by affecting infection rate of the midgut, intensity of infection, and dissemination from the midgut to secondary tissues, the most important for virus transmission being the salivary glands.

7.5.4. RNA interference (RNAi) as innate antiviral immune responses

The RNA interference (RNAi) pathway acts as an innate antiviral immune response modulating arbovirus infection *in mosquitoes* (Khoo et al 2010). RNA interference (RNAi) probably functions as an antiviral mechanism in most eukaryotic organisms. Variations in the activity of this antiviral pathway in mosquitoes could explain, in part, why some mosquitoes are competent vectors of medically important, arthropod-borne viruses (arboviruses) and others are not. Mosquito-borne arboviruses cause serious diseases in humans that are increasingly becoming public health problems, yet arbovirus infections cause minimal pathology in the mosquito vector, allowing persistent infections

Quote from Reeves et al., 2012

”Experimental releases of genetically modified (GM) insects are reportedly being evaluated in various countries, including Brazil, the Cayman Islands (United Kingdom), France, Guatemala, India, Malaysia, Mexico, Panama, Philippines, Singapore, Thailand, the United States of America, and Vietnam. GM mosquitoes (Aedes aegypti) have already been released for field trials into inhabited areas in the Cayman Islands (2009–?), Malaysia (2010–2011), and Brazil (2011– 2012). Here, we assess the regulatory process in the first three countries per- mitting releases (Malaysia, US, and the Cayman Islands) in terms of pre-release transparency and scientific quality. We find that, despite 14 US government– funded field trials over the last 9 years (on a moth pest of cotton), there has been no scientific publication of experimental data, and in only two instances have permit applications been published. The world’s first environmental impact statement (EIS) on GM insects, produced by US authorities in 2008, is found to be scientifically deficient on the basis that (1) most consideration of environmental risk is too generic to be scientifically meaningful; (2) it relies on unpublished data to establish central scientific points; and (3) of the approximately 170 scientific publications cited, the endorsement of the majority of novel transgenic approaches is based on just two laboratory studies in only one of the four species covered by the document. We find that it is not possible to determine from documents publically available prior to the start of releases if obvious hazards of the particular GM mosquitoes released in Malaysia, the Cayman Islands, and Brazil received expert examination. Simple regulatory measures are proposed that would build public confidence and stimulate the independent experimental studies that environmental risk assessments require. Finally, a checklist is provided to assist the general public, journalists, and lawmakers in determining, from documents issued by regulators prior to the start of releases, whether permit approval is likely to have a scientifically high quality basis.”

and lifelong virus transmission. The principal mosquito innate immune response to virus infections, RNAi, differs substantially from the human immune response and this difference could be the basis for the disparate outcomes of infection in the two hosts. Understanding the mosquito antiviral immune response could lead to strategies for interruption of arbovirus transmission and greatly reduce disease. Research focused on RNAi as the primary mosquito antiviral response has the greatest potential for developing a full understanding of mosquito innate immunity (Blair 2011). However, recent data suggest that some evolutionary conserved signaling pathways (Toll, Imd and Jak-Stat) also contribute to antiviral immunity. Moreover, symbionts, such as the intracellular bacterium Wolbachia and the gut microflora (microbiome), influence the course of virus infection in insects. These results add an additional level of complexity to antiviral immunity, but also provide novel opportunities to control the spread of arboviruses (Merkling and van Rij, 2013)

It has been hypothesized that genetically modified mosquitoes can be generated that transcribe a virus-specific dsRNA, triggering the RNAi response soon after ingestion of a blood meal. This could induce the RNAi pathway in the midgut prior to establishment of virus infection and profoundly change vector competence. Towards this goal transgenic *A. aegypti* lines that are refractory to DENV by exploiting the RNAi pathway are being developed (Sanchez-Vargas et al. 2004).

RNAi pathways are also part of the innate immune system of ticks, although tick RNAi pathways may differ from that of other arthropods such as insects (Kurscheid et al 2009).

Abdominal injection of dsRNA into unfed adult female ticks appeared to silence target gene expression even in the tick synganglia. The ability of dsRNA to cross the blood-brain barrier in ticks suggests that RNAi should prove to be a useful method for dissecting function of synganglia genes expressing specific neuropeptides in order to better assess their role in tick biology (Karim et al 2008). *It is possible that such approaches may also make ticks incompetent as arbovirus vectors.*

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