University of South Bohemia
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From the Robert Koch Institute, Berlin, Germany

Arboviral Encephalitis – Epidemiology, Diagnostics and Surveillance in the Face of Changing Environments

Habilitation thesis
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presented by

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Annotation:

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Anotace:

Donoso Mantke, O. (2011) Arbovirové encefalitidy – epidemiologie, diagnostika a surveillance ve světle měnícího se prostředí. Habilitační práce [v angličtině] – 236 s. Přírodovědecká fakulta Jihočeské univerzity v Českých Budějovicích, Česká republika.

Abstract :

The habilitation thesis focuses on arbovirus-induced disease of the central nervous system, with emphasis on those agents important and emerging in Europe, by giving an overview of their epidemiology, diagnostics, and surveillance. These are: West Nile virus (WNV), Toscana virus (TOSV), and tick-borne encephalitis virus (TBEV). The published studies selected are all part of activities within the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD) and/or the German Consultant Laboratory for Tick-borne encephalitis and further flaviviruses.

Abstrakt :

Předkládaná habilitační práce je zaměřena na arbovirová onemocnění centrální nervové soustavy. Zvláštní důraz je kladen na v Eropě emergentní virová agens a jejich epidemiologii, diagnostiku a surveillanci. Z nich pak zejména na virus západního Nilu, virus Toscana a virus klíšťové encefalitidy. Vybrané publikace, které jsou součástí habilitační práce, vznikly v rámci aktivit Evropské sítě pro diagnostiku importovaných virových infekcí (European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD)) a/nebo při činnosti Německé konzultační laboratoře pro klíšťovou encefalitidu a další flaviviry.

Prohlašuji, že jsem svou habilitační práci vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Přiložené práce vznikly v rámci aktivit Evropské sítě pro diagnostiku importovaných virových infekcí (European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD)) a/nebo při činnosti Německé konzultační laboratoře pro klíšťovou encefalitidu a další flaviviry.

Berlin, 20.01.2011 Dr. Oliver Donoso Mantke

“Science cannot solve the ultimate mystery of nature.
And that is because, in the last analysis, we ourselves are part of nature
and therefore part of the mystery that we are trying to solve.”

Max Planck

To my family, friends, and colleagues,
you always trusted in me.

Content

Chapter I.

Introduction and literature review

I.1 Arbovirus encephalitis

I.1.1 Arboviruses

The World Health Organization (WHO) defines arboviruses (arthropod-borne viruses) as a group of viruses which are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by hematophagous (blood feeding) arthropods including mosquitoes, ticks, and flies (http://www.who.int/vaccine_research/diseases/vector/en/). The term 'arbovirus' has no taxonomic significance. It is an informal term that refers to the biological-ecological similarities of these heterogeneous viruses. All arboviruses are maintained in complex life cycles [see examples in chapter I.2] involving at least one non-human primary vertebrate host and one primary arthropod vector. The viruses multiply and produce viremia in reservoir vertebrate hosts, multiply in the tissues of competent arthropod vectors, and are transmitted in saliva to new vertebrates by the bites of arthropods after a period of extrinsic incubation (Davis et al., 2008; Preiser, 2010; Weaver and Reisen, 2010). These cycles usually remain undetected until humans appear in a natural focus, or the virus escapes from this focus by a secondary vector or vertebrate host as the result of ecological changes. Humans and domestic animals can develop clinical symptoms but usually are 'dead-end' hosts because they do not produce significant viremia, and therefore do not contribute to the natural transmission cycle.

Over 500 different arboviruses are catalogued (Karabatsos, 1985; van Regenmortel et al., 2000; Tsai and Chandler, 2003; Hayes et al., 2008), of which some 100 are known to cause human disease, with clinical signs such as acute self-limiting fever (with or without rash), muscle and joint pain (e.g. chikungunya disease), hemorrhagic symptoms (e.g. dengue hemorrhagic fever) and/or neurological illness (e.g. tick-borne encephalitis). Not all arboviruses are restricted to the tropics, and only a subset of these involves the central nervous system (CNS), causing symptoms

ranging in severity from mild aseptic meningitis to encephalitis or flaccid paralysis. However, most human arboviral infections are clinically bland or result in an asymptomatic infection associated with seroconversion. Table 1 gives an overview of the most important encephalitic arboviruses worldwide which are formally classified in different families and genera based on their morphology, nature/structure of the genome, serological relationship, and biological properties. All encephalitic arboviruses are small, simple RNA viruses ranging in size from 40–60 nm for *Flaviviridae*, 60–80 nm for *Togaviridae* and *Reoviridae* to 80–120 nm for *Bunyaviridae* (Modrow et al., 2003). Alphaviruses and flaviviruses are enveloped spherical viruses whose genome consists of a positive-sense (+), single-stranded (ss) RNA molecule of 9–12 kb. *Bunyaviridae* are enveloped spherical/pleomorphic viruses whose genomes consist of three linear minus-sense (-) ssRNA segments of approximately 11 kb. And *Reoviridae* are non-enveloped icosahedral-shaped viruses that can contain up to 12 double-stranded linear RNA segments totaling 19–32 kb (Condit, 2007). All arboviruses, except for those belonging to the *Reoviridae*, have nucleocapsids surrounded by a host cell-derived lipid bilayer in which several viral envelope or membrane proteins are inserted that play an important role in viral attachment and cell entry, which is not well known at all. In the case of *Reoviridae*, the surface proteins are part of the outer capsid layer. Antibodies to these proteins form the basis for host immunity and serological diagnostics.

The habilitation thesis focuses on arboviruses inducing CNS disease, with emphasis on those agents important and emerging in Europe, by giving an overview of their epidemiology, diagnostics, and surveillance. These are: West Nile virus (WNV), Toscana virus (TOSV), and tick-borne encephalitis virus (TBEV). The published studies selected are all part of activities within the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD) and/or the German Consultant Laboratory for Tick-borne encephalitis and further flaviviruses. Fact sheets and further information concerning arbovirus encephalitis can be found on the respective websites of these institutions: http://www.enivd.org and http://www.rki.de/cln_169/nn_203794/EN/Content/Institute/DepartmentsUnits/NRC/TBE/TBE__node.html?__nnn=true.

Table 1 Important arboviruses that may cause encephalitis*

Virus *Family*/Genus	Arthropod vector°	Vertebrate reservoir	Distribution	Epidemiology	Incubation period†	Mortality	Seasonal predilection	Gender predilection‡	Age predilection	Other routes of transmission (human-to-human#)	References
***Togaviridae*/Alphavirus**											
Eastern equine encephalitis (EEEV)	Mosquitoes (*Culiseta, Aedes, Coquillettidia, Culex*)	Birds	Eastern US, Gulf coast, Caribbean, South America	CDC: average 6 cases per year; 260 confirmed cases (1964-2009)	4 to 10 days	>33%	Summer-fall	None	Persons over age 50 and younger than age 15	None reported	1-5
Western equine encephalitis (WEEV)	Mosquitoes (*Culex, Aedes* and others)	Birds	Western US and Canada, South America	CDC: 640 confirmed cases (1964-2009); but rare disease	2 to 10 days	3-8%	Summer-fall	None	Especially younger children (<4 years old)	Transplacental	1-5
Venezuelan equine encephalitis (VEEV)	Mosquitoes (*Culex* and others)	Rodents (enzootic)	South and Central America, Southern US (Florida)	Rare in the US; but important veterinary and public health problem in South/Central America	1 to 5 days	≤1% (epizootic)	Rainy season; summer-fall	None	Especially children	Suspected	1,3-5
***Flaviviridae*/Flavivirus**											
Japanese encephalitis serogroup											
Japanese encephalitis (JEV)	Mosquitoes (*Culex* and others)	Birds, pigs	Asia, Papua New Guinea, Torres Strait (Northern Australia)	WHO: 30,000 to 50,000 cases per year	5 to 15 days	Up to 40%	Rainy season; summer-fall	None	Especially children	None reported	1,5-9
West Nile (WNV)	Mosquitoes (*Culex* and others)	Birds	The Americas, Africa, Southern/southeastern Europe, the Middle East, Central/Southern Asia; Australia (subtype Kunjin virus)	CDC: 12,447 reported cases of WN encephalitis and meningitis (1999-present)	2 to 14 days	2-18% (in the US)	Rainy season; summer-fall	None	Especially adults (>50 years old)	Blood transfusion, organ transplantation, mother-to-child, breastfeeding?, by infected birds' feces?	1-3,5-8,10-12
St. Louis encephalitis (SLEV)	Mosquitoes (*Culex*)	Birds	The Americas	CDC: average 108 cases per year (2-1,967); 4,533 confirmed cases (1964-2009)	5 to 15 days	5-15%	Rainy season; summer-fall	None	Especially adults (>60 years old)	None reported	1-3,5,6
Murray Valley encephalitis (MVEV)	Mosquitoes (*Culex*)	Birds	Northern Australia and Papua New Guinea	Rare disease; last outbreak 1974 (58 cases)	7 to 28 days	~20%	Rainy season	None	Children and young adults	None reported	1,6,13
Rocio (ROCV)	Mosquitoes (*Psorophora, Aedes*?)	Birds?	Brazil	Rare disease; last outbreak 1973-1980 (1021 cases)	7 to 14 days	~10%	Sporadic?	Mostly male	Young adults	None reported	1,14,15
Mammalian tick-borne virus group											
Tick-borne encephalitis (TBEV)	Ticks (*Ixodes* and others)	Rodents, small mammals	Northern and Central Europe, Russia Northern Asia	Average of 8,755 cases per year in Europe (2,805) and Russia (1990-2007)	7 to 14 days	<2-40% (depends on subtype)	Summer-fall	None	Especially adults (>60 years old)	Blood transfusion, breastfeeding, consumption of non-pasteurized milk from viremic livestock	1,6,10,16-21
Louping ill (LIV)	Ticks (*Ixodes*)	Sheep, red grouse? (for UK)	British Island, Ireland, Norway, Iberian Peninsula, Turkey, Bulgaria, and possibly Japan (local variety Negishi virus)	Rare disease; known cases occurred mainly in individuals with occupational exposure	4 to 7 days	Rare	Summer-fall?	None	None reported	None, but direct contact with animals	1,6,10,17,20
Powassan (POWV)	Ticks (*Ixodes* and others)	Rodents, small mammals	Northern US and Canada; Far Eastern Russia (transmission: mosquitoes-birds?)	Rare disease in the US (CDC: 20 cases, 2001-2009)	8 to 34 days	10-15%	Summer-fall	None	Children (<15 years old)?	None reported	1-3,5,6,17,20
***Bunyaviridae*/Bunyavirus**											
California serogroup											
La Crosse (LACV)	Mosquitoes (*Aedes*)	Small mammals	Midwestern/mid-Atlantic and southeastern US states; other related viruses found in the US: California encephalitis-, Jamestown Canyon-, Snowshoe hare-, Trivitattus virus	CDC: average 80-100 cases per year; 3,590 confirmed cases (1964-2009)	5 to 15 days	Low (<2%)	Summer-fall	Mostly male	Children (≤15 years old)	None reported	1-3,5,22
Tahyna (TAHV)	Mosquitoes (*Aedes* and others)	Rodents, small mammals	Europe, Russia, Asia, and Africa; other related viruses found in Europe/Russia: Snowshoe hare-, Inkoo virus	Rare disease in Europe? (not notifiable)	5 to 15 days	None reported	Summer-fall	None	Children	None reported	1,10,11
***Bunyaviridae*/Phlebovirus**											
Sandfly fever (SFV)	Phlebotomine sandflies	Not well known	Mediterranean basin, the Middle East; and probably Caucasus and Central Asia (incl. Toscana, Sicilian and Naples viruses)	In Europe, Toscana virus is among the three most prevalent viruses associated with meningitis during warm season	4 to 8 days	None reported, but severe cases are described	Summer-fall	None	Especially children (in case of Toscana virus)	None reported	10,23,24
***Reoviridae*/Coltivirus**											
Colorado tick fever (CTFV)	Ticks (*Dermacentor*)	Small mammals	Western mountain regions of US and Canada	200-400 cases per year	3 to 6 days	Rare	Summer-fall	None	Children and adolescent	Blood transfusion	1-3

* Adapted from www.enivd.org (General fact sheet on viral encephalitis). ° Primary arthropod vectors are underlined. † The ranges reflect the usually incubation periods. ‡ Even if gender predilection is not reported in the table it must be remembered that people who engage in outdoor work and recreational activities are at higher risk (mostly male). # Even if person-to-person spread is not reported in the literature it must be remembered that all of these viruses manipulated in virology laboratories can be infectious to humans under circumstances implicating aerosols.

References:
1) Whitley and Gnann, 2002; 2) Romero and Newland, 2003; 3) Davis et al., 2008; 4) Zacks and Paessler, 2010; 5) CDC Division of Vector-Borne Infectious Diseases; 6) Gould and Solomon, 2008; 7) Tolle, 2009; 8) Preiser, 2010; 9) Misra and Kalita, 2010; 10) Kallio-Kokko et al., 2005; 11) Hubálek, 2008; 12) Gyure, 2009; 13) McCormack and Allworth, 2002; 14) Figueiredo, 2000; 15) Figueiredo, 2007; 16) Gritsun et al., 2003 a; 17) Charrel et al., 2004; 18) Lindquist and Vapalahti, 2008; 19) Mansfield et al., 2009; 20) Dobler, 2010; 21) Heyman et al., 2010; 22) Haddow and Odoi, 2009; 23) Brett-Major and Claborn, 2009; 24) Depaquit et al., 2010.

I.1.2 Encephalitis

Encephalitis is an irritation and inflammation of the brain parenchyma, associated with clinical evidence of brain dysfunction (Whitley and Gnann, 2002; Romero and Newland, 2003; Lewis and Glaser, 2005; Granerod and Crowcroft, 2007; Steiner et al., 2010). It often coexists with inflammation of the covering membranes of the brain and spinal cord ('meningoencephalitis'). Meningeal irritation (e.g. fever, headache, general malaise, vomiting) and somnolence are signs of meningitis, while behavioral, cognitive, and focal neurological symptoms and seizures are signs of the disruption of brain function. Like meningitis, encephalitis can be caused by a wide variety of infectious agents, including viruses, bacteria, fungi, and parasites as a result of direct CNS invasion (Whitley and Gnann, 2002; Lewis and Glaser, 2005; Granerod and Crowcroft, 2007; Donoso Mantke et al., 2008 a). Other types of encephalitis include acute parainfectious or post-vaccination encephalitis, i.e., acute disseminated encephalomyelitis (ADEM), sub-acute and chronic encephalitis, and encephalopathies due to non-infectious causes (e.g. intoxications, drugs, systemic organ dysfunction, or systemic infection sparing the brain). Those cases of aseptic encephalitis for which the etiology can be determined are most often caused by viral infections: herpes simplex viruses, varicella-zoster virus, Epstein-Barr virus, mumps virus, measles virus, and enteroviruses are considered to be the major causes of viral encephalitis in immunocompetent individuals worldwide (Nicolosi et al., 1986; Rantala and Uhari, 1989; Kamei and Takasu, 2000; Koskiniemi et al., 2001; Davison et al., 2003; Glaser et al., 2003; Lee et al., 2003; Glaser et al., 2006; Ilias et al., 2006; Mailles and Stahl, 2009). In addition to these common pathogens, which are responsible for 'acute sporadic encephalitis', arboviruses can cause 'acute epidemic encephalitis' with similar symptoms to those of herpes simplex encephalitis (HSE), being among the major etiologies associated with encephalitis, depending on regional/endemic situation and on environmental factors (Okuno et al., 1975 a; Okuno et al., 1975 b; Chunsuttiwat, 1989; Cizman and Jazbec, 1993; Günther et al., 1997; Wu et al., 1999; Solomon and Cardosa, 2000; Akiba et al., 2001; Khetsuriani et al., 2002; Kunze et al., 2004; Trevejo, 2004; Kari et al., 2006; Kupila et al., 2006; Tyler, 2009 a). Important non-arthropod-borne viral zoonotic pathogens affecting the CNS are lymphocytic choriomeningitis virus (transmitted by rodents), rabies virus (transmitted by terrestrial mammals or bats), and Nipah virus (transmitted by bats

and pigs) (Whitley and Gnann, 2002; Romero and Newland, 2003; Tyler, 2009 a; Tyler, 2009 b). Finally, non-viral infective causes of encephalitis may include such diseases as tuberculosis, rickettsial disease, and trypanosomiasis which should be taken into consideration for differential diagnosis (Steiner et al., 2010). However, it should be mentioned that encephalitis is a rare manifestation of human viral infection, but of public health importance worldwide due to its high morbidity and mortality.

I.1.3 Pathogenesis

The pathogenesis of acute viral encephalitis is diverse and not fully understood. Pathogens infecting the brain directly must gain access to the CNS first. This can occur by either hematogenous or neuronal routes (Johnson and Mims, 1968; Johnson, 1987; Johnson, 1998). Hematogenous spread is most common and can result in an altered blood–brain barrier (BBB), as exemplified by arboviral infections of which those caused by alphaviruses, flaviviruses, and *Bunyaviridae* are the ones best known (Chambers and Diamond, 2003; Dionisio et al., 2003; Cusi et al., 2005; Mandl, 2005; Davis et al., 2006; Diamond, 2009; Hollidge et al., 2010; Růžek et al., 2010).

In general, arboviral infections occur most frequently following the bite of an infected arthropod. The arboviruses are transmitted by saliva from the arthropod salivary glands. The amount of virus required to infect humans is unknown. The route of progression through the host is not clearly established, but it appears that upon inoculation of virus into the skin, initial infection and peripheral amplification occur in subcutaneous tissues and lymph nodes, allowing the virus to enter the bloodstream (primary viremia) (Malkova and Frankova, 1959). Dendritic cells in the skin are thought to be the first cells for viral replication and to transport the virus to nearby draining lymph nodes via the lymphatic system (Johnston et al., 1996; Johnston et al., 2000). Flaviviruses enter the cell via receptor-mediated endocytosis. Fusion of the viral membrane with the endosomal vesicle membrane occurs after exposure to low pH in the endosome, following internalization through a class II fusion mechanism identical to the one formed in alphaviruses and *Bunyaviridae* (Kielian, 2006; Plassmeyer et al., 2007). Viremia is further augmented by dissemination of virus to the reticuloendothelial system and sometimes to muscle (secondary viremia).

During the viremic phase, many extraneural tissues can be infected (e.g. spleen, liver, and bone marrow), and the release of the virus from these tissues enables the viremia to continue for several days (Albrecht, 1968). A main principle that applies is the relationship between peripheral virus burden and the tendency to cause neuroinvasion. Viruses with a low capacity to generate viremia in peripheral tissue can be classified as low in 'neuroinvasiveness' (capacity to enter the CNS) regardless of their intrinsic level of 'neurovirulence' (replication within the CNS) (Chambers and Diamond, 2003). A relationship between systemic virus burden and viremia is also apparent, with the potential of the virus to generate viremia being a correlate of neuroinvasion. Factors like time of onset, magnitude, and duration of viremia, as well as the integrity of the host's innate (complement and interferon), humoral, and cell-mediated immunity, also influence the viral dissemination and risk of entry into the CNS. Encephalitis tends to be a rare complication after viremia because access to the brain is carefully regulated at the BBB. The mechanisms used by the virus to cross the BBB and to enter the CNS are not completely understood. Several hypotheses have been postulated for mechanisms of CNS penetration: (i) transport (transcytosis) across the vascular endothelial cells or infection of these and other cells constituting the BBB (Dropulić and Masters, 1990; Lossinsky and Shivers, 2004); (ii) access to the CNS after loss of BBB integrity (Lustig et al., 1992; Lossinsky and Shivers, 2004); or (iii) entry through the highly susceptible olfactory bulb (Monath et al., 1983; Cook and Griffin, 2003). An alternative mechanism, typically used by herpes simplex virus, might be direct axonal retrograde transport from infected peripheral neurons. This strategy may be successful because pathogens travelling inside neurons avoid immune surveillance. Once a pathogen has entered the brain, a variety of anatomic sites can become infected. For example, WNV typically infects neurons in the brainstem (Gyure, 2009); TOSV predominantly affects the gray matter of deep nuclei and hippocampus (Cusi et al. 2005); and TBEV affects the spinal cord, brainstem, and cerebellum (Růžek et al., 2010). These observations might be characteristic but are not disease-specific due to substantial overlap of the topographic patterns of CNS infection between the viruses. At least, only laboratory diagnostics is reliable enough to identify the causative virus [see chapter I.3]. Neurologic signs and symptoms develop after infection as the result of direct necrosis, host inflammatory response, and/or apoptosis in certain regions of the CNS. Neuronal apoptosis is a hallmark of several viral encephalitides, including

those caused by WNV, TOSV, and TBEV (Shrestha et al., 2003; Cusi et al., 2005; Růžek et al., 2009). Knowledge of the natural history of neurological arboviral infections as well as of shared mechanisms of neuroinvasion and neurovirulence is important to control the spread of these pathogens and for the development of effective treatment measures and vaccines. Notably, pro-apoptotic, host-protein synthesis inhibitory, and type I interferon-blocking properties have been attributed to several non-structural proteins of arboviruses from different families. This suggests that the ability of these viruses to induce death and subvert the innate immune system is critical for their maintenance in nature and may hold the key to uncovering shared mechanisms of neuropathogenesis (Billecocq et al., 2004; Blakqori et al., 2007; Yin et al., 2009, Overby et al., 2010). Animal models have been established for providing insight into the way virus-specific and host factors influence the course of disease, with the mouse model having become a fundamental tool for studying viral pathology as well as for the development of vaccines and antivirals (Chambers and Diamond, 2003; Nalca et al., 2003; Shrestha et al., 2003; Cusi et al., 2005; Mandl, 2005; Holbrook and Gowen, 2008; Růžek et al., 2010).

I.1.4 Clinical features

Because encephalitis has so many underlying causes as described above, it is nearly impossible to make generalizations about clinical signs and symptoms. However, the clinical hallmark of acute viral encephalitis is fever accompanied by headache, a subsequently altered level of consciousness, and symptoms and signs of cerebral dysfunction. These may consist of abnormalities that can be categorized into cognitive dysfunction (acute disturbances of memory, speech, and orientation, etc.); behavioral changes (disorientation, hallucinations, psychosis, personality changes, agitation); focal neurological abnormalities (e.g. anomia, dysphasia, hemiparesis); and seizures. The presented signs and symptoms may suggest whether acute encephalitis is focal or diffuse (Chaudhuri and Kennedy, 2002; Steiner et al., 2010). Distribution and spread of histopathological changes in the CNS are important for etiologic considerations: four types of meningoencephalitis may be distinguished, affecting either only the meninges, or the gray matter or the white matter, or both, in a focal or a diffuse manner (Love and Wiley, 2008). For example, as a rough guide, mostly arboviral encephalitis has diffused brain involvement, and early fever,

vomiting, obtundation, and coma are typical. In contrast, herpes simplex encephalitis (HSE) can begin focally with hemiparesis, seizures, or cranial nerve defects. While acute encephalitis results from direct CNS invasion by an offending pathogen (with the gray matter often targeted), in post- or parainfectious encephalitis neurologic effects are the consequence of the host immune response (which often affects the white matter). From a clinician's point of view, a 'typical' arboviral infection runs a biphasic course. However, this only occurs in a minority of persons infected with an arbovirus. In most patients, the clinical presentation does not follow this pattern and is said to be 'atypical'. Usually, the incubation period is very short: 3 to 8 days, sometimes a little longer (Davis et al., 2008). Initially, there is a flu-like syndrome with fever, headache, fatigue, muscle and joint pain. Patients often have malaise and gastrointestinal signs (such as anorexia, nausea, vomiting, diarrhea, abdominal pain). Sometimes there is pharyngitis, rhinitis and swollen lymph nodes (as reviewed for different viruses by Figueiredo, 2000; McCormack and Allworth, 2002; Davis et al., 2008; Hubálek, 2008; Gyure, 2009; Depaquit et al., 2010; Dobler, 2010; Misra and Kalita, 2010; Růžek et al., 2010; Zacks and Paessler, 2010). A maculopapular rash on the chest, back, and upper extremities can occur with variable frequency as a common symptom in WNV infection. The initial phase of infection can also take a subclinical course. Most individuals infected with arboviruses develop an asymptomatic infection, although the exact ratio of neuroinvasive disease cases to asymptomatic infections varies for specific viruses, and is roughly estimated between 1:100 and 1:1000 (Davis et al., 2008). For most arboviruses, asymptomatic infections occur mainly in children and young adults. The resulting infection usually produces prolonged immunity. It should be remembered that asymptomatically infected individuals can still transmit the infection to others, e.g. by blood transfusion. The arboviral disease usually does not go further than the first described phase and thus does not come to the attention of the clinician. Then it is not possible to make a diagnosis unless there is an epidemic or if unusually good laboratory facilities are available. In a minority of patients, after a transient improvement of the general symptoms there is a second phase of the disease. Again there is fever (the pattern of fever is therefore biphasic, so the patient fulfils the criteria for a typical case). Again there are flu-like symptoms which may be more pronounced than in the variable prodromal stage. Patients presenting with headache, fever, and neck stiffness without focal weakness or altered mental status are classified as having meningitis.

Patients presenting with clinical or laboratory evidence of brain parenchymal involvement are classified as having encephalitis. Encephalitic signs and symptoms can include depressed concentration, memory impairment, somnolence, confusion, dizziness, disorientation, nausea, vomiting, lethargy, stupor, tremors, convulsions, myoclonus, photophobia, dysphagia, dysarthria, nystagmus, vertigo, limb or facial pains and weakness, paresthesia, alteration in sensory, behavioral abnormalities, and generalized or localized seizures (as reviewed for different viruses by Figueiredo, 2000; McCormack and Allworth, 2002; Davis et al., 2008; Hubálek, 2008; Gyure, 2009; Depaquit et al., 2010; Dobler, 2010; Misra and Kalita, 2010; Růžek et al., 2010; Zacks and Paessler, 2010). Extrapyramidal signs including Parkinsonism, increased muscle tone, and tremors occur with variable frequency in patients with alphavirus and flavivirus encephalitis. Altered mental status is a hallmark of all encephalitides. Development of coma and death also occur with variable frequency among arbovirus infections (see Table 1). In contrast to other arboviral encephalitides, neuromuscular weakness is often a prominent finding in WNV meningoencephalitis, occurring in up to 50% of patients. Clinical syndromes that have been described include an acute flaccid paralysis/poliomyelitis-like syndrome, a Guillain-Barré-like syndrome, and a generalized myeloradiculitis (Tyler, 2004; Sejvar et al., 2005; Debiasi and Tyler, 2006). The acute flaccid paralysis/poliomyelitis-like syndrome, as a result of anterior horn cell death in the spinal cord, has been characterized best clinically and presents as acute monoplegia, asymmetric upper or lower extremity weakness, or generalized asymmetric tetraplegia or quadriplegia. In general, the acute encephalitis phase lasts 1 to 3 weeks but convalescence is slow. Depending on the severity of the disease, neurological and/or neuropsychiatric symptoms may persist for months. However, it should be noted that clinical presentation and course of infection can be markedly variable, and that findings from a general clinical examination are not of sufficient diagnostic value to identify the causative virus.

I.1.5 Therapy and prevention

Currently, there are no specific antiviral therapies of proved efficacy available for any arbovirus infection (Steiner et al., 2010). Few drugs that have shown efficacy in experimental animal studies and infected cell cultures, particularly when administered

early after viral infection, include interferon (Brooks and Phillpotts, 1999; Pantelic et al., 2005), ribavirin (Morrey et al., 2002), monoclonal antibodies (Kimura-Kuroda and Yasui, 1988; Morrey et al., 2007), immune globulin (Ben-Nathan et al., 2003), and antisense oligomers that bind to arboviral RNA (Bia et al., 1980; Kumar et al., 2006). Ensuring the presence of the drugs in sufficient concentration throughout the brain will be crucial in a clinical setting, where drug administration can begin only after the appearance of clinical symptoms. A significant issue for the development of effective therapies for the treatment of viral encephalitis is the ability of the therapeutic agent to cross the BBB and limit virus replication or the host immune response (Strazielle and Ghersi-Egea, 2005; Pardridge, 2007). In the absence of viable specific antiviral therapy, treatment relies on supportive management. All cases of acute viral encephalitis require hospitalization and supportive care based on syndrome severity (Chaudhuri and Kennedy, 2002). Seizures are controlled with intravenous anticonvulsants. Careful attention must be paid to the maintenance of respiration, cardiac rhythm, fluid balance, prevention of deep vein thrombosis and aspiration pneumonia, and to the medical management of raised intracranial pressure and secondary bacterial infections. Because of the limited risk of human-to-human transmission of infection, isolation of patients with arbovirus encephalitis is usually not required, although general precautions should be taken because both blood and cerebrospinal fluid (CSF) are potentially infectious. Many patients require rehabilitation following encephalitis. Vaccines are available for a limited number of arboviruses with a potential to cause encephalitis, and there is a significant need for vaccine development (Nalca et al., 2003). Approved vaccines for humans are only available against Japanese encephalitis and tick-borne encephalitis (Mansfield et al., 2009; Halstead and Thomas, 2010; Heyman et al., 2010). A vaccine under ‘investigational new drug application’ for Venezuelan equine encephalitis is available in the United States, but exclusively for the US Army, and at great expense. There are also new vaccines under investigation for Eastern and Western equine encephalitis, but these are typically given only to laboratory workers, and also difficult to obtain (Holbrook and Gowen, 2008). For other causative pathogens (e.g. WNV), vaccines are in clinical trials (Martina et al., 2010). Otherwise, preventive measures against arbovirus infections are entirely environmental, including sanitation, vector control, education, and avoidance aspects.

I.1.6 Arboviruses as emerging threats

Arboviruses are distributed worldwide, representing nearly 30% of all emerging infectious diseases in the last decade (Jones et al., 2008; Tyler, 2009 a). While the variables contributing to the epidemiology of each virus are unique, influencing the pathogen transmission within the natural life cycle (related to the triad virus–vector–host), there are some common socio-economic, environmental, and ecological factors contributing to the emergence of arboviral diseases (Morens et al., 2004). In particular, (re-)emergent infectious viruses can form by variation of previously present agents (genetic mutation and/or recombination), changes in populations of reservoir hosts or intermediate insect vectors, microbial adaption from animal to human hosts, changes in human demographics and behavior (notably urbanization, travel, leisure activities), and environmental factors. Also, the introduction of new infective agents to a determined area can be significant (Weaver and Reisen, 2010). This may occur directly from person to person or indirectly through arthropod vectors or other carrier animals, and sometimes by the transport of goods (Sutherst, 2004). Finally, the effect of climate changes has been increasingly discussed to have an influence on the (re-) emergence of infectious diseases (Reiter, 2008; Gould and Higgs, 2009; Stark et al., 2009). In the near future climate and environmental changes in the form of rising temperatures and humidity will probably play a major role to promote vector-borne diseases, but it would be over-simplifying the complex interrelation by relating the emergence of arboviruses only to climate changes. This is a multi-variate system where microbial, host, and environmental factors interact to create opportunities for infectious agents to evolve in new ecological niches, reach and adapt to new hosts, and spread more easily between them (Table 2).

WNV, TOSV, and TBEV were identified to be among the main (re-) emergent viral infections in Europe (Pugliese et al., 2007 a; Vorou et al., 2007). Knowledge about the true prevalence and incidence of these pathogens needs to be expanded and regularly updated in order to identify the risk for the exposed population and to apply optimal preventive measures. From the public health view, it is important to enhance surveillance activities (reporting incidence of human diseases, vector surveillance, domestic and wild animal surveillance, monitoring of ecological factors); diagnostic capability (test establishment and quality control); and response to vector-borne diseases (vector control, personal protection, therapies, and vaccines) (Senior,

2008). Chapter I.4 deals with the key requirements for a European surveillance system, including improvement of diagnostic methods as valuable tools in the accurate estimation of the prevalence and incidence especially regarding WNV, TOSV, and TBEV as important emerging arboviral causes of encephalitis. Depending on the regional/endemic situation of European countries or on special incidents, other less common arboviral agents causing encephalitis, like Tahyna virus (Rudolf et al., 2008); 'imported' arboviral agents causing encephalitis, usually with other clinical hallmarks, like chikungunya virus or dengue virus (Donoso Mantke et al., 2004; Lemmer et al., 2004; Donoso Mantke et al., 2007 a; Niedrig et al., 2009; Panning et al., 2009; Domingo et al., 2010); or arboviruses recently identified to be human-pathogenic, like Usutu virus (Cavrini et al., 2009; Pecorari et al., 2009), might be considered for surveillance activities, but will not be further attended to in this thesis.

Table 2 Factors contributing to emergent and re-emergent viral infections*

- Global climate change
- Changes in human demographics and behavior (e.g. increasing leisure activities in nature)
- Environmental changes, incl. heavy rains followed by flood, and rehabilitation of wetlands (increased incidence of mosquito-vector populations)
- Introduction of exotic agents by human and animal migration
- Poverty
- Economic development, land use, and technology
- Tourism and business travels (travel-associated importation of rare and severe infections)
- Global trade in wildlife
- Animal smuggling (especially of birds)
- Genetic mutations and/or recombinations producing dangerous strains
- Adaptation of animal viruses to human host
- Human suscepility to infection
- Breakdown of public health measures

* Selected factors contributing to the emergence and re-emergence of infectious diseases, incl. genetic, biological, and socio-economic factors (Morens et al., 2004; Pugliese et al., 2007 a).

I.2 Epidemiology of encephalitic arboviruses important for Europe

I.2.1 West Nile virus

WNV is a mosquito-transmitted flavivirus which belongs to the Japanese encephalitis serogroup of the family *Flaviviridae*. It is by far one of the most widespread

arboviruses (Hayes et al., 2005; Gyure, 2009; Reiter, 2010). Based on signature amino acid substitutions or deletions in their envelope proteins, WNV isolates are assigned into at least two main lineages which are about 30% divergent (Berthet et al., 1997; Lanciotti et al., 1999). Lineage I, which can produce disease in humans, horses, and birds, includes virus strains from Africa, the Middle East, Europe, India, Australia (clade: Kunjin), and the Americas. In contrast, lineage II contains strains historically isolated in sub-Saharan Africa and Madagascar, and recently in Hungary, Russia, and Austria (Campbell et al., 2002; Bakonyi et al., 2006; Erdélyi et al., 2007; Platonov et al., 2008). So far, this lineage has been considered as non-pathogenic in humans and horses. Nevertheless, recent data show that South African strains belonging to lineage II were able to induce severe clinical symptoms in both humans and horses (Venter et al., 2009). At least six lineages have been proposed for strains isolated in Europe, Russia, and India (Lvov et al., 2004; Bakonyi et al., 2005; Bondre et al., 2007; Kramer et al., 2008; Vazquez et al., 2010), and in addition a distinct genotype lineage I was identified in the United States (US), probably representing the dominant strain in North America (Davis et al., 2005; Snapinn et al., 2007). WNV is mainly maintained in a life cycle between birds, especially passerines (perching birds) and charadriiformes (wader- or gull-like birds) as the most competent amplifying hosts judged by magnitude and duration of viremia, and ornithophilic mosquitoes, especially of the genus *Culex* as the preferred vectors (Hubálek and Halouzka, 1999; Dauphin et al., 2004; Reiter, 2010). Humans and other mammals, especially horses, can develop clinical illness but usually are incidental or dead-end hosts because they do not produce significant viremia, and thus do not contribute to the transmission cycle (Fig. 1).

The mosquito vector species vary in their abundance in different geographic regions, in their tendency to feed on mammals, and in their efficiency to transmit infection. In Europe, four mosquito species are considered to be the dominant vector species, i.e. *Culex pipiens*, *Cx. torrentium*, *Cx. modestus*, and *Coquillettidia richardii* (Hubálek, 2008; Reiter, 2010). Occasional vectors are *Ochlerotatus cantans* and *Anopheles maculipennis*. While natural foci are usually situated in a bird–mosquito cycle in wetland ecosystems, certain tick species may play a subsidiary role in dry and warm habitats.

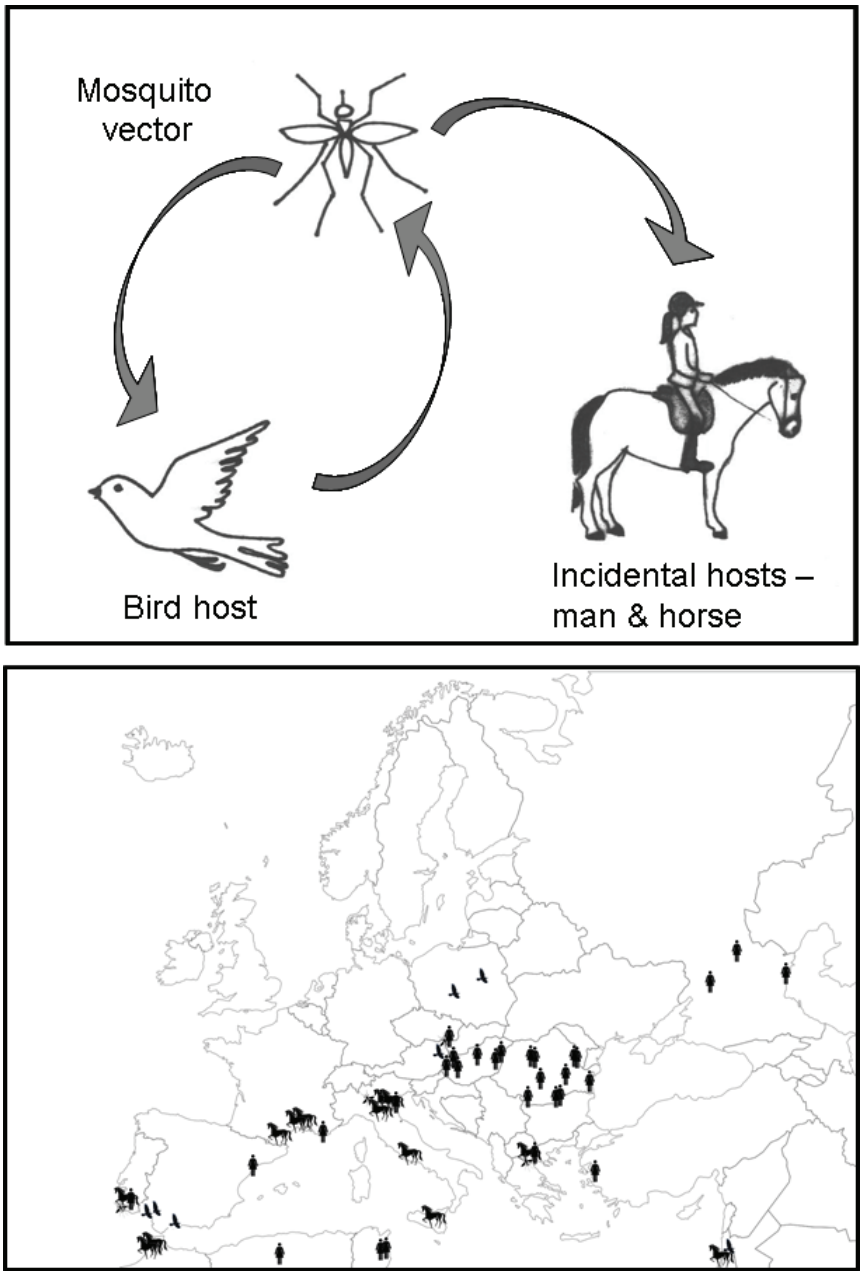

Figure 1 Natural transmission cycle and main geographical areas of WNV transmission

Upper panel: Mosquitoes acquire infection while feeding on a viremic bird host. The likelihood that a feeding mosquito will become infected increases as the level of viremia inside the amplifying host increases. The virus passes through the gut wall into the hemolymph of the insect, after which replication occurs in most of the internal tissues. Once the salivary glands are infected, the virus can pass to a new host via saliva injected into the skin when the mosquito takes the next blood meal. In nature, the virus cycles between mosquito vectors and bird hosts. It must survive the winter to initiate new annual cycles of infection (overwintering). Possible mechanisms of overwintering include survival in hibernating female mosquitoes, vertical transmission from infected females to their progeny, continued transmission in warmer latitudes, and chronic infections in migratory birds. Ornithophilic vectors that also bite and infect mammals, including humans, are termed 'bridge vectors'. Humans and horses are incidental hosts who can be infected by bridge vectors. Lower panel: Detection of WNV infection in different areas in Europe and in the Mediterranean basin (1962–present) has been done in: humans, birds, horses, and/or mosquitoes. There is further evidence of WNV transmission observed by past and recent serological surveys in humans, birds, or other animals in Albania, Eastern Bulgaria, Belarus, Ukraine, Moldavia, Eastern Croatia, and Poland.

Many species of wild birds might act as natural reservoir hosts for WNV. In Europe, this virus was isolated from *Ardeola ralloides*, *Plegadis falcinellus*, *Anas querquedula*, *Fulica atra*, *Tringa ochropus*, *Vanellus vanellus*, *Larus ridibundus*, *Streptopelia turtur*, *Corvus corone*, *Corvus frugilegus*, and *Sturnus vulgaris*, but the range of bird species might be much broader, as experimental and serological studies could show (Hubálek, 2008; Calistri et al., 2010; Reiter, 2010). Birds can be infected by a variety of routes other than mosquito bites (e.g. oral and fecal-oral infection), and different species may have different potential for virus maintenance and dispersion of the virus in nature. Migratory birds are considered to play an important role in the geographic introduction and spread of the virus.

WNV was first isolated in 1937 from the blood of a febrile woman who lived in the West Nile sub-region in Uganda (Smithburn et al., 1940). Since then, both sporadic cases in humans and horses as well as major outbreaks have been reported in many countries of the Old World (Bernkopf et al., 1953; Hubálek et al., 1999; Murgue et al., 2001 a; Murgue et al., 2002). This includes a series of WNV outbreaks that occurred in several countries in southern/eastern Europe, Russia and around the Mediterranean basin during the last three decades (Calistri et al., 2010). Many ecologic aspects of WNV infection, involving mosquitoes, birds, and humans, have been well documented since the early 1950s in Egypt (Taylor et al., 1956), in the 1960s in the Camargue, southern France (Joubert et al., 1970), and in the 1970s in South Africa (McIntosh et al., 1976). By far the largest outbreaks in Europe occurred in Bucharest, Romania, in 1996 (393 human cases; 17 deaths) and Volgograd, Russia, in 1999 (826 human cases; 40 deaths). Both outbreaks happened in urban areas and were associated with cellars flooded with sewage-polluted water in poorly maintained apartment blocks, resulting in optimal breeding sites for *Culex pipiens* (Tsai et al., 1998; Platonov et al., 2001). Despite the fact that WNV circulation has been known in Europe for many decades, awareness of the disease increased with the introduction of WNV to the US in 1999 (Nash et al., 2001) and its tremendous spread from the East to the West Coast within only five years (Debiasi and Tyler, 2006). To date, WNV is widely established in the Americas being the leading cause of arboviral encephalitis in the US with 12,447 cases of encephalitis and meningitis, and 1,185 deaths reported in humans (data: CDC, 1999–present). The majority of the strains responsible for the outbreaks in Europe and the US belong to lineage I and

are grouped into two different clusters which might have an important impact on the strain pathogenicity (Lanciotti et al., 2002; Charrel et al., 2003). Strains belonging to the European Mediterranean/Kenyan cluster are characterized by a moderate pathogenicity for horses and humans and limited or no pathogenicity for birds. In contrast, strains grouped in the Israeli/American cluster are characterized by high rates of avian deaths and, in the US, also by high rates of illness and death in humans and horses (Komar et al., 2001; Schuffenecker et al., 2005). Compared to the US situation, the incidence of WNV in Europe is poorly studied and the risk of a similar epidemic, although low, cannot be precisely estimated. However, following the large urban outbreaks in Bucharest and Volgograd, further evidence of WNV transmission has been identified in humans and/or horses in the last years: in the Czech Republic (1997), in France (2000, 2003, 2004, 2006), in Italy (1998, 2008–2009), in Hungary (2003–2008), in Romania (1997–2000, 2008–2009), in Spain (2004), and in Portugal (2004) (Hubálek et al., 1999; Ceianu et al., 2001; Murgue et al., 2001 b; Connell et al., 2004; Del Giudice et al., 2004; Jourdain et al., 2007; Kaptoul et al., 2007; Krisztalovics et al., 2008; Macini et al., 2008; Popovici et al., 2008; Alonso-Padilla et al., 2009; Rizzo et al., 2009; Sotelo et al., 2009; Angelini et al., 2010). Figure 1 shows the areas in Europe and in the Mediterranean basin where WNV transmission occurred since 1962. More recently, in 2009, human cases of WNV infection were reported from Hungary (7 cases), Romania (2 cases) and Italy (16 cases). In July 2010, Portugal reported a probable case of WNV infection which was the first (probable) case of WNV infection reported in the European Union (EU) in 2010 (ECDC, 2010). WNV-specific titers for IgM and IgG between two paired samples suggested a recent infection with WNV, but confirmatory tests by neutralization conducted in the Robert Koch Institute (RKI), Germany, were negative. Since July 2010, the WHO Regional Office for Europe, together with key partners – such as the European Centre for Disease Prevention and Control (ECDC), the ENIVD, and the EpiSouth network for communicable disease control in Southern Europe and Mediterranean countries – has been closely monitoring the regional situation of WNV due to a number of reports of neuroinvasive disease. As of early September 2010, over 600 cases of human WNV disease have been officially or unofficially reported from Greece, Hungary, Israel, Italy, Portugal, Romania, Russia, and Turkey. After the recorded outbreak of WNV infection in humans in Bucharest in 1996, this is the second largest outbreak of the disease in the EU. The current

outbreak shows very well that WNV circulation is a fact in several countries of the EU and beyond, with new areas of transmission identified in Romania and Italy, and for the first time in Greece and Turkey. Furthermore, lineage II WNV seemed to be involved in this outbreak, which commonly has not been identified in humans or horses in the EU.

I.2.2 Toscana virus

TOSV belongs to the genus Phlebovirus within the family *Bunyaviridae*. This genus currently comprises 68 antigenically distinct serotypes and contains the majority of known sandfly-borne viruses (Liu et al., 2003; Depaquit et al., 2010). Phleboviruses have been isolated in the Americas from sandflies belonging to the genus *Lutzomyia*, and in Africa, Southern Europe, and Central Asia mainly from *Phlebotomus* and also from *Sergentomyia* (Brett-Major and Claborn, 2009; Depaquit et al., 2010). However, mosquitoes and ticks can also transmit phleboviruses (Elliott et al., 2000; Chevalier et al., 2010; Stone, 2010). Eight phleboviruses (Alenquer virus, Candiru virus, Chagres virus, sandfly fever Naples virus, Punta Toro virus, Rift Valley fever virus, sandfly fever Sicilian virus, and sandfly fever Toscana virus) have been linked to diseases in humans of which in Europe only TOSV, sandfly fever Naples, and Sicilian viruses are of importance. While the last two ones cause a febrile illness, TOSV is characterized by its neurotropism and the clinical characteristics of meningitis, meningoencephalitis, and flu-like disease (Charrel et al., 2005). The sandfly fever viruses (SFV) are widely distributed in the Mediterranean basin by several phlebotomine sandfly species as their main vectors and reservoirs (*P. papatasi*, *P. perniciosus*, *P. ariasi*, *P. perfiliewi*, and *P. neglectus*). There is evidence of the presence of different viruses within the same sandfly population. As animal reservoirs have not been confirmed until today, it is suggested that these viruses are maintained in their arthropod vectors by transovarial transmission and that vertebrate hosts play little or no role in the maintenance of these pathogens in the natural life cycle (Tesh, 1988). This could be underlined by presence of TOSV in wild-caught phlebotomine males (which do not take blood meals), demonstration of vertical transmission under laboratory conditions, and detection of venereal transmission from infected males to uninfected females (Tesh and Modi, 1984; Ciufolini et al., 1989; Tesh et al., 1992). However, experimental evidence suggests that an

amplifying vertebrate host is needed to sustain the viruses in the arthropod populations (Ciufolini et al., 1985; Tesh and Modi, 1987; Ciufolini et al., 1989).

In 2008, the 100th anniversary was celebrated of an Austrian expedition sent to characterize 'Pappatacifieber' in the Balkans (Doerr and Taussig, 1909; Flamm, 2008). Even though pappataci fever (also known as three-day fever, sandfly fever, dengue Mediterranean, evil dog "Hundskrankheit", and phlebotomus fever) was first described clinically by Pick in 1886, and the first manuscript on phlebotomine sandflies by Pym dates back to 1804, Doerr and his colleagues understood much of what is known today about sandfly fever (Dionisio et al., 2003). Interest in this disease increased and virus isolation (SFV Naples and Sicilian) happened during World War II as large numbers of troops serving in the Mediterranean basin suffered a flu-like, incapacitating illness well known to the locals (Sabin et al., 1944). Seroepidemiological studies have shown that the prevalence of antibodies against SFV Naples and Sicilian is more than 50%, especially in Mediterranean countries (Tesh et al., 1976; Nicoletti et al., 1996). However, there are only a few reports of clinical cases among the native population and some virus isolation from sandflies, and human cases have often been found in people visiting Mediterranean countries (Filipe, 1974; Saidi et al., 1977; Niklasson and Eitrem, 1985; Darwish et al., 1987; Eitrem et al., 1991 a; Eitrem et al., 1991 b; Cohen et al., 1999; Batieha et al., 2000; Mehrabi Tavana, 2001; Dionisio et al., 2003; Papa et al., 2006; Konstantinou et al., 2007; Izri et al., 2008). Recent research interest in sandfly fever has increased because of outbreaks of acute meningitis/meningoencephalitis in several European countries bordering the Mediterranean Sea and cases of severe neurologic disease in European travelers linked to TOSV (Mendoza-Montero et al., 1998; Valassina et al., 2000; Dionisio et al., 2001; Echevarría et al., 2003; Hemmersbach-Miller et al., 2004; Navarro et al., 2004; Charrel et al., 2005; Defuentes et al., 2005; Di Nicuolo et al., 2005; Kuhn et al., 2005; Peyrefitte et al., 2005; Sanbonmatsu-Gámez et al., 2005; Charrel et al., 2007; De Lamballerie et al., 2007; Santos et al., 2007; Sanbonmatsu-Gámez et al., 2009; Sonderegger et al., 2009; Gabriel et al., 2010). Based on these studies and case reports, TOSV is circulating not only in Italy (where it was first isolated in 1971) but also in Portugal, Spain, France, Greece, Cyprus, and Turkey. Furthermore, in Italy (Francisci et al., 2003; Valassina et al., 2003; Pugliese et al., 2007 b), in southeastern France (De Lamballerie et al., 2007), and in Spain (Mendoza-Montero et al., 1998; Echevarría et al., 2003; Sanbonmatsu-Gámez et al.,

2005) seroprevalence studies were conducted, with prevalences ranging from 3% (in Northern Italy) up to 26% (in Southern Spain). TOSV must be considered as an emerging pathogen in the Mediterranean basin and therefore a significant public health issue in Europe (Charrel et al., 2005). Current data regarding the distribution of sandfly fever viruses and their main vectors in the EU and neighboring countries around the Mediterranean Sea can be obtained from the VBORNET-project of the ECDC. Due to the fact that sandflies are typical Mediterranean faunal elements that do not spread widely, any occurrence of phlebovirus infections was restricted to southern parts of Europe until recently. Since 1999, sandflies have been found in Central Europe north of the Alps, and so the introduction and establishment of certain sandfly-borne pathogens like TOSV or SFV Naples and Sicilian in biological cycles inside temperate regions can no longer be excluded (Aspöck et al., 2008; Depaquit et al., 2010). Finally, new phleboviruses of unclear pathogenicity, such as the Granada virus, that circulate among phlebotomine sandflies may cause infections in Europe (Collao et al., 2010), and highly pathogenic phleboviruses like Rift Valley fever virus may be introduced to Europe (Chevalier et al., 2010).

I.2.3 Tick-borne encephalitis virus

TBEV is a medically important member of the genus Flavivirus, family *Flaviviridae* (Thiel et al., 2005). It is classified as one species with three subtypes, namely the European subtype, the Siberian subtype, and the Far Eastern subtype, which are associated with varying degrees of disease severity (Růžek et al., 2010). Besides TBEV, three other tick-borne flaviviruses, i.e. Louping ill virus, Langat virus, and Powassan virus, also trigger encephalitis in mammals, but these infections occur infrequently and the viruses do not cause significant outbreaks (Gritsun et al., 2003 a; Charrel et al., 2004; Lindquist and Vapalahti, 2008; Mansfield et al., 2009; Dobler, 2010). The principal vector as well as reservoir of the European TBEV subtype is *Ixodes ricinus*, and *I. persulcatus* for the other two subtypes (Rampas and Gallia, 1949; Gritsun et al., 2003 b; Süss, 2003; Golovljova et al., 2004). Although the virus has been isolated from several other tick species (Gresíková and Noseck, 1966; Křivanec et al., 1988; Zlobin and Gorin, 1996; Gresíková and Kaluzová, 1997; Gritsun et al., 2003 a), in nature only the two ixodid tick species mentioned appear to play an important role in virus maintenance and contribute significantly to the epidemiology of human disease (Pavlovskij, 1939; Labuda and Randolph, 1999).

Horizontal TBEV transmission between ticks and their vertebrate reservoir hosts is necessary for virus endemism (Nuttall and Labuda, 2003). The duration of viremia in hosts is crucial for TBEV transmission to ticks, because the virus is mostly ingested by ticks at the moment while engorging on a viremic host. Generally, the hosts are divided into three groups: reservoir, indicator, and accidental hosts (Charrel et al., 2004). Natural reservoir hosts of TBEV, i.e. animals that are sensitive to the virus, exhibiting viremia for long periods of time without becoming clinically ill and thus important for the transmission of the virus to ticks, include rodents (*Clethrionomys*, *Apodemus*, *Mus*, *Microtus*, *Micromys*, *Pitymys*, *Arvicola*, *Glis*, *Sciurus*, and *Citellus*) (Kozuch et al., 1967), insectivores (*Sorex*, *Talpa*, *Erinaceus*) (Kozuch et al., 1967), and carnivores (*Vulpes*, *Mustela*) (Karabatsos, 1985; Süss, 2003). Insectivores and rodents also harbor the virus during the winter. Indicator hosts are unable to transmit the virus to other vectors, either due to only brief viremia with low virus production, or due to lack of necessary cell-based mechanisms to support non-viremic transmission during co-feeding (Labuda et al., 1996). Accidental hosts of TBEV are humans who can be infected by a bite of an infected tick, or by consumption of non-pasteurized milk from viremic livestock, as well as large animals such as goats, cows, sheep, roe deer, dogs, and swine. They can develop a disease with viremia, but they do not participate in virus circulation in nature and are, therefore, a dead end of the natural TBEV cycle (Fig. 2). In addition, seroprevalence in these large vertebrates may represent an indirect means of measuring the intensity of TBEV transmission within a geographical region and make them valuable sentinels for epidemiological risk assessment (Gritsun et al., 2003 a; Donoso Mantke et al., 2008 b).

Although the first hints of the existence of the disease date back to Scandinavian church records from the 18th century (Åland islands, Finland), what is known today as tick-borne encephalitis (TBE) was first recognized and medically described by the Austrian physician H. Schneider in 1931 as ‘meningitis serosa epidemica’ of unknown etiology (Schneider, 1931). A disease with similar clinical symptoms had been observed in the Far East since 1914, but has occurred more frequently since 1933, and shortly thereafter – during three successive expeditions in 1937–1939 – the etiologic agent was isolated in Russia and its transmission by ticks could also be demonstrated (Zilber, 1939). The disease was called ‘Russian spring–summer

encephalitis' (or Far East or taiga encephalitis), and the virus became known as Russian spring–summer encephalitis virus and lately TBEV. In Finland, TBE was initially described as 'Kumlinge disease' in the 1940s (Oker-Blom, 1956), and the first European TBEV was isolated in Czechoslovakia after the Second World War in 1948 (Gallia et al., 1949; Krejčí, 1949 a), when the incidence of clinical manifestations caused by the virus was so high that it was noticed by infectiologists in affected regions (Krejčí, 1949 b). Simultaneously, the virus was also isolated from *I. ricinus,* suggesting the role of the tick as a vector of the disease (Rampas and Gallia, 1949).

Today, the distribution of TBEV correlates with the ixodid tick vectors' occurrence (Fig. 2). *I. ricinus* occurs in most parts of Europe, and the distribution extends to the southeast (Turkey, Northern Iran, and Caucasus) (Nuttall and Labuda, 1998). *I. persulcatus* is seen in the vast area extending from Eastern Europe to China and Japan (Jaenson et al., 1994). Parallel occurrence of both tick species was reported in northeastern Europe and the east of Estonia and Latvia, as well as in several European regions of Russia (Haglund et al., 2003; Bormane et al., 2004; Golovljova et al., 2004; Jääskeläinen et al., 2006 a). The prevalence of TBEV-infected *I. ricinus* ticks varies from 0.5% to 5%, whereas in *I. persulcatus* in certain regions of Russia prevalence up to 40% was recorded (Charrel et al., 2004). It should be noted that methods for measuring virus prevalence in ticks or animal reservoirs have not been standardized, and reliable tools should be introduced to translate epizootic prevalence data into infection risk for humans. However, TBE occurs in many parts of Central Europe and Scandinavia, particularly in Austria, the Czech Republic, Estonia, Finland, Germany, Hungary, Latvia, Lithuania, Poland, Russia, the Slovak Republic, Slovenia, Sweden, Switzerland, and also Northern Asia (Bröker, 2008; Donoso Mantke et al., 2008 b; Lu et al., 2008; Süss, 2008).

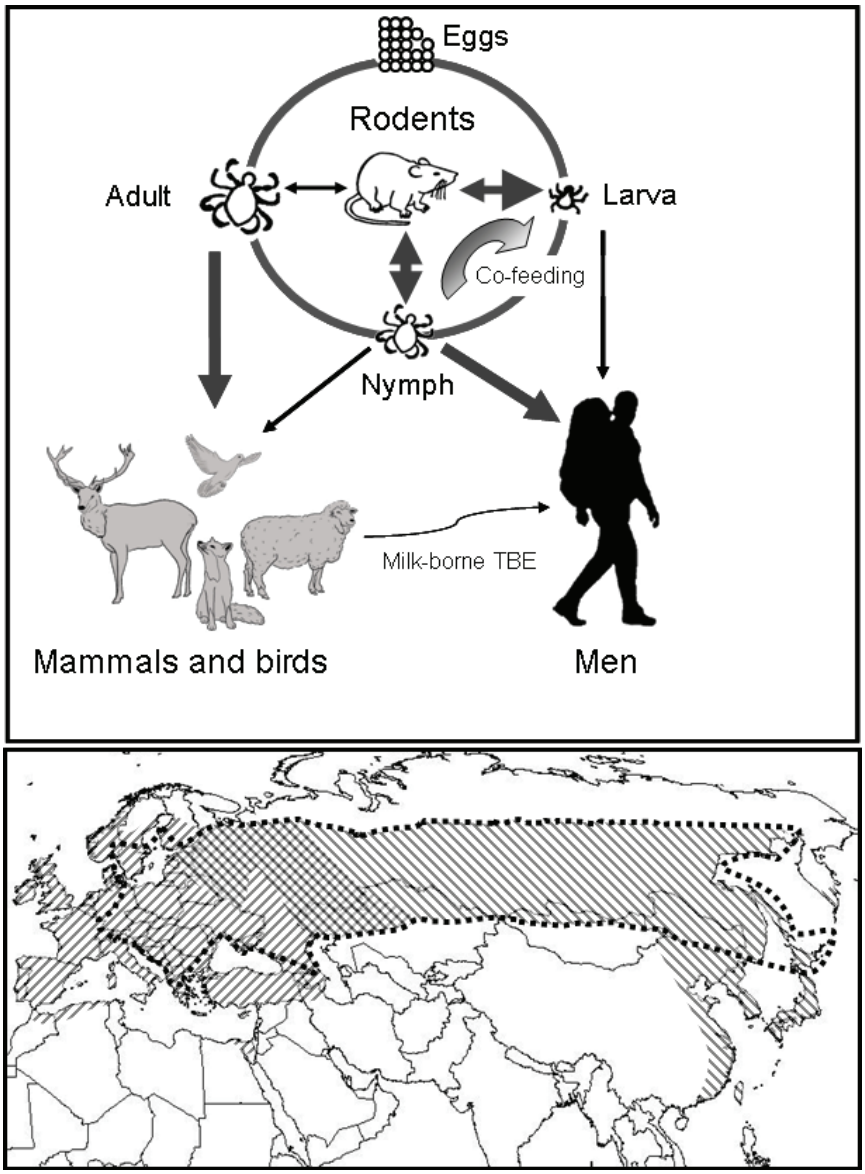

Figure 2 Life cycle and distribution of ixodid ticks and TBEV

Upper panel: The life cycle of ixodid ticks consists of three developmental stages (larva, nymph, and adult). Each stage needs to take a blood meal on a suitable vertebrate host, usually for a period of a few days, to develop into the next stage. Further, adult female ticks need a blood meal for egg production. Each stage of *I. ricinus* takes approximately one year to develop into the next stage. Thus, it takes 3 years on average for the completion of the shortest life cycle. TBEV infects ticks chronically for the duration of their life while feeding on infected animal reservoir hosts. It can be transmitted transstadially (from one stage to a subsequent one), transovarially, or primarily from nymphs to naïve larvae while co-feeding on the same rodent host. TBEV can be transmitted to man or other animal hosts by all three tick stages with different prevalence. Lower panel: Different shadings show the western distribution of *I. ricinus* and eastern distribution of *I. persulcatus*. The distribution of both vectors overlaps in the checkered area. The dotted line shows the border of the TBE-endemic area. Note that TBE is distributed in an endemic pattern of so-called natural foci. The distribution of ixodid ticks in China is uncertain and, therefore, not shown in detail.

The number of human cases of TBE in all endemic regions of Europe has increased by almost 400% in the last 30 years. In 2006, 3,914 cases were reported in Europe when excluding Russia (7,424 cases when including Russia), the highest number since 1995 (Kunze et al., 2010). It is important to note that fluctuations are a typical phenomenon in the TBE epidemiology which depend on various factors, e.g. weather and climate, rodent prevalence, or human behavior. Furthermore, new TBE foci are emerging and latent ones re-emerging in several European countries (Bröker and Gniel, 2003; Donoso Mantke et al., 2008 b; D'Agaro et al., 2009; Fomsgaard et al., 2009; Ergünay et al., 2010). In Russia, the highest TBE incidence is reported in Western Siberia and Ural (Gresíková and Kaluzová, 1997). No TBE cases have been reported in e.g. Great Britain, Ireland, Iceland, Belgium, the Netherlands, Luxemburg, Spain, and Portugal. In contrast, Bulgaria, Croatia, Denmark, France, Greece, Italy, Norway, Romania, Serbia, China, and Japan are countries with only sporadic TBE occurrence. Because of the increased mobility of people travelling to risk areas, TBE has become an international public health problem with relation to travel medicine. The risk of an infection is especially high for people living in endemic areas or visiting them for leisure activities in nature (Bröker and Gniel, 2003). Recently, the ECDC launched an internet-based spotlight on tick-borne diseases including fact sheets and relevant information on TBE.

I.3 Diagnostics of arboviruses

I.3.1 Clinical diagnosis

After suspecting viral encephalitis, the approach to clarify the etiology of the disease should consist of obtaining a meticulous history of the patient and of a careful general and neurological examination (Steiner et al., 2010). Early diagnosis is vital, as symptoms can appear suddenly and escalate to brain damage, hearing and/or speech loss, blindness, or even death. The history is mandatory in the assessment of the patient. Therefore, it could be important to obtain relevant information from an accompanying person (relative, friend, etc.) if the patient is in a confused, agitated, or disoriented state. To consider an arboviral etiology, the patient’s history should include questions related to the geographical location of exposure and recent travel history (to identify endemic/regionally prevalent pathogens); seasonal occurrence

(summer–fall, rainy season); occupation and outdoor activities (forestry worker, farmer, camping, hiking, etc.); contact with wild or domestic animals (e.g. birds' feces); history of arthropod bite (e.g. tick bite); and mode of the course of disease up to the appearance of the neurological signs (like bleeding tendency outside the CNS, indicating viral hemorrhagic fever). Viral infection of the CNS is almost always part of a generalized systemic infectious disease. Thus, other organs may be involved prior to or in association with the CNS manifestations, as described e.g. for TBE and West Nile disease (Misić-Majerus et al., 2005; Gyure, 2009). A neurological examination involves a series of tests designed to assess motor and sensory function, nerve function, hearing and speech, vision, coordination and balance, mental status, and changes in mood or behavior. Neuroimaging can reveal signs of brain inflammation, internal bleeding or hemorrhage, or other brain abnormalities. The findings relate to those of meningitis and disruption of brain parenchyma function. An electroencephalogram (EEG) may help demonstrate cerebral involvement during the early stage of disease. Only in rare instances does the EEG show specific features that may give clues to the diagnosis (e.g. HSE). Magnetic resonance imaging (MRI) is a more sensitive and specific application than computerized tomography (CT) when evaluating viral encephalitis. However, in practice these methods are of little help in the differential diagnosis.

I.3.2 Laboratory diagnosis

Examination of peripheral blood and/or CSF (incl. cell count and cellular morphology) gives a first conclusive clue to the nature of disease. A lymphocytosis in peripheral blood or lymphocytic pleocytosis in CSF with elevated protein and normal glucose levels are non-specific findings in viral encephalitis, and separate from other infective non-viral forms of meningitis or encephalitis (Debiasi and Tyler, 2004; Steiner et al., 2010). Ultimately, in a patient with suspected viral encephalitis the core diagnostics of choice is to obtain serum and CSF for virological tests. Diagnostic testing for arboviruses is mainly limited to patients who are suspected to be infected by these viruses based on the patients' history. Different measures for virus diagnosis can be initiated to confirm the clinical diagnosis. During acute infection, it is only possible to directly detect the viral pathogen itself. This can be achieved only with a limited

number of diagnostic assays focusing either on the detection of virus-specific proteins or the virus genome (Fig. 3).

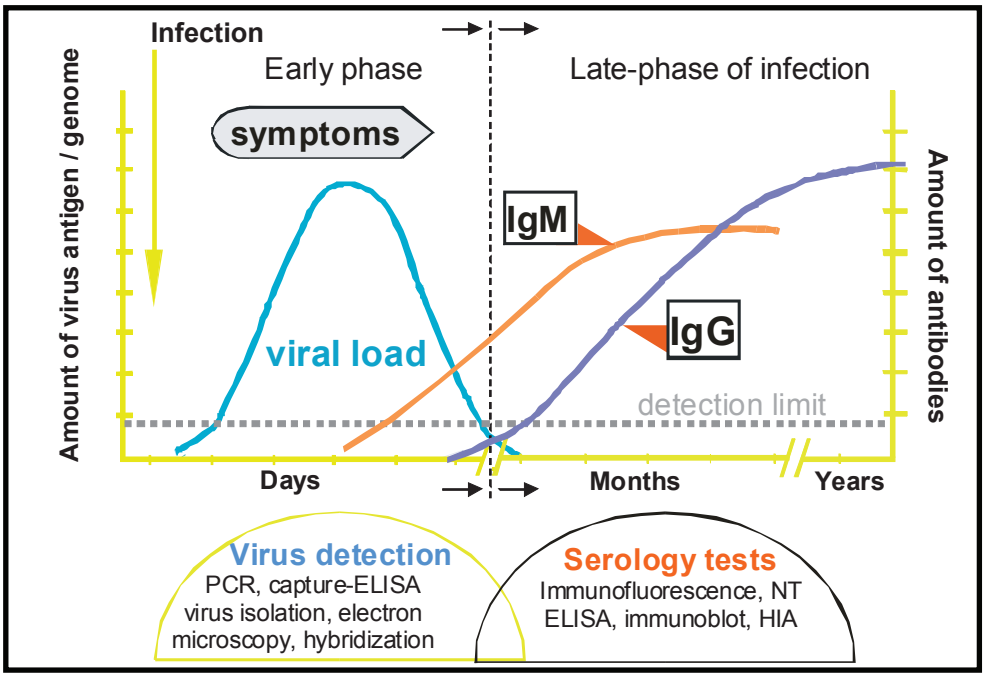

Figure 3 Schema for diagnosis of viral infections

For particle detection by electron microscopy, virus loads have to exceed 10^6/mL, which is largely dependent on the virus, the course of infection, and the time point of investigation. Table 3 gives a brief overview on the sensitivity and specificity of the different diagnostic methods. The estimated time required for the different diagnostic assays also gives important information on the practicality of these methods in the acutely infected patient.

Table 3 Basic features of different diagnostic methods*

Method	Time for diagnosis	Sensitivity	Specificity
Virus detection			
Virus isolation	1-7 days	High[a]	High[b]
Hybridization	3-4 hr	High[c]	Good
PCR	2-4 hr	High[d]	High
Pyrosequencing	1 hr	High[e]	High
Electron microscopy	30 min	Low[f]	High[g]
Capture ELISA	3-5 hr	Good[h]	High
Serology			
ELISA	3-4 hr	High	Low
Immunofluorescence	2-4 hr	Good	Good
Immunoblot	2-4 hr	Good	Good
Neutralization	4-7 days	Good	High
HIA	2-4 hr	Low	Good

* Adapted from Niedrig et al., book chapter in press.
[a] Depending on cultivation system
[b] Depending on detection system
[c] ca. 10^4 particle/mL
[d] ca. 200 genome equivalent/mL
[e] Requires PCR
[f] ≥10^6 particle/mL
[g] Detection of the virus family
[h] ca. 0.01 µg antigen/mL

Because of the low commercial impact of most of the arboviruses, there are no specific assays for protein detection, but some assays are available as in-house assays in only a few laboratories (Table 4). Some assays may involve immunohistochemical staining of tissue samples with monoclonal antibodies directed against the suspected virus (Bae et al., 2005). Other assays applied for the detection of virus antigens are specific capture enzyme immunoassays (EIA), but these are rather uncommon and not very sensitive. Owing to the unique genetic code of RNA and DNA, the detection of virus-specific sequences by PCR methods has been the major technology for diagnostic testing for many years. In general, PCR approaches are very rapid, enable extremely low detection limits, and provide genetic material for further characterization (e.g. by sequencing). On the other hand, the high specificity due to the exact base pairing of primer and template may lead to false-negative

results even after minor changes in the target sequence. Since flaviviruses are prone to frequent sequence aberrations the PCR assays used should be selected carefully. Finally, the exact target virus sequences have to be available for the proper design of reliable assays, a prerequisite that is hard to achieve for flaviviruses. However, nearly all recent cases of acute viral hemorrhagic fevers imported to the Western World were diagnosed by PCR, before a confirmation by virus cultivation was performed successfully. Virus cultivation on suitable cells is an additional option for virus detection in an acute phase of disease. Cultivation is laborious and can take one to seven days depending on the susceptible cells used and the amount of virus in the patient's serum. However, a specific method for the unequivocal identification of samples showing even typical cytopathic effects is essential. Since nearly all of these viruses (except for the sandfly fever viruses) are highly pathogenic and have to be handled in a high containment laboratory, virus cultivation is limited to a few highly experienced facilities.

The introduction of WNV into North America in 1999 as a new pathogen caused serious problems, including the initial misdiagnosis as St. Louis encephalitis (SLE). Up to then SLE had been a common infection in the US, caused by SLEV, a virus also belonging to the *Flaviviridae* with high potential for cross-reactivity in other serological flavivirus assays. It turned out very soon that WNV easily spreads by infected migrating birds and can also occasionally be transmitted to humans by infected mosquito vectors. This newly emerging infection made it necessary to develop diagnostic tools for detecting WNV in acute encephalitis patients and for providing safe blood and plasma for medical care. Accidental transmission by transplanting organs of an infected donor clearly showed that reliable diagnostic assays are required to meet established biological safety standards (Iwamoto et al., 2003). Tremendous efforts were made by introducing routine screening of blood and blood products by EIA and RT-PCR to avoid any further transmission. Such a routine testing is always based on evaluated and standardized diagnostic assays. However, the use of certified EIAs does not overcome the general problem of cross-reactivity among the flaviviruses. As found in evaluation studies for different commercially available EIAs for WNV-specific IgG, all of them also showed positive reactivity with anti-YF and anti-TBE sera (Malan et al., 2004; Pfleiderer et al., 2006; Linke et al., 2008). Even when applying the plaque reduction neutralization technique usually

providing the highest specificity, certain high-titer sera displaying anti-YF and/or anti-TBE reactivity showed cross-neutralization activity to WNV as well.

Table 4 Overview of the most used diagnostic methods for selected arbovirus infections*

Viral pathogen	Diagnostic		
	Acute/early phase	Acute/late phase	Convalescent phase
Dengue virus 1-4	PCR[a,b], VI[b], IH, EIA[a] (NS-1)	IgM EIA[a], IFA[a], HIA, rapid tests	IgM/IgG EIA[a], IgM/IgG IFA[a]
Yellow fever virus	PCR[b], VI[b], IH	IgM EIA , IFA[a]	IgM/IgG EIA, IgM/IgG IFA[a]
West Nile virus	PCR[a,b], VI[b], IH	IgM EIA[a], IgM IFA[a], HIA	IgM/IgG EIA[a], IgM/IgG IFA[a], HIA
Tick-borne encephalitis virus	PCR[b], VI[b], IH	IgM EIA[a], IFA[a], HIA	IgM/IgG EIA[a], IgM/IgG IFA[a], HIA
Chikungunya virus	PCR[b], VI[b], IH	IgM/IgG EIA, IgM IFA[a], HIA	IgM/IgG IFA[a], HIA
Sandfly fever virus	PCR[a,b], VI[b]	IB[a], IgM IFA[a]	IB[a], IgM IFA[a], IgM/IgG EIA[a]
Crimean Congo hemorrhagic fever virus	PCR[b], VI[b]	IgM/IgG EIA[a], IgM IFA	IgM/IgG EIA, IgG IFA
Rift Valley fever virus	PCR[b], VI[b]	IgM/IgG EIA[a], IgM IFA, HIA, inhibition EIA	IgM/IgG EIA, IgG IFA, HIA

* Adapted from Niedrig et al., book chapter in press.
[a] Commercial assays available.
[b] Methods useful for analysis of enviromental or vector (mosquitoes, ticks, phlebotomes) samples.

Abbreviations:
EIA- Enzyme immunoassay; HIA- Hemagglutination inhibition assay; IB- immunoblot; IFA- immunofluorescence assay; IH- Immunohistological analysis of tissue samples; PCR- Polymerase chain reaction; VI- Virus isolation on cells.

In an external quality assurance (EQA) for the serological detection of WNV-positive sera, it turned out that only 8/27 (29.6%) of the participating laboratories reached the proficiency criteria for correct IgM and IgG detection. These data demonstrate the challenge to achieve a reliable serological diagnosis of WNV infection (Niedrig et al., 2007 a). To overcome these cross-reactivity problems, the analysis of antibody avidity might be a rapid and simple option. As shown for differentiation of primary from previous WNV infection, the IgG avidity assay provides an additional diagnostic

certainty (Levett et al., 2005). This also needs to be evaluated for cross-reactivity with other flavivirus-reactive sera. A reverse ELISA based on the B domain of the E-protein seems to be more specific (Ludolfs et al., 2007). With this assay it was possible to avoid the frequent cross-reaction caused by sera directed against other flaviviruses. For the diagnosis of WNV by PCR, several assays have been published (Lanciotti and Kerst, 2001; Linke et al., 2007). By epidemiological studies it could be shown that the two lineages found in Africa have a very distinct pattern of distribution (Campbell et al., 2002). In the US, due to the exclusive introduction of one virus strain, only variants of lineage 1 originally deriving from an Israeli prototype are present (Lanciotti et al., 1999; Hayes, 2001). The introduction of WNV lineage 2 into Hungary, Russia, Austria, and Greece was demonstrated recently (Bakonyi et al., 2006; Erdélyi et al., 2007; Platonov et al., 2008; ECDC, 2010). This information has immediate consequences for the design of WNV-specific RT-PCR systems to be used in Europe, which should react with sequences of both lineages. As shown in an EQA, only 11/30 (36.7%) laboratories were able to detect lineage 2 in high concentration, which demonstrates the necessity for improving the other assays by adaptation of primers to lineage 2 of WNV (Niedrig et al., 2006).

The SFV transmitted by phlebotomine flies is widely distributed in all countries of the Mediterranean region. The sandfly fever group comprises several different strains named after the regions where they were first isolated during local outbreaks: Toscana, Naples, Sicily, Corfu, and Cyprus (Xu et al., 2007). While the sandfly fever normally takes a very mild and aseptic course, meningitis and meningoencephalitis are rare complications due to TOSV. The number of reported infections is rather low (De Lamballerie et al., 2007). However, in recent years several studies demonstrated the presence of TOSV in Italy, Portugal, Spain, France, Greece, Cyprus, and Turkey, thus underlining the importance of the infectious agent (Hemmersbach-Miller et al., 2004; Charrel et al., 2005; Sanbonmatsu-Gámez et al., 2005; Santos et al., 2007). Most of the diagnoses were performed by in-house assays, although commercial assays for serology such as EIA, IFA, and IB are available (see Table 4). The recently developed RT-PCRs for virus genome detection support the commercially available nested PCR assay and can be used for screening of phlebotomine flies as well as for analysis of patient specimens in acute infection (Pérez-Ruiz et al., 2007; Weidmann et al., 2008). The introduction of new and more sensitive PCR-based

tools will help improve the diagnosis of viral meningitis patients in sandfly-endemic areas. Seroneutralization assays using early convalescent sera remain the reference method used to specifically identify the sandfly fever viruses or to assess the antibody response specificity. Reference tools, reagents, and quality control are not widely available. However, the ENIVD is able to provide some of these reagents.

TBE is the most important flavivirus infection in Europe and Russia, transmitted by infected ixodid ticks. The highest incidence is found in the area of Kemerovo in Siberia but ticks are also present in Central and Eastern Europe, with high prevalences causing a large number of encephalitis cases (Charrel et al., 2004; Korenberg and Likhacheva, 2006). The biphasic course of the disease caused by European strains starts with uncharacteristic influenza-like symptoms, followed by a symptom-free interval before a second phase with neurological symptoms develops in about 20–30% of the patients, with an occasionally fatal outcome (<2%) (Heyman et al., 2010). Very often the patients do not recognize or remember a tick bite which results in a late diagnosis for the patient. Serology is the most frequently used diagnostic approach in suspected cases, although some retrospective studies have shown that the TBEV could be detected by PCR in the early phase of the disease (Saksida et al., 2005; Donoso Mantke et al., 2007 b; Schultze et al., 2007). In the majority of cases, the first-line diagnosis is performed by testing for IgM using commercial TBE EIAs available from different manufacturers with reasonable quality. Although these EIAs cannot overcome the problem of serological cross-reactivity between the different flaviviruses, their quality improved after an extensive assay evaluation some years ago (Niedrig et al., 2001). In an EQA study for TBE serology, it turned out that correct results were obtained for at least 90% of the samples by 33/40 (82.5%) participating laboratories for IgM and 16/42 (38.1%) laboratories for IgG, with testing often based on commercial EIAs (Niedrig et al., 2007 b). In contrast, it was shown that only 35–44% of the laboratories correctly detected the Far Eastern and/or the Siberian TBEV subtypes with PCR (Donoso Mantke et al., 2007 c), despite the fact that these subtypes are already present in northeastern Europe and the Baltic countries (Golovljova et al., 2004; Jääskeläinen et al., 2006 a; Donoso Mantke et al., 2008 b). For the first time Růžek et al. have developed a multiplex RT-PCR that is able to discriminate among all three TBEV subtypes (Růžek et al., 2007). Recently, a further development of molecular diagnostic assays for TBEV now brings

together the advantages of quantitative real-time PCR (improved rapidity, sensitivity, reproducibility, reduced risk of cross-contamination) and the advantage of multiplex PCR (discrimination of different amplicons within a single reaction). This new PCR method is based on highly degenerated primers and probe targeting the NS1 protein region in a one-step assay and is followed by pyrosequencing (Achazi et al., 2010). Pyrosequencing is a new technique in which the enzymatic incorporation of the four different nucleotides, which are added separately and are complementary to the sequenced DNA strand, is monitored in real-time. For each incorporated nucleotide, a clear light signal is generated and presented as a peak histogram, called a pyrogram. Pyrosequencing has its strength in the sequencing of fragments of up to 80 bases and the identification of single nucleotide polymorphisms (SNPs). It can be speculated that with the increasing requirement of diagnosing suspected acute TBEV infections, rapid diagnosis by PCR will become more important for the analysis of serum and/or liquor specimens. Unfortunately, because of the short viremia, a negative PCR result does not rule out a TBEV infection with certainty (Donoso Mantke et al., 2007 b). One argument for physicians not requesting PCR testing is that no specific medical treatment is available for TBE patients. However, this will hopefully change. Nevertheless, often other medical products such as antibiotics are given which might additionally be harmful for the patient.

I.4 Surveillance of arbovirus encephalitis

Despite improvements in the diagnosis of viral encephalitis, including CSF-PCR (Debiasi and Tyler, 2004; Romero and Newland, 2006; Steiner et al., 2010), the etiology of up to 85% of acute encephalitis cases has remained unknown in recent epidemiological surveys (Granerod and Crowcroft, 2007; Donoso Mantke et al., 2008 a; Jmor et al., 2008; Mailles and Stahl, 2009). This issue is challenging, especially when considering the early detection of new and (re-) emerging pathogens such as WNV, TOSV, and TBEV (Pugliese et al., 2007 a; Vorou et al., 2007; Depaquit et al., 2010; ECDC, 2010; Kunze et al., 2010), or potential outbreaks caused by a deliberate release of pathogens (Bossi et al., 2004). An accurate etiological diagnosis is important for surveillance activities aimed at clarifying the etiological pattern of viral encephalitis/meningitis. However, this is impossible to achieve as long as routine

clinical/laboratory investigations do not include the most common pathogens in a standardized manner. Moreover, a correct (differentiated) immediate diagnosis and the introduction of symptomatic or specific therapy may be of vital importance for the survival of patients and may reduce the extent of brain injury.

Based on certain ENIVD activities and several European studies, data were obtained on the surveillance situation for viral encephalitis and meningitis in Europe, as well as on the possible etiologies in order to clarify the incidence and causes of viral encephalitis/meningitis at the national level (Donoso Mantke et al., 2008 a). It could be shown that comparative data for the incidence of most viral agents of human (meningo-) encephalitis are missing. In the presented studies, the proportion of cases with unknown etiology ranged between 30% and 80%. The reasons for unknown diagnosis could be traced to either a failure of the diagnostic tests or an inappropriate case definition, resulting in under-ascertainment of both known viruses and “new” viruses. Furthermore, the surveillance of emerging zoonotic diseases in European countries is not uniform. For example, although endemic in several countries, TBEV surveillance is not standardized concerning case definitions, laboratory diagnostic practice, and risk mapping, nor always mandatory in Europe (Donoso Mantke et al., 2008 b). In order to increase the benefit of surveillance of acute encephalitis in Europe, it was developed that a broad standard case definition and harmonized/standardized diagnostic algorithm, using a multiplex-microarray system validated for a wide range of viruses, may help to discover the true incidence and etiological pattern of viral encephalitis/meningitis within each country. Currently it is still expensive to use microarrays that enable the detection of several microbes’ nucleic acids simultaneously, but they have the potential to become a useful diagnostic technique (Jokela et al., 2005; Jääskeläinen et al., 2006 b). This would offer a unique analysis platform for all participating laboratories in a European surveillance system for viral encephalitis/meningitis by including both the more common pathogens of viral CNS diseases (e.g. herpes simplex-, varicella-zoster-, enteroviruses) and relevant viral zoonotic agents (e.g. TBEV, WNV, rabies virus) according to the regional/endemic situation of the European countries or on special request. The proposed basis for a future surveillance system at the European level would guarantee high performance and comparability of the results consistent with EQA programs. The ENIVD has a huge expertise in organizing EQA studies for the

diagnostics of arboviruses, and has pointed out the importance of quality control/standardization of laboratory procedures for the detection, surveillance, and control of viral infections (Donoso Mantke et al., 2005; Niedrig et al., 2007 c). To improve surveillance, it is also important to quantify the extent of cases of unknown etiology in order to allow a comparison of the data from each country and to identify possible weaknesses in the surveillance data. Therefore, clinicians must be motivated to report all cases of viral encephalitis/meningitis and to reach a definitive etiological diagnosis. Some clinicians may not see confirmation as a priority, especially if in the clinical practice all suspected cases of acute encephalitis are routinely treated with acyclovir.

Finally, the surveillance of clinical cases should be further backed up by vector surveillance (virological investigation of mosquitoes, ticks, flies, etc.) and indicator host surveillance (virological investigation/seroepidemiological surveys of humans, birds, horses, rodents, etc.) in order to expand and regularly update the knowledge about the circulation of certain arboviruses. This would facilitate the timely assessment of the risk for the exposed population and the application of public health measures in an optimal way. So far, such surveillance activities are not fully implemented in national surveillance systems for communicable diseases. And furthermore, methods to measure virus prevalence in vectors and animal hosts have not been standardized, and reliable tools should be introduced to translate epizootic prevalence data into infection risk for humans. A multidisciplinary approach is required involving professionals trained in human medicine, veterinary medicine, epidemiology, microbiology, parasitology, entomology, wildlife biology, and similar disciplines (Dufour et al., 2008; Stark et al., 2009).

As one of the activities of the German Consultant Laboratory for Tick-borne encephalitis and further flaviviruses, we are working on the implementation of alternative indicators for risk assessment of tick-borne diseases in the national surveillance system. Therefore, in a pilot study we collected larvae, nymphs, and adult ticks in seven representative sampling sites around Berlin in a monthly planned routine from September 2008 to December 2009. Based on the collected ticks, the tick-density factor (TDF) for each area was estimated followed by the additional analysis of the presence of different tick-borne pathogens (*Borrelia burgdorferi sensu lato*, *Rickettsia spp.*, *Babesia spp.*, and *Anaplasma spp.*) in the collected ticks, using

new commercial assays for this purpose. The results provide valuable data for the estimation of the presence of ticks and the risk of infection by tick-borne pathogens in the Berlin area, where little is known about the distribution of tick species and the harbored pathogens (Hagedorn et al., 2010; submitted). In another study we investigated the suitability of rodents as a surrogate marker for virus spread. Here we could show that in experimentally infected *Microtus arvalis* voles TBEV was detectable in different organs for at least three months and in blood for one month. 10% of all rodents analyzed were positive for TBEV. Therefore, we tested the suitability of rodents as sentinels for the virus prevalence in the field. Based on data from wild-caught rodents in several areas of Germany, we found that in TBE risk areas the infection rate of rodents was higher compared to that of areas with only single human cases or non-risk areas. TBEV was detected in six rodent species: *Apodemus agrarius*, *A. flavicollis*, *A. sylvaticus*, *Microtus agrestis*, *M. arvalis*, and *Myodes glareolus*. However, *M. glareolus* showed a high infection rate in all areas investigated (Achazi et al., 2010; submitted).

I.5 Objectives of published studies

The objectives of the published studies selected are all part of activities within the ENIVD network and/or the German Consultant Laboratory for Tick-borne encephalitis and further flaviviruses. These involve a preliminary survey regarding the epidemiological situation of viral encephalitis in EU Member States (also including a survey on TBE surveillance in Europe). The objectives are to identify the requirements for a possible future surveillance study at European level, as well as to improve the diagnostic methods and to carefully monitor the present situation especially regarding WNV, TOSV, and TBEV as potential emerging arboviral causes of encephalitis.

I.6 References

Achazi K, Nitsche A, Patel P, Radonić A, Donoso Mantke O, Niedrig M. Detection and differentiation of tick-borne encephalitis virus subtypes by a reverse transcription quantitative real-time PCR and pyrosequencing. J Virol Methods. 2010 Oct 7. [Epub ahead of print]

Akiba T, Osaka K, Tang S, et al. Analysis of Japanese encephalitis epidemic in Western Nepal in 1997. Epidemiol Infect. 2001; 126(1):81-8.

Albrecht P. Pathogenesis of neurotropic arbovirus infections. Curr Top Microbiol Immunol. 1968; 43:44-91.

Alonso-Padilla J, Loza-Rubio E, Escribano-Romero E, et al. The continuous spread of West Nile virus (WNV): seroprevalence in asymptomatic horses. Epidemiol Infect. 2009; 137(8):1163-8.

Angelini P, Tamba M, Finarelli AC, et al. West Nile virus circulation in Emilia-Romagna, Italy: the integrated surveillance system 2009. Euro Surveill. 2010; 15(16). pii: 19547.

Aspöck H, Gerersdorfer T, Formayer H, Walochnik J. Sandflies and sandfly-borne infections of humans in Central Europe in the light of climate change. Wien Klin Wochenschr. 2008; 120(19-20 Suppl 4):24-9.

Bae HG, Drosten C, Emmerich P, et al. Analysis of two imported cases of yellow fever infection from Ivory Coast and The Gambia to Germany and Belgium. J Clin Virol. 2005; 33(4):274-80.

Bakonyi T, Hubálek Z, Rudolf I, Nowotny N. Novel flavivirus or new lineage of West Nile virus, central Europe. Emerg Infect Dis. 2005; 11(2):225-31.

Bakonyi T, Ivanics E, Erdélyi K, et al. Lineage 1 and 2 strains of encephalitic West Nile virus, central Europe. Emerg Infect Dis. 2006; 12(4):618-23.

Batieha A, Saliba EK, Graham R, Mohareb E, Hijazi Y, Wijeyaratne P. Seroprevalence of West Nile, Rift Valley, and sandfly arboviruses in Hashimiah, Jordan. Emerg Infect Dis. 2000; 6(4):358-62.

Ben-Nathan D, Lustig S, Tam G, Robinzon S, Segal S, Rager-Zisman B. Prophylactic and therapeutic efficacy of human intravenous immunoglobulin in treating West Nile virus infection in mice. J Infect Dis. 2003; 188(1):5-12.

Bernkopf H, Levine S, Nerson R. Isolation of West Nile virus in Israel. J Infect Dis. 1953; 93(3):207-18.

Berthet FX, Zeller HG, Drouet MT, Rauzier J, Digoutte JP, Deubel V. Extensive nucleotide changes and deletions within the envelope glycoprotein gene of Euro-African West Nile viruses. J Gen Virol. 1997; 78 (Pt 9):2293-7.

Bia FJ, Thornton GF, Main AJ, Fong CK, Hsiung GD. Western equine encephalitis mimicking herpes simplex encephalitis. JAMA. 1980; 244(4):367-9.

Billecocq A, Spiegel M, Vialat P, et al. NSs protein of Rift Valley fever virus blocks interferon production by inhibiting host gene transcription. J Virol. 2004; 78(18):9798-806.

Blakqori G, Delhaye S, Habjan M, et al. La Crosse bunyavirus nonstructural protein NSs serves to suppress the type I interferon system of mammalian hosts. Virology. 2007; 81(10):4991-9.

Bondre VP, Jadi RS, Mishra AC, Yergolkar PN, Arankalle VA. West Nile virus isolates from India: evidence for a distinct genetic lineage. J Gen Virol. 2007; 88(Pt 3):875-84.

Bormane A, Lucenko I, Duks A, et al. Vectors of tick-borne diseases and epidemiological situation in Latvia in 1993-2002. Int J Med Microbiol. 2004; 293 Suppl 37:36-47.

Bossi P, Tegnell A, Baka A, et al. BICHAT guidelines for the clinical management of bioterrorism-related viral encephalitis. Euro Surveill 2004; 9(12): http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=509

Brett-Major DM, Claborn DM. Sand fly fever: what have we learned in one hundred years? Mil Med. 2009; 174(4):426-31.

Bröker M. Frühsommermeningoenzephalitis (FSME) in Ostasien. ImpfDialog. 2008; 2:52-5. German.

Bröker M, Gniel D. New foci of tick-borne encephalitis virus in Europe: consequences for travellers from abroad. Travel Med Infect Dis. 2003; 1(3):181-4.

Brooks TJ, Phillpotts RJ. Interferon-alpha protects mice against lethal infection with St Louis encephalitis virus delivered by the aerosol and subcutaneous routes. Antiviral Res. 1999; 41(1):57-64.

Calistri P, Giovannini A, Hubalek Z, et al. Epidemiology of west nile in europe and in the mediterranean basin. Open Virol J. 2010; 4:29-37.

Campbell GL, Marfin AA, Lanciotti RS, Gubler DJ. West Nile virus. Lancet Infect Dis. 2002; 2(9):519-29.

Cavrini F, Gaibani P, Longo G, et al. Usutu virus infection in a patient who underwent orthotropic liver transplantation, Italy, August-September 2009. Euro Surveill. 2009; 14(50). pii: 19448.

CDC Division of Vector-Borne Infectious Diseases, http://www.cdc.gov/ncidod/dvbid/index.html

Ceianu CS, Ungureanu A, Nicolescu G, et al. West nile virus surveillance in Romania: 1997-2000. Viral Immunol. 2001;14(3):251-62.

Chambers TJ, Diamond MS. Pathogenesis of flavivirus encephalitis. Adv Virus Res. 2003; 60:273-342.

Charrel RN, Brault AC, Gallian P, et al. Evolutionary relationship between Old World West Nile virus strains. Evidence for viral gene flow between Africa, the Middle East, and Europe. Virology. 2003; 315(2):381-8.

Charrel RN, Attoui H, Butenko AM, et al. Tick-borne virus diseases of human interest in Europe. Clin Microbiol Infect. 2004; 10(12):1040-55.

Charrel RN, Gallian P, Navarro-Mari JM, et al. Emergence of Toscana virus in Europe. Emerg Infect Dis. 2005; 11(11):1657-63.

Charrel RN, Izri A, Temmam S, et al. Cocirculation of 2 genotypes of Toscana virus, southeastern France. Emerg Infect Dis. 2007; 13(3):465-8.

Chaudhuri A, Kennedy PG. Diagnosis and treatment of viral encephalitis. Postgrad Med J. 2002; 78(924):575-83.

Chevalier V, Pépin M, Plée L, Lancelot R. Rift Valley fever--a threat for Europe? Euro Surveill. 2010; 15(10):19506.

Chunsuttiwat S. Japanese encephalitis in Thailand. Southeast Asian J Trop Med Public Health. 1989; 20(4):593-7.

Ciufolini MG, Maroli M, Verani P. Growth of two phleboviruses after experimental infection of their suspected sand fly vector, Phlebotomus perniciosus (Diptera: Psychodidae). Am J Trop Med Hyg. 1985; 34(1):174-9.

Ciufolini MG, Maroli M, Guandalini E, Marchi A, Verani P. Experimental studies on the maintenance of Toscana and Arbia viruses (Bunyaviridae: Phlebovirus). Am J Trop Med Hyg. 1989; 40(6):669-75.

Cizman M, Jazbec J. Etiology of acute encephalitis in childhood in Slovenia. Pediatr Infect Dis J. 1993; 12(11):903-8.

Cohen D, Zaide Y, Karasenty E, et al. Prevalence of antibodies to West Nile fever, sandfly fever Sicilian, and sandfly fever Naples viruses in healthy adults in Israel. Public Health Rev. 1999; 27(1-3):217-30.

Collao X, Palacios G, de Ory F, et al. Granada virus: a natural phlebovirus reassortant of the sandfly fever Naples serocomplex with low seroprevalence in humans. Am J Trop Med Hyg. 2010; 83(4):760-5.

Condit RC. Principles of virology. In: Knipe DM, Howley PM (eds.): Field's virology, 5th Edition. Philadelphia, PA: Wolters Kluwer-Lippincott William & Wilkins, 2007:25-57.

Connell J, Garvey P, Cotter S, et al. Two linked cases of West Nile virus (WNV) acquired by Irish tourists in the Algarve, Portugal. Euro Surveill. 2004; 8(32).

Cook SH, Griffin DE. Luciferase imaging of a neurotropic viral infection in intact animals. Virology. 2003; 77(9):5333-8.

Cusi MG, Gori Savellini G, Terrosi C, et al. Development of a mouse model for the study of Toscana virus pathogenesis. Virology. 2005; 333(1):66-73.

D'Agaro P, Martinelli E, Burgnich P et al. Prevalence of tick-borne encephalitis virus in Ixodes ricinus from a novel endemic area of North Eastern Italy. J Med Virol. 2009; 81(2):309-16.

Darwish MA, Feinsod FM, Scott RM, et al. Arboviral causes of non-specific fever and myalgia in a fever hospital patient population in Cairo, Egypt. Trans R Soc Trop Med Hyg. 1987; 81(6):1001-3.

Dauphin G, Zientara S, Zeller H, Murgue B. West Nile: worldwide current situation in animals and humans. Comp Immunol Microbiol Infect Dis. 2004; 27(5):343-55.

Davis CT, Ebel GD, Lanciotti RS, et al. Phylogenetic analysis of North American West Nile virus isolates, 2001-2004: evidence for the emergence of a dominant genotype. Virology. 2005; 342(2):252-65.

Davis LE, DeBiasi R, Goade DE, et al. West Nile virus neuroinvasive disease. Ann Neurol. 2006; 60(3):286-300.

Davis LE, Beckham JD, Tyler KL. North American encephalitic arboviruses. Neurol Clin. 2008; 26(3):727-57.

Davison KL, Crowcroft NS, Ramsay ME, Brown DW, Andrews NJ. Viral encephalitis in England, 1989-1998: what did we miss? Emerg Infect Dis. 2003; 9(2):234-40.

Debiasi RL, Tyler KL. Molecular methods for diagnosis of viral encephalitis. Clin Microbiol Rev. 2004; 17(4):903-25.

Debiasi RL, Tyler KL. West Nile virus meningoencephalitis. Nat Clin Pract Neurol. 2006; 2(5):264-75.

Defuentes G, Rapp C, Imbert P, Durand JP, Debord T. Acute meningitis owing to phlebotomus fever Toscana virus imported to France. J Travel Med. 2005; 12(5):295-6.

De Lamballerie X, Tolou H, Durand JP, Charrel RN. Prevalence of Toscana virus antibodies in volunteer blood donors and patients with central nervous system infections in southeastern France. Vector Borne Zoonotic Dis. 2007; 7(2):275-7.

Del Giudice P, Schuffenecker I, Vandenbos F, Counillon E, Zeller H. Human West Nile virus, France. Emerg Infect Dis. 2004; 10(10):1885-6.

Depaquit J, Grandadam M, Fouque F, Andry PE, Peyrefitte C. Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review. Euro Surveill. 2010; 15(10):19507.

Diamond MS. Virus and host determinants of West Nile virus pathogenesis. PLoS Pathog. 2009; 5(6):e1000452.

Di Nicuolo G, Pagliano P, Battisti S, et al. Toscana virus central nervous system infections in southern Italy. J Clin Microbiol. 2005; 43(12):6186-8.

Dionisio D, Valassina M, Ciufolini MG, et al. Encephalitis without meningitis due to sandfly fever virus serotype toscana. Clin Infect Dis. 2001; 32(8):1241-3.

Dionisio D, Esperti F, Vivarelli A, Valassina M. Epidemiological, clinical and laboratory aspects of sandfly fever. Curr Opin Infect Dis. 2003; 16(5):383-8.

Dobler G. Zoonotic tick-borne flaviviruses. Vet Microbiol. 2010; 140(3-4):221-8.

Doerr RFK, Taussig S. Das Pappatacifieber. Leipzig: Verlag Franz Deuticke, 1909.

Domingo C, Niedrig M, Teichmann A, et al. 2nd International External Quality Control Assessment for the Molecular Diagnosis of Dengue Infections. PLoS Negl Trop Dis. 2010; 4(10): e833.

Donoso Mantke O, Lemmer K, Biel SS, et al. Quality control assessment for the serological diagnosis of dengue virus infections. J Clin Virol. 2004; 29(2):105-12.

Donoso Mantke O, Schmitz H, Zeller H, Heyman P, Papa A, Niedrig M; European Network for Diagnostics of Imported Viral Diseases (ENIVD). Quality assurance for the diagnostics of viral diseases to enhance the emergency preparedness in Europe. Euro Surveill. 2005; 10(6):102-6.

Donoso Mantke O, Niedrig M; ENIVD members. Laboratory capacity for detection of chikungunya virus infections in Europe. Euro Surveill. 2007 a; 12(9):E070913.2.

Donoso Mantke O, Achazi K, Niedrig M. Serological versus PCR methods for the detection of tick-borne encephalitis virus infections in humans. Future Virology. 2007 b; 2(6):565-72.

Donoso Mantke O, Aberle SW, Avsic-Zupanc T, Labuda M, Niedrig M. Quality control assessment for the PCR diagnosis of tick-borne encephalitis virus infections. J Clin Virol. 2007 c; 38(1):73-7.

Donoso Mantke O, Vaheri A, Ambrose H, et al. Analysis of the surveillance situation for viral encephalitis and meningitis in Europe. Euro Surveill. 2008 a; 13(3). pii: 8017.

Donoso Mantke O, Schädler R, Niedrig M. A survey on cases of tick-borne encephalitis in European countries. Euro Surveill. 2008 b; 13(17). pii: 18848.

Dropulić B, Masters CL. Entry of neurotropic arboviruses into the central nervous system: an in vitro study using mouse brain endothelium. J Infect Dis. 1990; 161(4):685-91.

Dufour B, Moutou F, Hattenberger AM, Rodhain F. Global change: impact, management, risk approach and health measures--the case of Europe. Rev Sci Tech. 2008; 27(2):529-50.

ECDC THREAT ASSESSMENT. Outbreak of West Nile virus infection in Greece. 02 Sep 2010. http://www.ecdc.europa.eu/en/healthtopics/Documents/1009_Threat%20assessment_West_Nile_Virus.pdf

Echevarría JM, de Ory F, Guisasola ME, et al. Acute meningitis due to Toscana virus infection among patients from both the Spanish Mediterranean region and the region of Madrid. J Clin Virol. 2003; 26(1):79-84.

Eitrem R, Stylianou M, Niklasson B. High prevalence rates of antibody to three sandfly fever viruses (Sicilian, Naples, and Toscana) among Cypriots. Epidemiol Infect. 1991 a; 107(3):685-91.

Eitrem R, Niklasson B, Weiland O. Sandfly fever among Swedish tourists. Scand J Infect Dis. 1991 b; 23(4):451-7.

Elliott RM, Bouloy M, Calisher CH, et al. Genus Phlebovirus. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, et al. (eds.): Virus taxonomy: classification and nomenclature of viruses; seventh report of the International Committee on Taxonomy of Viruses. San Diego, CA: Academic Press, 2000:614–6.

Erdélyi K, Ursu K, Ferenczi E, et al. Clinical and pathologic features of lineage 2 West Nile virus infections in birds of prey in Hungary. Vector Borne Zoonotic Dis. 2007; 7(2):181-8.

Ergünay K, Saygan MB, Aydoğan S, et al. Confirmed Exposure to Tick-Borne Encephalitis Virus and Probable Human Cases of Tick-Borne Encephalitis in Central/Northern Anatolia, Turkey. Zoonoses Public Health. 2010 Jun 30. [Epub ahead of print]

Figueiredo LT. The Brazilian flaviviruses. Microbes Infect. 2000; 2(13):1643-9.

Figueiredo LT. Emergent arboviruses in Brazil. Rev Soc Bras Med Trop. 2007; 40(2):224-9.

Filipe AR. Serological survey for antibodies to arboviruses in the human population of Portugal. Trans R Soc Trop Med Hyg. 1974; 68(4):311-4.

Flamm H. Aufklärung des Pappataci-Fiebers durch österreichische Militärärzte. Wien Klin Wochenschr. 2008; 120/7-8:198-208. German.

Fomsgaard A, Christiansen C, Bodker R. First identification of tick-borne encephalitis in Denmark outside of Bornholm, August 2009. Euro Surveill. 2009; 14(36). pii: 19325.

Francisci D, Papili R, Camanni G, et al. Evidence of Toscana virus circulation in Umbria: first report. Eur J Epidemiol. 2003; 18(5):457-9.

Gabriel M, Resch C, Günther S, Schmidt-Chanasit J. Toscana virus infection imported from Elba into Switzerland. Emerg Infect Dis. 2010; 16(6):1034-6.

Gallia F, Rampas J, Hollender L. [Laboratory infection caused by tick-borne encephaltis virus.] Čas. Lék Čes. 1949; 88:224-9. Czech.

Glaser CA, Gilliam S, Schnurr D, et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998-2000. Clin Infect Dis. 2003; 36(6):731-42.

Glaser CA, Honarmand S, Anderson LJ, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. Clin Infect Dis. 2006; 43(12):1565-77.

Golovljova I, Vene S, Sjölander KB, Vasilenko V, Plyusnin A, Lundkvist A. Characterization of tick-borne encephalitis virus from Estonia. J Med Virol. 2004; 74(4):580-8.

Gould EA, Higgs S. Impact of climate change and other factors on emerging arbovirus diseases. Trans R Soc Trop Med Hyg. 2009; 103(2):109-21.

Gould EA, Solomon T. Pathogenic flaviviruses. Lancet. 2008; 371(9611):500-9.

Granerod J, Crowcroft NS. The epidemiology of acute encephalitis. Neuropsychol Rehabil. 2007; 17(4-5):406-28.

Gresíková M, Kaluzová M. Biology of tick-borne encephalitis virus. Acta Virol. 1997; 41(2):115-24.

Gresíková M, Noseck J. Isolation of tick-borne encephalitis virus from Haemaphysalis inermis ticks. Acta Virol. 1966; 10:359-61.

Gritsun TS, Lashkevich VA, Gould EA. Tick-borne encephalitis. Antiviral Res. 2003 a; 57(1-2):129-46.

Gritsun TS, Frolova TV, Zhankov AI, et al. Characterization of a siberian virus isolated from a patient with progressive chronic tick-borne encephalitis. J Virol. 2003 b; 77(1):25-36.

Günther G, Haglund M, Lindquist L, Forsgren M, Sköldenberg B. Tick-borne encephalitis in Sweden in relation to aseptic meningo-encephalitis of other etiology: a prospective study of clinical course and outcome. J Neurol. 1997; 244(4):230-8.

Gyure KA. West Nile virus infections. Neuropathol Exp Neurol. 2009; 68(10):1053-60.

Haddow AD, Odoi A. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003-2007. PLoS One. 2009; 4(7):e6145.

Haglund M, Vene S, Forsgren M, et al. Characterisation of human tick-borne encephalitis virus from Sweden. J Med Virol. 2003; 71(4):610-21.

Halstead SB, Thomas SJ. Japanese encephalitis: new options for active immunization. Clin Infect Dis. 2010; 50(8):1155-64.

Hayes CG. West Nile virus: Uganda, 1937, to New York City, 1999. Ann N Y Acad Sci. 2001; 951:25-37.

Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. Epidemiology and transmission dynamics of West Nile virus disease. Emerg Infect Dis. 2005; 11(8):1167-73.

Hayes E, Mackenzie J, Shope R, et al. Arthropod-borne viral diseases. In: Heymann DL (ed.): Control of Communicable Diseases Manual, 19th Edition. Washington, DC: WHO/American Public Health Association, 2008:34-62.

Hemmersbach-Miller M, Parola P, Charrel RN, Paul Durand J, Brouqui P. Sandfly fever due to Toscana virus: an emerging infection in southern France. Eur J Intern Med. 2004; 15(5):316-317.

Heyman P, Cochez C, Hofhuis A, et al. A clear and present danger: tick-borne diseases in Europe. Expert Rev Anti Infect Ther. 2010; 8(1):33-50.

Holbrook MR, Gowen BB. Animal models of highly pathogenic RNA viral infections: encephalitis viruses. Antiviral Res. 2008; 78(1):69-78.

Hollidge BS, González-Scarano F, Soldan SS. Arboviral encephalitides: transmission, emergence, and pathogenesis. J Neuroimmune Pharmacol. 2010; 5(3):428-42.

Hubálek Z. Mosquito-borne viruses in Europe. Parasitol Res. 2008; 103 Suppl 1:S29-43.

Hubálek Z, Halouzka J. West Nile fever--a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis. 1999; 5(5):643-50.

Hubálek Z, Halouzka J, Juricová Z. West Nile fever in Czechland. Emerg Infect Dis. 1999; 5(4):594-5.

Ilias A, Galanakis E, Raissaki M, Kalmanti M. Childhood encephalitis in Crete, Greece. Child Neurol. 2006; 21(10):910-2.

Iwamoto M, Jernigan DB, Guasch A, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med. 2003; 348(22):2196-203.

Izri A, Temmam S, Moureau G, Hamrioui B, de Lamballerie X, Charrel RN. Sandfly fever Sicilian virus, Algeria. Emerg Infect Dis. 2008; 14(5):795-7.

Jaenson TG, Tälleklint L, Lundqvist L, Olsen B, Chirico J, Mejlon H. Geographical distribution, host associations, and vector roles of ticks (Acari: Ixodidae, Argasidae) in Sweden. J Med Entomol. 1994; 31(2):240-56.

Jääskeläinen AE, Tikkakoski T, Uzcátegui NY, Alekseev AN, Vaheri A, Vapalahti O. Siberian subtype tickborne encephalitis virus, Finland. Emerg Infect Dis. 2006 a; 12(10):1568-71.

Jääskeläinen AJ, Piiparinen H, Lappalainen M, Koskiniemi M, Vaheri A. Multiplex-PCR and oligonucleotide microarray for detection of eight different herpesviruses from clinical specimens. J Clin Virol. 2006 b; 37(2):83-90.

Jmor F, Emsley HC, Fischer M, Solomon T, Lewthwaite P. The incidence of acute encephalitis syndrome in Western industrialised and tropical countries. Virol J. 2008; 5:134.

Johnson RT. The pathogenesis of acute viral encephalitis and postinfectious encephalomyelitis. Infect Dis. 1987; 155(3):359-64.

Johnson RT (ed.). Viral infections of the nervous system, 2nd Edition. Philadelphia, PA: Lippincott-Raven, 1998.

Johnson RT, Mims CA. Pathogenesis of viral infections of the nervous system. N Engl J Med. 1968; 278(1):23-30, 84-92.

Johnston LJ, Halliday GM, King NJ. Phenotypic changes in Langerhans' cells after infection with arboviruses: a role in the immune response to epidermally acquired viral infection? J Virol. 1996; 70(7):4761-6.

Johnston LJ, Halliday GM, King NJ. Langerhans cells migrate to local lymph nodes following cutaneous infection with an arbovirus. J Invest Dermatol. 2000; 114(3):560-8.

Jokela P, Joki-Korpela P, Maaronen M, Glumoff V, Hyypiä T. Detection of human picornaviruses by multiplex reverse transcription-PCR and liquid hybridization. J Clin Microbiol. 2005; 43(3):1239-45.

Jones KE, Patel NG, Levy MA, et al. Global trends in emerging infectious diseases. Nature. 2008; 451(7181):990-3.

Joubert L, Oudar J, Hannoun C, et al. [Epidemiology of the West Nile virus: study of a focus in Camargue. IV. Meningo-encephalomyelitis of the horse]. Ann Inst Pasteur (Paris). 1970; 118(2):239-47. French.

Jourdain E, Schuffenecker I, Korimbocus J, et al. West Nile virus in wild resident birds, Southern France, 2004. Vector Borne Zoonotic Dis. 2007; 7(3):448-52.

Kallio-Kokko H, Uzcategui N, Vapalahti O, Vaheri A. Viral zoonoses in Europe. FEMS Microbiol Rev. 2005; 29(5):1051-77.

Kamei S, Takasu T. Nationwide survey of the annual prevalence of viral and other neurological infections in Japanese inpatients. Intern Med. 2000; 39(11):894-900.

Kaptoul D, Viladrich PF, Domingo C, et al. West Nile virus in Spain: report of the first diagnosed case (in Spain) in a human with aseptic meningitis. Scand J Infect Dis. 2007; 39(1):70-1.

Karabatsos N (ed.). International catalogue of arboviruses, including certain other viruses of vertebrates, 3rd Edition. San Antonio, TX: Am Soc Trop Med Hyg., 1985.

Kari K, Liu W, Gautama K, et al. A hospital-based surveillance for Japanese encephalitis in Bali, Indonesia. BMC Med. 2006; 4:8.

Khetsuriani N, Holman RC, Anderson LJ. Burden of encephalitis-associated hospitalizations in the United States, 1988-1997. Clin Infect Dis. 2002; 35(2):175-82.

Kielian M. Class II virus membrane fusion proteins. Virology. 2006; 344(1):38-47.

Kimura-Kuroda J, Yasui K. Protection of mice against Japanese encephalitis virus by passive administration with monoclonal antibodies. J Immunol. 1988; 141(10):3606-10.

Komar N, Panella NA, Boyce E. Exposure of domestic mammals to West Nile virus during an outbreak of human encephalitis, New York City, 1999. Emerg Infect Dis. 2001; 7(4):736-8.

Konstantinou GN, Papa A, Antoniadis A. Sandfly-fever outbreak in Cyprus: are phleboviruses still a health problem? Travel Med Infect Dis. 2007; 5(4):239-42.

Korenberg E, Likhacheva T. Analysis of the long-term dynamics of tick-borne encephalitis (TBE) and ixodid tick-borne borrelioses (ITBB) morbidity in Russia. Int J Med Microbiol. 2006; 296 Suppl 40:54-8.

Koskiniemi M, Rantalaiho T, Piiparinen H, et al. Infections of the central nervous system of suspected viral origin: a collaborative study from Finland. J Neurovirol. 2001; 7(5):400-8.

Kozuch O, Gresíková M, Nosek J, Lichard M, Sekeyová M. The role of small rodents and hedgehogs in a natural focus of tick-borne encephalitis. Bull World Health Organ. 1967; 36 Suppl:61-6.

Kramer LD, Styer LM, Ebel GD. A global perspective on the epidemiology of West Nile virus. Annu Rev Entomol. 2008; 53:61-81.

Krejčí J. Isolement d'un virus noveau en course d'un epidémie de meningoencephalite dans la region de Vyškov (Moraviae). Presse Méd. (Paris). 1949 a; 74:1084. French.

Krejčí J. Epidemie virusových encefalitid na Vyškovsku. Lék. Listy (Brno). 1949 b; 4: 73-5; 112-6; 132-4. Czech.

Krisztalovics K, Ferenczi E, Molnar Z, et al. West Nile virus infections in Hungary, August-September 2008. Euro Surveill. 2008; 13(45):pii: 19030.

Křivanec K, Kopecký J, Tomková E, Grubhoffer L. Isolation of TBE virus from the tick Ixodes hexagonus. Folia Parasitol (Praha). 1988; 35(3):273-6.

Kuhn J, Bewermeyer H, Hartmann-Klosterkoetter U, Emmerich P, Schilling S, Valassina M. Toscana virus causing severe meningoencephalitis in an elderly traveller. Neurol Neurosurg Psychiatry. 2005; 76(11):1605-6.

Kumar P, Lee SK, Shankar P, Manjunath N. A single siRNA suppresses fatal encephalitis induced by two different flaviviruses. PLoS Med. 2006; 3(4):e96.

Kunze U, Asokliene L, Bektimirov T, et al. Tick-borne encephalitis in childhood--consensus 2004. Wien Med Wochenschr. 2004; 154(9-10):242-5.

Kunze U; ISW TBE. Tick-borne encephalitis: from childhood to golden age does increased mobility mean increased risk? Conference report of the 11th meeting of the International Scientific Working Group on Tick-Borne Encephalitis (ISW-TBE). Vaccine. 2010; 28(4):875-6.

Kupila L, Vuorinen T, Vainionpää R, Hukkanen V, Marttila RJ, Kotilainen P. Etiology of aseptic meningitis and encephalitis in an adult population. Neurology. 2006; 66(1):75-80.

Labuda M, Randolph SE. Survival strategy of tick-borne encephalitis virus: cellular basis and environmental determinants. Zentralbl Bakteriol. 1999; 289(5-7):513-24.

Labuda M, Austyn JM, Zuffova E, et al. Importance of localized skin infection in tick-borne encephalitis virus transmission. Virology. 1996; 219(2):357-66.

Lanciotti RS, Kerst AJ. Nucleic acid sequence-based amplification assays for rapid detection of West Nile and St. Louis encephalitis viruses. J Clin Microbiol. 2001; 39(12):4506-13.

Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Science. 1999; 286(5448):2333-7.

Lanciotti RS, Ebel GD, Deubel V, et al. Complete genome sequences and phylogenetic analysis of West Nile virus strains isolated from the United States, Europe, and the Middle East. Virology. 2002; 298(1):96-105.

Lee TC, Tsai CP, Yuan CL, et al. Encephalitis in Taiwan: a prospective hospital-based study. Jpn J Infect Dis. 2003; 56(5-6):193-9.

Lemmer K, Donoso Mantke O, Bae HG, Groen J, Drosten C, Niedrig M. External quality control assessment in PCR diagnostics of dengue virus infections. J Clin Virol. 2004; 30(4):291-6.

Levett PN, Sonnenberg K, Sidaway F, et al. Use of immunoglobulin G avidity assays for differentiation of primary from previous infections with West Nile virus. J Clin Microbiol. 2005; 43(12):5873-5.

Lewis P, Glaser CA. Encephalitis. Pediatr Rev. 2005; 26(10):353-63.

Lindquist L, Vapalahti O. Tick-borne encephalitis. Lancet. 2008; 371(9627):1861-71.

Linke S, Ellerbrok H, Niedrig M, Nitsche A, Pauli G. Detection of West Nile virus lineages 1 and 2 by real-time PCR. J Virol Methods. 2007; 146(1-2):355-8.

Linke S, Muehlen M, Niedrig M, et al. Assessing the exposure of German and Austrian bird ringers to West Nile virus (Flavivirus) and evaluating their potential risk of infection. J Ornithol. 2008; 149:271–75.

Liu DY, Tesh RB, Travassos Da Rosa AP, et al. Phylogenetic relationships among members of the genus Phlebovirus (Bunyaviridae) based on partial M segment sequence analyses. J Gen Virol. 2003; 84(Pt 2):465-73.

Lossinsky AS, Shivers RR. Structural pathways for macromolecular and cellular transport across the blood-brain barrier during inflammatory conditions. Histol Histopathol. 2004; 19(2):535-64.

Love S, Wiley CA. Viral infections. In: Love S, Louis DN, Ellison DW (eds.): Greenfield's Neuropathology, 8th Edition. Vol 2. London: Hodder Arnold, 2008:8.

Lu Z, Bröker M, Liang G. Tick-borne encephalitis in mainland China. Vector Borne Zoonotic Dis. 2008; 8(5):713-20.

Ludolfs D, Niedrig M, Paweska JT, Schmitz H. Reverse ELISA for the detection of anti West Nile virus IgG antibodies in humans. Eur J Clin Microbiol Infect Dis. 2007; 26(7):467-73.

Lustig S, Danenberg HD, Kafri Y, Kobiler D, Ben-Nathan D. Viral neuroinvasion and encephalitis induced by lipopolysaccharide and its mediators. J Exp Med. 1992; 176(3):707-12.

Lvov DK, Butenko AM, Gromashevsky VL, et al. West Nile virus and other zoonotic viruses in Russia: examples of emerging-reemerging situations. Arch Virol Suppl. 2004; (18):85-96.

Macini P, Squintani G, Finarelli AC, et al. Detection of West Nile virus infection in horses, Italy, September 2008. Euro Surveill. 2008; 13(39). pii: 18990.

Mailles A, Stahl JP; Steering Committee and Investigators Group. Infectious encephalitis in France in 2007: a national prospective study. Clin Infect Dis. 2009; 49(12):1838-47.

Malan AK, Martins TB, Hill HR, Litwin CM. Evaluations of commercial West Nile virus immunoglobulin G (IgG) and IgM enzyme immunoassays show the value of continuous validation. Clin Microbiol. 2004; 42(2):727-33.

Malkova D, Frankova V. The lymphatic system in the development of experimental tick-borne encephalitis in mice. Acta Virol. 1959; 3:210-4.

Mandl CW. Steps of the tick-borne encephalitis virus replication cycle that affect neuropathogenesis. Virus Res. 2005; 111(2):161-74.

Mansfield KL, Johnson N, Phipps LP, Stephenson JR, Fooks AR, Solomon T. Tick-borne encephalitis virus - a review of an emerging zoonosis. J Gen Virol. 2009; 90(Pt 8):1781-94.

Martina BE, Koraka P, Osterhaus AD. West Nile Virus: is a vaccine needed? Curr Opin Investig Drugs. 2010; 11(2):139-46.

McCormack JG, Allworth AM. Emerging viral infections in Australia. Med J Aust. 2002; 177(1):45-9.

McIntosh BM, Jupp PG, Dos Santos I, Meenehan GM. Epidemics of West Nile and Sindbis viruses in South Africa with Culex (Culex) univittatus Theobald as vector. S Afr J Sci 1976; 72:295–300.

Mehrabi Tavana A. The seroepidemiological studies of sand fly fever in Iran during imposed war. Iranian J Publ Health 2001; 30/3-4:145-6.

Mendoza-Montero J, Gámez-Rueda MI, Navarro-Marí JM, de la Rosa-Fraile M, Oyonarte-Gómez S. Infections due to sandfly fever virus serotype Toscana in Spain. Clin Infect Dis. 1998; 27(3):434-6.

Misić-Majerus L, Bujić N, Madarić V, Avsić-Zupanc T. [Hepatitis caused by tick-borne meningoencephalitis virus (TBEV)--a rare clinical manifestation outside the central nervous system involvement]. Acta Med Croatica. 2005; 59(4):347-52. Croatian.

Misra UK, Kalita J. Overview: Japanese encephalitis. Prog Neurobiol. 2010; 91(2):108-20.

Modrow S, Falke D, Truyen U (eds.). Molekulare Virologie, 2nd Edition. Heidelberg: Spektrum Akademischer Verlag, 2003.

Monath TP, Cropp CB, Harrison AK. Mode of entry of a neurotropic arbovirus into the central nervous system. Reinvestigation of an old controversy. Lab Invest. 1983; 48(4):399-410.

Morens DM, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. Nature. 2004; 430(6996):242-9.

Morrey JD, Smee DF, Sidwell RW, Tseng C. Identification of active antiviral compounds against a New York isolate of West Nile virus. Antiviral Res. 2002; 55(1):107-16.

Morrey JD, Siddharthan V, Olsen AL, et al. Defining limits of treatment with humanized neutralizing monoclonal antibody for West Nile virus neurological infection in a hamster model. Antimicrob Agents Chemother. 2007; 51(7):2396-402.

Murgue B, Murri S, Triki H, Deubel V, Zeller HG. West Nile in the Mediterranean basin: 1950-2000. Ann N Y Acad Sci. 2001 a; 951:117-26.

Murgue B, Murri S, Zientara S, Durand B, Durand JP, Zeller H. West Nile outbreak in horses in southern France, 2000: the return after 35 years. Emerg Infect Dis. 2001 b; 7(4):692-6.

Murgue B, Zeller H, Deubel V. The ecology and epidemiology of West Nile virus in Africa, Europe and Asia. Curr Top Microbiol Immunol. 2002; 267:195-221.

Nalca A, Fellows PF, Whitehouse CA. Vaccines and animal models for arboviral encephalitides. Antiviral Res. 2003; 60(3):153-74.

Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med. 2001; 344(24):1807-14.

Navarro JM, Fernández-Roldán C, Pérez-Ruiz M, Sanbonmatsu S, de la Rosa M, Sánchez-Seco MP. [Meningitis by Toscana virus in Spain: description of 17 cases]. Med Clin (Barc). 2004; 122(11):420-2. Spanish.

Nicoletti L, Ciufolini MG, Verani P. Sandfly fever viruses in Italy. Arch Virol Suppl. 1996; 11:41-7.

Nicolosi A, Hauser WA, Beghi E, Kurland LT. Epidemiology of central nervous system infections in Olmsted County, Minnesota, 1950-1981. J Infect Dis. 1986; 154(3):399-408.

Niedrig M, Vaisviliene D, Teichmann A, Klockmann U, Biel SS. Comparison of six different commercial IgG-ELISA kits for the detection of TBEV-antibodies. J Clin Virol. 2001; 20(3):179-82.

Niedrig M, Linke S, Zeller H, Drosten C. First international proficiency study on West Nile virus molecular detection. Clin Chem. 2006; 52(10):1851-4.

Niedrig M, Donoso Mantke O, Altmann D, Zeller H. First international diagnostic accuracy study for the serological detection of West Nile virus infection. BMC Infect Dis. 2007 a; 7:72.

Niedrig M, Avsic T, Aberle SW, et al. Quality control assessment for the serological diagnosis of tick borne encephalitis virus infections. J Clin Virol. 2007 b; 38(3):260-4.

Niedrig M, Donoso-Mantke O, Schädler R; ENIVD members. The European Network for Diagnostics of Imported Viral Diseases (ENIVD)--12 years of strengthening the laboratory diagnostic capacity in Europe. Euro Surveill. 2007 c; 12(4):E070419.5.

Niedrig M, Zeller H, Schuffenecker I, et al. International diagnostic accuracy study for the serological detection of chikungunya virus infection. Clin Microbiol Infect. 2009; 15(9):880-4.

Niedrig M, Nitsche A, Donoso Mantke O. Chapter 26: Arthropod-Borne Viruses. In: Jerome KR (ed.): Lennette's Laboratory Diagnosis of Viral Infections, 4th Edition. New York, NY: Informa Healthcare, 2010:451-9, in press.

Niklasson B, Eitrem R. Sandfly fever among Swedish UN troops in Cyprus. Lancet. 1985; 1(8439):1212.

Nuttall PA, Labuda M. Tick-borne encephalitides. In: Palmer SR, Lord Soulsby, Simpson DIH (eds.): Zoonoses. Oxford: Oxford University Press, 1998:469-86.

Nuttall PA, Labuda M. Dynamics of infection in tick vectors and at the tick-host interface. Adv Virus Res. 2003; 60:233-72.

Oker-Blom N. Kumlinge disease; a meningo-encephalitis occurring in the Aaland Islands. Ann Med Exp Biol Fenn. 1956; 34(3):309-18.

Okuno T, Tseng PT, Hsu ST, Huang CT, Kuo CC. Japanese encephalitis surveillance in China (Province of Taiwan) during 1968-1971. I. Geographical and seasonal features of case outbreaks. Jpn J Med Sci Biol. 1975 a; 28(5-6):235-53.

Okuno T, Tseng PT, Hsu ST, Huang CT, Kuo CC. Japanese encephalitis surveillance in China (Province of Taiwan) during 1968-1971. II. Age-secific incidence in connection with Japanese encephalitis vaccination program. Jpn J Med Sci Biol. 1975 b; 28(5-6):255-67.

Overby AK, Popov VL, Niedrig M, Weber F. Tick-borne encephalitis virus delays interferon induction and hides its double-stranded RNA in intracellular membrane vesicles. Virology. 2010; 84(17):8470-83.

Panning M, Charrel RN, Donoso Mantke O, Landt O, Niedrig M, Drosten C. Coordinated implementation of chikungunya virus reverse transcription-PCR. Emerg Infect Dis. 2009; 15(3):469-71.

Pantelic L, Sivakumaran H, Urosevic N. Differential induction of antiviral effects against West Nile virus in primary mouse macrophages derived from flavivirus-susceptible and congenic resistant mice by alpha/beta interferon and poly(I-C). J Virol. 2005; 79(3):1753-64.

Papa A, Konstantinou G, Pavlidou V, Antoniadis A. Sandfly fever virus outbreak in Cyprus. Clin Microbiol Infect. 2006; 12(2):192-4.

Pardridge WM. Blood-brain barrier delivery. Drug Discov Today. 2007; 12(1-2):54-61.

Pavlovskij EN. [On natural focality of infectious and parasitic diseases.] Vestn Akad Nauk SSSR. 1939; 10:98-108. Russian.

Pecorari M, Longo G, Gennari W, et al. First human case of Usutu virus neuroinvasive infection, Italy, August-September 2009. Euro Surveill. 2009; 14(50). pii: 19446.

Pérez-Ruiz M, Collao X, Navarro-Marí JM, Tenorio A. Reverse transcription, real-time PCR assay for detection of Toscana virus. J Clin Virol. 2007; 39(4):276-81.

Peyrefitte CN, Devetakov I, Pastorino B, et al. Toscana virus and acute meningitis, France. Emerg Infect Dis. 2005; 11(5):778-80.

Pfleiderer C, König C, Chudy M, Schmidt M, Roth WK, Nübling CM. Molecular epidemiology of West Nile Virus in humans. Dev Biol (Basel). 2006; 126:197-201; discussion 326-7.

Plassmeyer ML, Soldan SS, Stachelek KM, Roth SM, Martín-García J, González-Scarano F. Mutagenesis of the La Crosse Virus glycoprotein supports a role for Gc (1066-1087) as the fusion peptide. Virology. 2007; 358(2):273-82.

Platonov AE, Shipulin GA, Shipulina OY, et al. Outbreak of West Nile virus infection, Volgograd Region, Russia, 1999. Emerg Infect Dis. 2001; 7(1):128-32.

Platonov AE, Fedorova MV, Karan LS, Shopenskaya TA, Platonova OV, Zhuravlev VI. Epidemiology of West Nile infection in Volgograd, Russia, in relation to climate change and mosquito (Diptera: Culicidae) bionomics. Parasitol Res. 2008; 103 Suppl 1:S45-53.

Popovici F, Sarbu A, Nicolae O, et al. West Nile fever in a patient in Romania, August 2008: case report. Euro Surveill. 2008; 13(39). pii: 18989.

Preiser W. [Tropical virus not only in the tropics. Treatment, epidemiology and diagnosis of tropical viral infections]. Pharm Unserer Zeit. 2010; 39(1):34-40.German.

Pugliese A, Beltramo T, Torre D. Emerging and re-emerging viral infections in Europe. Cell Biochem Funct. 2007 a; 25(1):1-13.

Pugliese A, Beltramo T, Torre D. Seroprevalence study of Tick-borne encephalitis, Borrelia burgdorferi, Dengue and Toscana virus in Turin Province. Cell Biochem Funct. 2007 b; 25(2):185-8.

Rampas J, Gallia F. Isolation of tick-borne encephalitis virus from ticks Ixodes ricinus. Čas Lék Čes. 1949; 88:1179-80. Czech.

Rantala H, Uhari M. Occurrence of childhood encephalitis: a population-based study. Pediatr Infect Dis J. 1989; 8(7):426-30.

Reiter P. Climate change and mosquito-borne disease: knowing the horse before hitching the cart. Rev Sci Tech. 2008; 27(2):383-98.

Reiter P. West Nile virus in Europe: understanding the present to gauge the future. Euro Surveill. 2010; 15(10). pii: 19508.

Rizzo C, Vescio F, Declich S, et al. West Nile virus transmission with human cases in Italy, August - September 2009. Euro Surveill. 2009; 14(40). pii: 19353.

Romero JR, Newland JG. Viral meningitis and encephalitis: traditional and emerging viral agents. Semin Pediatr Infect Dis. 2003; 14(2):72-82.

Romero JR, Newland JG. Diagnosis of viral encephalitides: zoonotic-associated viruses. Pediatr Infect Dis J. 2006; 25(8):741-2.

Rudolf I, Hubálek Z, Sikutová S, Svec P. [Neglected arthropod-borne viral infections in the Czech Republic]. Epidemiol Mikrobiol Imunol. 2008; 57(3):80-9. Czech.

Růzek D, Stastná H, Kopecký J, Golovljova I, Grubhoffer L. Rapid subtyping of tick-borne encephalitis virus isolates using multiplex RT-PCR. Virol Methods. 2007; 144(1-2):133-7.

Růžek D, Vancová M, Tesarová M, Ahantarig A, Kopecký J, Grubhoffer L. Morphological changes in human neural cells following tick-borne encephalitis virus infection. J Gen Virol. 2009; 90(Pt 7):1649-58.

Růžek D, Dobler G, Donoso Mantke O. Tick-borne encephalitis: Pathogenesis and clinical implications. Travel Med Infect Dis. 2010; 8(4):223-32.

Sabin AB, Philip CB, Paul JR. Phlebotomus (pappataci or sandfly) fever: a disease of military importance; summary of existing knowledge and preliminary report of original investigations. JAMA. 1944; 125:603-6, 693-9.

Saidi S, Tesh R, Javadian E, Sahabi Z, Nadim A. Studies on the epidemiology of sandfly fever in Iran. II. The prevalence of human and animal infection with five phlebotomus fever virus serotypes in Isfahan province. Am J Trop Med Hyg. 1977; 26(2):288-93.

Saksida A, Duh D, Lotric-Furlan S, Strle F, Petrovec M, Avsic-Zupanc T. The importance of tick-borne encephalitis virus RNA detection for early differential diagnosis of tick-borne encephalitis. J Clin Virol. 2005; 33(4):331-5.

Sanbonmatsu-Gámez S, Pérez-Ruiz M, Collao X, et al. Toscana virus in Spain. Emerg Infect Dis. 2005; 11(11):1701-7.

Sanbonmatsu-Gámez S, Pérez-Ruiz M, Palop-Borrás B, Navarro-Marí JM. Unusual manifestation of toscana virus infection, Spain. Emerg Infect Dis. 2009; 15(2):347-8.

Santos L, Simões J, Costa R, Martins S, Lecour H. Toscana virus meningitis in Portugal, 2002-2005. Euro Surveill. 2007; 12(6):E3-4.

Schneider H. Über epidemische akute „Meningitis serosa“. Wien Klin Woch. 1931; 44:350-2. German.

Schuffenecker I, Peyrefitte CN, el Harrak M, Murri S, Leblond A, Zeller HG. West Nile virus in Morocco, 2003. Emerg Infect Dis. 2005; 11(2):306-9.

Schultze D, Dollenmaier G, Rohner A, Guidi T, Cassinotti P. Benefit of detecting tick-borne encephalitis viremia in the first phase of illness. J Clin Virol. 2007; 38(2):172-5.

Sejvar JJ, Bode AV, Marfin AA, et al. West Nile virus-associated flaccid paralysis. Emerg Infect Dis. 2005; 11(7):1021-7.

Senior K. Vector-borne diseases threaten Europe. Lancet Infect Dis. 2008; 8(9):531-2.

Shrestha B, Gottlieb D, Diamond MS. Infection and injury of neurons by West Nile encephalitis virus. Virology. 2003; 77(24):13203-13.

Smithburn KC, Hughes TP, Burke A, Paul J. A neurotropic virus isolated from the blood of a native of Uganda. Am J Trop Med. 1940; 20:471-92.

Snapinn KW, Holmes EC, Young DS, Bernard KA, Kramer LD, Ebel GD. Declining growth rate of West Nile virus in North America. J Virol. 2007; 81(5):2531-4.

Solomon T, Cardosa MJ. Emerging arboviral encephalitis. Newsworthy in the West but much more common in the East. BMJ. 2000; 321(7275):1484-5.

Sonderegger B, Hachler H, Dobler G, Frei M. Imported aseptic meningitis due to Toscana virus acquired on the island of Elba, Italy, August 2008. Euro Surveill. 2009; 14(1). pii: 19079.

Sotelo E, Fernandez-Pinero J, Llorente F, et al. Characterization of West Nile virus isolates from Spain: new insights into the distinct West Nile virus eco-epidemiology in the Western Mediterranean. Virology. 2009; 395(2):289-97.

Stark K, Niedrig M, Biederbick W, Merkert H, Hacker J. [Climate changes and emerging diseases. What new infectious diseases and health problem can be expected?] Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2009; 52(7):699-714. German.

Steiner I, Budka H, Chaudhuri A, et al. Viral meningoencephalitis: a review of diagnostic methods and guidelines for management. Eur J Neurol. 2010; 17(8):999-e57.

Stone R. Infectious diseases. Rival teams identify a virus behind deaths in central China. Science. 2010 ; 330(6000):20-1.

Strazielle N, Ghersi-Egea JF. Factors affecting delivery of antiviral drugs to the brain. Rev Med Virol. 2005; 15(2):105-33.

Süss J. Epidemiology and ecology of TBE relevant to the production of effective vaccines. Vaccine. 2003; 21 Suppl 1:S19-35.

Süss J. Tick-borne encephalitis in Europe and beyond--the epidemiological situation as of 2007. Euro Surveill. 2008; 13(26). pii: 18916.

Sutherst RW. Global change and human vulnerability to vector-borne diseases. Clin Microbiol Rev. 2004; 17(1):136-73.

Taylor RM, Work TH, Hurlbut HS, Rizk F. A study of the ecology of West Nile virus in Egypt. Am J Trop Med Hyg. 1956; 5(4):579-620.

Tesh RB. The genus Phlebovirus and its vectors. Annu Rev Entomol. 1988; 33:169-81.

Tesh RB, Saidi S, Gajdamovic SJ, Rodhain F, Vesenjak-Hirjan J. Serological studies on the epidemiology of sandfly fever in the Old World. Bull World Health Organ. 1976; 54(6):663-74.

Tesh RB, Modi GB. Studies on the biology of phleboviruses in sand flies (Diptera: Psychodidae). I. Experimental infection of the vector. Am J Trop Med Hyg. 1984; 33(5):1007-16.

Tesh RB, Modi GB. Maintenance of Toscana virus in Phlebotomus perniciosus by vertical transmission. Am J Trop Med Hyg. 1987; 36(1):189-93.

Tesh RB, Lubroth J, Guzman H. Simulation of arbovirus overwintering: survival of Toscana virus (Bunyaviridae:Phlebovirus) in its natural sand fly vector Phlebotomus perniciosus. Am J Trop Med Hyg. 1992; 47(5):574-81.

Thiel HJ, Collett MS, Gould EA, et al. Family Flaviviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds.): Virus taxonomy: classification and nomenclature; eighth report of the International Committee on Taxonomy of Viruses. San Diego, CA: Academic Press, 2005:981-98.

Tolle MA. Mosquito-borne diseases. Curr Probl Pediatr Adolesc Health Care. 2009; 39(4):97-140.

Trevejo RT. Acute encephalitis hospitalizations, California, 1990-1999: unrecognized arboviral encephalitis? Emerg Infect Dis. 2004; 10(8):1442-9.

Tsai TF, Chandler LJ. Arboviruses. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (eds.): Manual of Clinical Microbiology, 8th Edition. Washington, DC: ASM Press, 2003:1553-69.

Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. Lancet. 1998; 352(9130):767-71.

Tyler KL. West Nile virus infection in the United States. Arch Neurol. 2004; 61(8):1190-5.

Tyler KL. Emerging viral infections of the central nervous system: part 1. Arch Neurol. 2009 a; 66(8):939-48.

Tyler KL. Emerging viral infections of the central nervous system: part 2. Arch Neurol. 2009 b; 66(9):1065-74.

Valassina M, Meacci F, Valensin PE, Cusi MG. Detection of neurotropic viruses circulating in Tuscany: the incisive role of Toscana virus. J Med Virol. 2000; 60(1):86-90.

Valassina M, Valentini M, Pugliese A, Valensin PE, Cusi MG. Serological survey of Toscana virus infections in a high-risk population in Italy. Clin Diagn Lab Immunol. 2003; 10(3):483-4.

van Regenmortel MHV, Fauquet CM, Bishop DHL, et al. (eds.). Virus taxonomy: classification and nomenclature of viruses; seventh report of the International Committee on Taxonomy of Viruses. San Diego, CA: Academic Press, 2000.

Vazquez A, Sanchez-Seco MP, Ruiz S, et al. Putative new lineage of west nile virus, Spain. Emerg Infect Dis. 2010; 16(3):549-52.

Venter M, Human S, Zaayman D, et al. Lineage 2 west nile virus as cause of fatal neurologic disease in horses, South Africa. Emerg Infect Dis. 2009; 15(6):877-84.

Vorou RM, Papavassiliou VG, Tsiodras S. Emerging zoonoses and vector-borne infections affecting humans in Europe. Epidemiol Infect. 2007; 135(8):1231-47.

Weaver SC, Reisen WK. Present and future arboviral threats. Antiviral Res. 2010; 85(2):328-45.

Weidmann M, Sanchez-Seco MP, Sall AA, et al. Rapid detection of important human pathogenic Phleboviruses. J Clin Virol. 2008; 41(2):138-42.

Whitley RJ, Gnann JW. Viral encephalitis: familiar infections and emerging pathogens. Lancet. 2002; 359(9305):507-13.

Wu YC, Huang YS, Chien LJ, et al. The epidemiology of Japanese encephalitis on Taiwan during 1966-1997. Am J Trop Med Hyg. 1999; 61(1):78-84.

Xu F, Chen H, Travassos da Rosa AP, Tesh RB, Xiao SY. Phylogenetic relationships among sandfly fever group viruses (Phlebovirus: Bunyaviridae) based on the small genome segment. J Gen Virol. 2007; 88(Pt 8):2312-9.

Yin J, Gardner CL, Burke CW, Ryman KD, Klimstra WB. Similarities and differences in antagonism of neuron alpha/beta interferon responses by Venezuelan equine encephalitis and Sindbis alphaviruses. Virol. 2009; 83(19):10036-47.

Zacks MA, Paessler S. Encephalitic alphaviruses. Vet Microbiol. 2010; 140(3-4):281-6.

Zilber LA. Spring (spring–summer) epidemical tick-borne encephalitis. Arch Biol Nauk. 1939; 56:9-37. Russian.

Zlobin VI, Gorin OZ. Tick-borne encephalitis: etiology, epidemiology and prophylactics in Siberia. In: Philippova LB (ed.): [Kleshchevoi entsefalit: Etiologiia, Epidemiologiia I profilaktika v sibiri.] Novosibirsk: Nauka, 1996:177. Russian.

Links to selected websites:

CDC Division of Vector-Borne Infectious Diseases: http://www.cdc.gov/ncidod/dvbid/index.html

European Centre for Disease Prevention and Control (ECDC):

http://ecdc.europa.eu/en/Pages/home.aspx

European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD): http://www.enivd.org

German Consultant Laboratory for Tick-borne encephalitis and further flaviviruses:

http://www.rki.de/cln_169/nn_203794/EN/Content/Institute/DepartmentsUnits/NRC/TBE/TBE__node.html?__nnn=true

World Health Organization (WHO): http://www.who.int/en/

Chapter II.

List of published studies included in this thesis

The following publications cannot be displayed here for copyright reasons, but for further search the references are indicated as follows.

Chapter II.1

Epidemiology and surveillance of acute encephalitis

II.1.1

Analysis of the surveillance situation for viral encephalitis and meningitis in Europe

Donoso Mantke O, Vaheri A, Ambrose H, Koopmans M, de Ory F, Zeller H, Beyrer K, Windorfer A, Niedrig M; European Network for Diagnostics of Imported Viral Diseases (ENIVD) Working Group for Viral CNS Diseases

Euro Surveill. 2008; 13(3). pii: 8017

II.1.2

A survey on cases of tick-borne encephalitis in European countries

Donoso Mantke O, Schädler R, Niedrig M

Euro Surveill. 2008; 13(17). pii: 18848

Chapter II.2
Diagnostics of arboviruses

II.2.1
Chapter 26: Arthropod-borne viruses

Niedrig M, Nitsche A, Donoso Mantke O

In: Jerome KR (ed.): Lennette's Laboratory Diagnosis of Viral Infections, 4th Edition. New York, NY: Informa Healthcare, 2010:451-9, in press.

II.2.2
Serological versus PCR methods for the detection of tick-borne encephalitis virus infections in humans

Donoso Mantke O, Achazi K, Niedrig M

Future Virology. 2007; 2(6):565-72

II.2.3
Detection and differentiation of tick-borne encephalitis virus subtypes by a reverse transcription quantitative real-time PCR and pyrosequencing

Achazi K, Nitsche A, Patel P, Radonić A, Donoso Mantke O, Niedrig M

J Virol Methods. 2010 Oct 7. [Epub ahead of print]

Chapter II.3
Improvement of arboviral diagnostics

II.3.1

The European Network for Diagnostics of Imported Viral Diseases (ENIVD) – 12 years of strengthening the laboratory diagnostic capacity in Europe

Niedrig M, Donoso-Mantke O, Schädler R; ENIVD members

Euro Surveill. 2007; 12(4):E070419.5

II.3.2

Quality assurance for the diagnostics of viral diseases to enhance the emergency preparedness in Europe

Donoso Mantke O, Schmitz H, Zeller H, Heyman P, Papa A, Niedrig M; European Network for Diagnostics of Imported Viral Diseases (ENIVD)

Euro Surveill. 2005; 10(6):102-6

II.3.3

First international diagnostic accuracy study for the serological detection of West Nile virus infection

Niedrig M, Donoso Mantke O, Altmann D, Zeller H

BMC Infect Dis. 2007; 7:72

II.3.4

Quality control assessment for the serological diagnosis of tick-borne encephalitis virus infections

Niedrig M, Avsic T, Aberle SW, Ferenczi E, Labuda M, Rozentale B, Donoso Mantke O

J Clin Virol. 2007; 38(3):260-4

II.3.5

Quality control assessment for the PCR diagnosis of tick-borne encephalitis virus infections

Donoso Mantke O, Aberle SW, Avsic-Zupanc T, Labuda M, Niedrig M

Clin Virol. 2007; 38(1):73-7

Chapter II.4

Special reviews on tick-borne diseases

II.4.1

A clear and present danger: tick-borne diseases in Europe

Heyman P, Cochez C, Hofhuis A, van der Giessen J, Sprong H, Porter SR, Losson B, Saegerman C, Donoso-Mantke O, Niedrig M, Papa A

Expert Rev Anti Infect Ther. 2010; 8(1):33-50

II.4.2

Tick-borne encephalitis: Pathogenesis and clinical implications

Růžek D, Dobler G, Donoso Mantke O

Travel Med Infect Dis. 2010 Jul; 8(4):223-32

Chapter II.5
Submitted manuscripts

II.5.1
Analysis of Ixodes and Dermacentor ticks for tick-borne pathogens around Berlin

Peter Hagedorn, Timo Hillebrand, Elmara Graser, Oliver Donoso-Mantke, Doreen Vollandt, Cristina Domingo Carrasco, Matthias Niedrig

Submitted to Ticks and Tick Borne Diseases

II.5.2
Rodents as sentinels for the prevalence of tick-borne encephalitis virus

Katharina Achazi, Daniel Růžek, Oliver Donoso-Mantke, Mathias Schlegel, Hanan Sheikh Ali, Mathias Wenk, Jonas Schmidt-Chanasit, Lutz Ohlmeyer, Ferdinand Rühe, Torsten Vor, Christian Kiffner, René Kallies, Rainer G. Ulrich, Matthias Niedrig

Submitted to Vector-Borne and Zoonotic Diseases

Chapter III.
Shrnutí/Summary

Shrnutí

Infekce mozku, míchy a mozkových plen představují hlavní příčiny encefalitidy, myelitidy a meningitidy u člověka. Nicméně jisté procento těchto onemocnění zůstává bez určení etiologického původce z důvodu neschopnosti jej identifikovat.

Většina případů encefalitidy je zapříčiněna virovou infekcí: virus herpes simplex, virus varicella-zoster, virus Epsteina a Barrové, virus příušnic, virus spalniček a enteroviry jsou pokládány za hlavní původce encefalitidy u imunokompetentních jedinců. Vedle těchto klinicky častých případů mohou být příčinou encefalitidy i viry přenášené členovci (komáry nebo klíšťaty). Tyto infekce mohou být zaměněny nejčastěji za herpetické encefalitidy. V Evropě jsou nejvýznamnějšími encefalitickými arboviry virus západního Nilu, virus Toscana a virus klíšťové encefalitidy.

Způsob, jakým evropské země provádějí monitoring a diagnostiku virových encefalitid, vykazuje značnou variabilitu a tudíž data z různých zemí nejsou porovnatelná. Navzdory pokrokům PCR diagnostiky virových encefalitid zůstává etiologická příčina až v 80% případů neurčena. Význam tohoto problému narůstá díky potřebě rychlé a spolehlivé detekce nových a (re-)emergentních patogenů, jako je virus západního Nilu, či potenciálních epidemií způsobených záměrným šířením patogenů. Kvalitní diagnostika je zcela kritická pro monitoring etiologických agens virových encefalitid/meningitid. Toho však nelze dosáhnout dokud nebudou nejběžnější výše uvedení patogeny součástí rutinního testování. Kromě toho správná diagnostika a včasné zahájení odpovídající symptomatické nebo specifické terapie může omezit poškození mozku infekcí a v konečném důsledku rozhodnou o samotném výsledku onemocnění.

Evropská síť pro diagnostiku importovaných virových onemocnění (The European Network for Diagnostics of ‘Imported’ Viral Diseases - ENIVD) provedla několik studií za účelem shrnutí dostupných dat a zlepšení jejich získávání a pro posílení diagnostiky a monitoringu virových encefalitid v Evropě. Tato skupina určila

požadavky pro budoucí monitorující studie na evropské úrovni a přispěla ke zlepšení kvality prováděných diagnostických metod způsobem externího hodnocení kvality. Kromě toho důkladně sledovala současnou situaci výskytu viru západního Nilu, viru Toscana a viru klíšťové encefalitidy, čili potenciálních emergentních původců virových encefalitid.

ENIVD analyzovala současnou situaci monitoringu virových encefalitid a meningitid v Evropě, čímž shrnula dostupné zdroje epidemiologických dat u jednotlivých států Evropské unie. Dála mapovala laboratorní kapacity a identifikovala klíčové požadavky pro budoucí studie zaměřené na monitoring v evropském měřítku. Doporučili a zavedli jsme široce platné definice onemocnění a standardizovali diagnostické postupy. Jejich pomocí s využitím multiplex-microarray systémů validovaných pro široké spektrum virů, bude možné odhalit skutečnou incidenci a etiologický pattern virových encefalitid/meningitid v každé evropské zemi. Toto bude zaručovat dobrou srovnatelnost výsledků, konzistentních se systémem externího hodnocení kvality. Pro zlepšení monitoringu je rovněž důležité kvantifikovat množství případů nejasné/neznámé etiologie. Díky tomu bude snazší srovnávat data z jednotlivých zemí, případně určit slabiny monitoringových programů. Kliničtí lékaři musí být dostatečně motivováni hlásit všechny případy virových encefalitid/meningitid a snažit se vždy určit přesnou etiologickou diagnózu. Evropská studie založená na těsné spolupráci klinických lékařů, epidemiologů a mikrobiologů poskytne přesnější a časově dostupnější data o virových onemocněních centrální nervové soustavy. Taková iniciativa by mohla napomoci zvýšení frekvence správné diagnózy, snížit množství případů onemocnění neznámé etiologie, vyvinout a validovat nové diagnostické metody, zlepšit doporučení a návody, a celkově vytěžit více hodnotná data z klinických a epidemiologických programů.

Studie externího hodnocení kvality provedené sítí ENVID ukázaly, že metody užívané ve většině klinických laboratoří fungují dobře při použití virového prototypového kmene, jsou ale méně spolehlivé v případě užití recentně se vyskytujících virových kmenů/izolátů. Příkladem budiž detekce nově se objevujících kmenů viru západního Nilu, linie II, běžně se vyskytujících v Africe, či detekce sibiřských nebo dálnovýchodních kmenů viru klíšťové encefalitidy. Výskyt kmenů viru západního Nilu, linie II, byl v současné době zaznamenán v Maďarsku a Řecku.

Sibiřské a dálnovýchodní kmeny viru klíšťové encefalitidy se běžně vyskytují v některých zemích Evropské unie, jako např. v pobaltských republikách a Finsku. Výsledky externího hodnocení kvality přinesly přehled o současných diagnostických možnostech evropských laboratoří. Kromě toho byla laboratořím se slabšími výsledky nabídnuta pomoc a poskytnuty instrukce pro zvýšení kvality výsledku. V budoucnu bude existovat díky naším studiím lepší vhled do situace týkající se monitoringu virových encefalitid v Evropě.

Summary

Infectious processes in the brain, spinal cord, and meninges are considered to be the main causes of encephalitis, myelitis, and meningitis. However, a certain part of these neurological diseases remains without etiological diagnosis because of lack of understanding and/or use of unsuitable assays.

Most cases of encephalitis are caused by viral infections: herpes simplex viruses, varicella-zoster virus, Epstein-Barr virus, mumps virus, measles virus, and enteroviruses are considered to be the major causes of viral encephalitis in immuno-competent individuals. In addition to these clinically common pathogens, arthropod-borne viruses (transmitted through the bite of insects and ticks) may cause arboviral encephalitis that can be confounded with herpes simplex encephalitis. In Europe, the most important types of arboviral pathogens causing encephalitis are West Nile virus (WNV), Toscana virus (TOSV), and tick-borne encephalitis virus (TBEV).

The way in which European countries handle the surveillance and diagnostics of viral encephalitic diseases varies widely and, therefore, these issues are not comparable. Despite improvements in the PCR diagnosis of viral encephalitis, comprising analysis of cerebrospinal fluid, the etiology of up to 80% of encephalitis cases remained unknown in recent surveys. This issue is challenging when considering early detection of new and (re-) emerging pathogens such as WNV or potential outbreaks caused by deliberate release of pathogens. The reliable diagnosis is important for surveillance activities aimed at clarifying the etiological pattern of viral encephalitis/meningitis. However, this is impossible to achieve as long as routine

investigations do not include the most common pathogens (see above) in a standardized manner. Moreover, a correct (differentiated) immediate diagnosis and introduction of symptomatic or specific therapy may have a decisive influence on survival of patients, and may reduce the extent of brain injury.

The European Network for Diagnostics of ‘Imported’ Viral Diseases (ENIVD) has completed studies in order to improve compiling and collecting data and to enhance the diagnostics and monitoring of encephalitis viruses in Europe. Our group has identified requirements for a future surveillance study at the European level and intended to improve diagnostic methods by external quality assurance (EQA). Also, it is our aim to carefully monitor the present situation especially regarding WNV, TOSV, and TBEV as potential emerging arboviral causes of encephalitis.

The ENIVD has analyzed the present surveillance situation for viral encephalitis/meningitis in Europe by giving an overview of the existing epidemiological sources of information in the European Union (EU) Member States, mapping the laboratory capacity, and identifying key requirements for a possible future surveillance study at the European level. Therefore, we recommended that the introduction of a broad standard case definition and harmonized/standardized diagnostic algorithm using a multiplex-microarray system validated for a wide range of viruses may help discover the true incidence and etiological pattern of viral encephalitis/meningitis within each country. This would guarantee high performance and comparability of the results consistent with EQA programs. To improve surveillance, it is also important to quantify the extent of cases of unknown etiology, in order to allow a comparison of the data from each country and to identify possible weaknesses in the surveillance data. Therefore, clinicians must be motivated to report all cases of viral encephalitis/meningitis and to reach a definitive etiological diagnosis. A European study based on a close cooperation between clinicians, epidemiologists, and microbiologists will provide more accurate and timely data on viral CNS diseases which are of public health interest. Such an initiative could help increase case ascertainment, reduce the rate of unknown etiologies, develop and validate new diagnostic methods, improve recommendations and guidelines, and gain more valuable clinical and epidemiological data for research purposes than in the current situation.

The EQA studies performed by ENIVD showed that the assays used in most laboratories are focusing on the virus prototype strains that were predominant in recent years. Consequently, newly circulating strains like WNV lineage II, normally occurring in Africa, or the Siberian and Far Eastern subtypes of TBEV are not detected by these assays. Recently, WNV lineage II was detected in Hungary and Greece, while TBEV subtypes other than the European subtype are circulating in European countries, e.g. in the Baltic States and Finland. Based on the results of the EQA studies, an overview of the current diagnostic capacity of European laboratories was obtained and the laboratories with weaker performance received help and instructions to improve their work. For the future, there will be better insight into the European surveillance situation of viral encephalitic diseases.

Acknowledgements

Over the last five years, a huge number of people have contributed to the published studies included in this thesis and the work described therein. Without their help, support, and encouragement it would not have been possible for me to complete the objectives of the different studies. Therefore, I would like to take this opportunity to thank them all!

I am very grateful to my boss and colleague Professor Matthias Niedrig who expertly guided me through my PhD studies in the past and gave me many projects and tasks on interesting topics related to arboviruses to work on them independently during my post-doc times. With his support I was able to complete my work for this thesis, despite our full agendas and responsibilities for the laboratory. As his deputy I am much obliged for his calm character and good team spirit.

My heartfelt thanks belong to Professor Libor Grubhoffer who offered me the opportunity to do the habilitation at the University of South Bohemia and gave me guidance concerning the rules of the habilitation procedures. I hope that we will continue the good collaboration between our laboratory groups and that I can enjoy more of your guided tourist tours in beautiful South Bohemia.

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I will not forget my colleagues from my working group since you have become like a little family for me over the last years, day by day having a good atmosphere and lots of fun. Accept my profuse thanks!

Of course my deepest thanks belong to my mother, my family and my friends for their continuous support and love.

List of abbreviations

Viruses

SFV	Sandfly fever virus
TBEV	Tick-borne encephalitis virus
TOSV	Toscana virus
WNV	West Nile virus

Other abbreviations, characters or units

ADEM	Acute disseminated encephalomyelitis
approx.	Approximately
BBB	Blood–brain barrier
CDC	Centers for Disease Control and Prevention, USA
CNS	Central nervous system
CSF	Cerebrospinal fluid
CT	Computerized tomography
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
EEG	Electroencephalogram
e.g.	Exempli gratia (for example)
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
ENIVD	European Network for Diagnostics of ‘Imported’ Viral Diseases
EQA	External quality assurance
et al.	Et aliae (and others)
etc.	Et cetera (and other things, and so forth)
EU	European Union
HIA	Hemagglutination inhibition assay
HSE	Herpes simplex encephalitis
IB	Immunoblot
i.e.	Id est (that is, namely)
IFA	Immunofluorescence assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M

IH	Immunohistological analysis
incl.	Including, inclusively, included
MRI	Magnetic resonance imaging
NT	Neutralization test
PCR	Polymerase chain reaction
RKI	Robert Koch Institute
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
SLE	St. Louis encephalitis
SNP	Single nucleotide polymorphism
ss	Single-stranded
TBE	Tick-borne encephalitis
TDF	Tick-density factor
UK	United Kingdom
US	United States
VI	Virus isolation
WHO	World Health Organization
YF	Yellow fever
%	Per cent
hr	Hour
kb	Kilobase (10^3 base pair)
µg	Microgram (10^{-6} gram)
mL	Milliliter (10^{-3} liter)
min	Minute
nm	Nanometer (10^{-9} meter)
pH	Pondus hydrogenii (measure of the acidity)

Curriculum vitae

Oliver Donoso Mantke

Personal data:

- Date of birth: 23 September 1972
- Place of birth: Berlin, Germany
- Nationality: German
- Family status: Single

Formation:

20 June 2005 Graduation to PhD with „summa cum laude“ at the Free University of Berlin, Germany
Study work: „Prevalence of cardiotropic viruses in human heart tissue – Analysis of explanted hearts of heart transplant recipients and heart donors.”
(http://www.diss.fu-berlin.de/diss/receive/FUDISS_thesis_000000001656?lang=en)

13 June 2000 Diploma in Biology and Environmental Management at the Free University of Berlin, Germany. Study work: „Ice-minus bacteria (Isolation-Mutation-Application) – an alternative frost control for the agriculture.”

1997-1998 1-year-scholarship at the Universidad Complutense de Madrid, Spain
(sponsored by the German Academic Exchange Service – DAAD)

1993-2000 Study of Biology and Environmental Management at the Free University of Berlin, Germany

Professional experience:

Since 09/09 Supervisor for the European Public Health Microbiology Training Programme (EUPHEM) at the Robert Koch Institute (RKI) and Facilitator inside the EPIET Introductory Course; Organisation and Facilitation of a Laboratory Module at the RKI as part of the Master of Science for Applied Epidemiology (MSAE) study at the Charité University Hospital Berlin

Since 02/09 Facilitator inside the EPIET “Laboratory Essentials in Field Epidemiology” Training Module

Since 11/08 Lecturer in the virology practical training (block: inflammation/infection) of the reform study path as well as the F10 practical course for hygiene, microbiology, and virology of the Charité University Hospital Berlin and examiner in the objective structured clinical examination for physicians (OSCE)

Since 01/08 Deputy Head of the German Consultant Laboratory for Tick-borne encephalitis

Since 07/04 Scientific co-worker at the Robert Koch Institute (RKI) for the research and co-ordination of the European Network for Diagnostics of ‘Imported’ Viral Diseases (ENIVD)

10/00-06/04 PhD-position at the Virology department of the Robert Koch Institute (RKI), Laboratory working group of Prof. Matthias Niedrig

1998-2000 Tutor for students training in Animal Physiology at the Free University of Berlin

1996-2000 Industrial trainee at the Phytobacter GmbH (Company for microbiological products)

Supervision of diploma or PhD studies:

Under review Katharina Achazi/ PhD thesis
"Identification of an indicator for the distribution of tick-borne encephalitis virus and development of an RNA-interference strategy against tick-borne encephalitis."

29.07.05 René Kallies/ Diploma thesis
"Study of the prevalence and pathogenicity of Ljunganviruses in mice."

Further students/fellows under supervision: 2 PhD students, 1 EUPHEM fellow

Consultancy and reviews:

2010 Review expert of the Belgian project on National Reference Centers (NRC) for Human Microbiology to officially select an NRC for Tick-borne encephalitis virus

2010 Evaluator in EUPHEM training site appraisals (HPA, UK and RIVM, The Netherlands)

Since 2009 Member of the International Scientific Working group on Tick-borne encephalitis (ISW-TBE)

2009 International project reviewer for the Czech Science Foundation (GA CR)

2008 International reviewer (Assistant professor) for PhD thesis at the University of South Bohemia, Ceske Budejovice, Czech Republic

2007 Member of the Working Group on the ECDC Surveillance System (TESSy)

Since 2006 ECDC expert for infectious diseases (OJ/2005/26-10-PROC/2005/012)

2005-2007 Member of the Network Forum at the European Centre for Disease Prevention and Control (ECDC)

Reviewer for the following international scientific journals:

- Emerging Infectious Diseases
- Eurosurveillance
- Future Virology
- Intervirology
- Journal of General Virology
- Journal of Infection
- Journal of Medical Virology
- Journal of Virological Methods
- Liver Transplantation
- Vaccine
- Virus Research

Further qualifications and certifications:

Certification in tropical and travel medicine (CRM)

Certification to work with genetically modified organisms according to GenTSV §15 (BioMedConcept)

Training in small laboratory animal experimental procedures (RKI)

Training in laboratory quality management according to DIN EN ISO/IEC 17025 (DIN/Beuth)

Training of Trainers workshop „Designing Case Studies“ (ECDC, CDC)

Invited national expert in the ECDC Preparedness and Response briefing sessions

List of Publications

Published scientific and expert papers with impact factor (IF) and citation response (according to SCOPUS)

1. Rumer L, Sheshukova O, Dautel H, Donoso Mantke O, Niedrig M. Differentiation of Medically Important Euro-Asian Tick Species Ixodes ricinus, Ixodes persulcatus, Ixodes hexagonus, and Dermacentor reticulatus by Polymerase Chain Reaction. Vector Borne Zoonotic Dis. 2010 Oct 28. [Epub ahead of print] **IF: 2.607; citations: not available**

2. Růžek D, Dobler G, Donoso Mantke O. Tick-borne encephalitis: pathogenesis and clinical implications. Travel Med Infect Dis. 2010 Jul; 8(4):223-32. **IF: not available; citations: n/a**

3. Achazi K, Nitsche A, Patel P, Radonić A, Donoso Mantke O, Niedrig M. Detection and differentiation of tick-borne encephalitis virus subtypes by a reverse transcription quantitative real-time PCR and pyrosequencing. J Virol Methods. 2010 Oct 7. [Epub ahead of print] **IF: 2.133; citations: n/a**

4. Domingo C, Niedrig M, Teichmann A, Kaiser M, Rumer L, Jarman RG, Donoso-Mantke O. 2nd International external quality control assessment for the molecular diagnosis of dengue infections. PLoS Negl Trop Dis. 2010 Oct 5; 4(10). pii: e833. **IF: 4.693; citations: n/a**

5. Heyman P, Cochez C, Hofhuis A, van der Giessen J, Sprong H, Porter SR, Losson B, Saegerman C, Donoso-Mantke O, Niedrig M, Papa A. A clear and present danger: tick-borne diseases in Europe. Expert Rev Anti Infect Ther. 2010 Jan; 8(1):33-50. **IF: 2.857; citations: n/a**

6. Kunze U; ISW TBE (for the German Consultant Laboratory: Oliver Donoso Mantke). Tick-borne encephalitis: from childhood to golden age does increased mobility mean increased risk? Conference report of the 11th meeting of the International Scientific Working Group on Tick-Borne Encephalitis (ISW-TBE). Vaccine. 2010 Jan 22; 28(4):875-6. **IF: 3.616; citations: n/a**

7. Niedrig M, Zeller H, Schuffenecker I, Drosten C, Emmerich P, Rumer L, Donoso-Mantke O. International diagnostic accuracy study for the serological detection of chikungunya virus infection. Clin Microbiol Infect. 2009 Sep; 15(9):880-4. **IF: 4.014; citations: n/a**

8. Panning M, Charrel RN, Donoso Mantke O, Landt O, Niedrig M, Drosten C. Coordinated implementation of chikungunya virus reverse transcription-PCR. Emerg Infect Dis. 2009 Mar; 15(3):469-71. **IF: 6.794; citations: 2**

9. Donoso Mantke O, Schädler R, Niedrig M. A survey on cases of tick-borne encephalitis in European countries. Euro Surveill. 2008 Apr 24; 13(17). pii: 18848. **IF: n/a; citations: 17**

10. Donoso Mantke O, Vaheri A, Ambrose H, Koopmans M, de Ory F, Zeller H, Beyrer K, Windorfer A, Niedrig M; European Network for Diagnostics of Imported Viral Diseases (ENIVD) Working Group for Viral CNS Diseases. Analysis of the surveillance situation for viral encephalitis and meningitis in Europe. Euro Surveill. 2008 Jan 17; 13(3). pii: 8017. **IF: n/a; citations: 3**

11. Donoso Mantke O, Achazi K, Niedrig M. Serological versus PCR methods for the detection of tick-borne encephalitis virus infections in humans. Future Virol. 2007; 2(6):565-72. **IF: 0.713; citations: 0**

12. Donoso Mantke O, Niedrig M; ENIVD members. Laboratory capacity for detection of chikungunya virus infections in Europe. Euro Surveill. 2007 Sep 13; 12(9):E070913.2. **IF: n/a; citations: 2**

13. Niedrig M, Donoso-Mantke O, Schädler R; ENIVD members. The European Network for Diagnostics of Imported Viral Diseases (ENIVD)--12 years of strengthening the laboratory diagnostic capacity in Europe. Euro Surveill. 2007 Apr 19; 12(4):E070419.5. **IF: n/a; citations: 1**

14. Niedrig M, Donoso Mantke O, Altmann D, Zeller H. First international diagnostic accuracy study for the serological detection of West Nile virus infection. BMC Infect Dis. 2007 Jul 3; 7:72. **IF: 2.550; citations: 5**

15. Niedrig M, Avsic T, Aberle SW, Ferenczi E, Labuda M, Rozentale B, Donoso Mantke O. Quality control assessment for the serological diagnosis of tick borne encephalitis virus infections. J Clin Virol. 2007 Mar; 38(3):260-4. **IF: 3.124; citations: 6**

16. Donoso Mantke O, Kallies R, Niklasson B, Nitsche A, Niedrig M. A new quantitative real-time reverse transcriptase PCR assay and melting curve analysis for detection and genotyping of Ljungan virus strains. J Virol Methods. 2007 Apr; 141(1):71-7. **IF: 2.133; citations: 14**

17. Donoso Mantke O, Aberle SW, Avsic-Zupanc T, Labuda M, Niedrig M. Quality control assessment for the PCR diagnosis of tick-borne encephalitis virus infections. J Clin Virol. 2007 Jan; 38(1):73-7. **IF: 3.124; citations: 6**

18. Donoso Mantke O, Meyer R, Prösch S, Nitsche A, Leitmeyer K, Kallies R, Niedrig M. High prevalence of cardiotropic viruses in myocardial tissue from explanted hearts of heart transplant recipients and heart donors: a 3-year retrospective study from a German patients' pool. J Heart Lung Transplant. 2005 Oct; 24(10):1632-8. **IF: 3.541; citations: 11**

19. Donoso Mantke O, Schmitz H, Zeller H, Heyman P, Papa A, Niedrig M; European Network for Diagnostics of Imported Viral Diseases (ENIVD). Quality assurance for the diagnostics of viral diseases to enhance the emergency preparedness in Europe. Euro Surveill. 2005 Jun; 10(6):102-6. **IF: n/a; citations: 9**

20. Hattermann K, Müller MA, Nitsche A, Wendt S, Donoso Mantke O, Niedrig M. Susceptibility of different eukaryotic cell lines to SARS-coronavirus. Arch Virol. 2005 May; 150(5):1023-31. **IF: 1.909; citations: 14**

21. Donoso Mantke O, Nitsche A, Meyer R, Klingel K, Niedrig M. Analysing myocardial tissue from explanted hearts of heart transplant recipients and multi-organ donors for the presence of parvovirus B19 DNA. J Clin Virol. 2004 Sep; 31(1):32-9. **IF: 3.124; citations: 10**

22. Lemmer K, Donoso Mantke O, Bae HG, Groen J, Drosten C, Niedrig M. External quality control assessment in PCR diagnostics of dengue virus infections. J Clin Virol. 2004 Aug; 30(4):291-6. **IF: 3.124; citations: 11**

23. Donoso Mantke O, Meyer R, Prösch S, Niedrig M. Frequent detection of viral nucleic acids in heart valve tissue. J Clin Microbiol. 2004 May; 42(5):2298-300. **IF: 4.162; citations: 1**

24. Donoso Mantke O, Lemmer K, Biel SS, Groen J, Schmitz H, Durand JP, Zeller H, Niedrig M. Quality control assessment for the serological diagnosis of dengue virus infections. J Clin Virol. 2004 Feb; 29(2):105-12. **IF: 3.124; citations: 5**

25. Niklasson B, Hörnfeldt B, Nyholm E, Niedrig M, Donoso-Mantke O, Gelderblom HR, Lernmark A. Type 1 diabetes in Swedish bank voles (Clethrionomys glareolus): signs of disease in both colonized and wild cyclic populations at peak density. Ann N Y Acad Sci. 2003 Nov; 1005:170-5. **IF: 2.670; citations: 21**

26. Biel SS, Donoso Mantke O, Lemmer K, Vaheri A, Lundkvist A, Emmerich P, Hukic M, Niedrig M. Quality control measures for the serological diagnosis of hantavirus infections. J Clin Virol. 2003 Dec; 28(3):248-56. **IF: 3.124; citations: 5**

Book chapter

1. Niedrig M, Nitsche A, Donoso Mantke O. Chapter 26: Arthropod-Borne Viruses. In: Jerome KR (ed.): Lennette's Laboratory Diagnosis of Viral Infections, 4th Edition. New York, NY: Informa Healthcare, 2010:451-9, in press.

2. Donoso Mantke O, Domingo C, Radonic A, Hagedorn P, Achazi K, Niedrig M. Chapter 24: Tick-borne encephalitis virus. In: Liu D (ed.): Molecular detection of human viral pathogens. Boca Raton, FL: CRC Press, 2011: 271-82.

List of oral presentations at scientific conferences

1. Donoso Mantke O, Meyer R, Pregla R, Hetzer R, Prösch S, Schreier E, Niedrig M. Prevalence of cardiotropic viruses in cardiac transplant recipients and donor hearts – What does it mean for heart valve banking? 1st BIS-Symposium on the Future of Heart Valve Banking, Berlin, 2001.

2. Donoso Mantke O, Lemmer K, Niedrig M and members of the ENIVD. Quality control measures for the sero-diagnosis of Dengue virus infections. Winter Meeting/Joint Meeting of the Sociedade Portuguesa de Virologia and the European Society for Clinical Virology, Estoril (Portugal), 2003: Abstracts #10.

3. Donoso Mantke O, Meyer R, Prösch S, Hetzer R, Niedrig M. Detection of viral genome sequences in myocardial tissue of heart valve donors – A risk to the health of homograft recipients? Infection 2003; 31 (Suppl 1):69. Tagungsband der Deutschen Gesellschaft für Infektionskrankheiten und der Paul Ehrlich Gesellschaft für Chemotherapie. 7. Kongress für Infektionskrankheiten und Tropenmedizin (KIT 2003), 27 February – 1 March 2003, Berlin.

4. Donoso Mantke O, Sonnenberg K, Yan H, Lehmann C, Pfeffer T, Stöcker W, Niedrig M. Antibody response in patients infected with the new coronavirus causing severe acute respiratory syndrome (SARS). [Round table: “SARS y otros virus emergentes”] VIII Congreso Nacional de Virologia, Barcelona (Spain), 2003.

5. Donoso Mantke O. Proposal for a Research Training Network of the ENIVD group. 14th Meeting of the European Network for Diagnostics of ‘Imported’ Viral Diseases (ENIVD), Rome (Italy), 2005.

6. Donoso Mantke O, Meyer R, Prösch S, Nitsche A, Leitmeyer K, Kallies R, Niedrig M. High prevalence of cardiotropic viruses in myocardial tissue from explanted hearts of heart trans-

plant recipients and heart donors: a 3-year retrospective study from a German patients' pool. Infection 2005; 33:191. Tagungsband der Deutschen Gesellschaft für Infektionskrankheiten und der Paul Ehrlich Gesellschaft für Chemotherapie. 8. Kongress für Infektionskrankheiten und Tropenmedizin (KIT 2005), 9 – 11 June 2005, Hamburg.

7. Donoso Mantke O, Niedrig M. Prevalence of Parvovirus B19 and other myocardiotropic viruses in myocardial tissue from explanted hearts of heart transplant recipients and heart donors: A 3-year retrospective study from a German patients' pool. International Parvovirus Meeting, Leipzig, 2005: Abstracts S. 22-23.

8. Donoso Mantke O. Summary report of the meeting on diagnostic and surveillance of viral encephalitis in European countries. 15th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD), Warsaw (Poland), 2006.

9. Donoso Mantke O. Evaluation report of the Research Training Network of the ENIVD group (ENIVD RTN). 15th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD), Warsaw (Poland), 2006.

10. Donoso Mantke O. The European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD) – Improving the preparedness for diagnostic of biological threats in Europe and worldwide. Meeting on Molecular Diagnostics in the Clinical Laboratory – Present and future of EQAP Networks in Molecular Diagnostics, Mannheim, 2006.

11. Donoso Mantke O. Report on the activities from Work Package 3 – Establish basis for the development of a European surveillance system for human encephalitis. 16th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD), Limassol (Cyprus), 2007.

12. Donoso Mantke O. The Research Training Network of the ENIVD group – Initial training and research on the diagnostic and pathogenesis of rodent- and arthropod-borne viral diseases. 16th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD), Limassol (Cyprus), 2007.

13. Donoso Mantke O. Presentation of the Robert Koch Institute, Berlin. Seminar at the Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Ceske Budejovice (Czech Republic), 10 January 2008.

14. Donoso Mantke O. Improving the diagnostic and monitoring of encephalitis viruses in Europe with the support of the ENIVD – Survey on tick-borne encephalitis. Seminar at the Institute of

Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, Ceske Budejovice (Czech Republic), 10 January 2008.

15. Donoso Mantke O. Survey on tick-borne encephalitis. 17th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD), Madrid (Spain), 2008.

16. Donoso Mantke O. Draft situation report on the diagnostic capacity and quality assurance activities in Europe for the detection of emerging and re-emerging viral diseases. 17th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD), Madrid (Spain), 2008.

17. Donoso Mantke O. Flaviviruses: New Insights on Old Foes. 1st FEBS International Summer School 2008 "Pathogen-Host Interplay", Berlin-Potsdam, 25 July 2008.

18. Donoso Mantke O. Tick-borne diseases. 1st EPIET Laboratory Essentials in Field Epidemiology Module, Bilthoven (The Netherlands), 23-27 February 2009.

19. Donoso Mantke O. Virusübertragungen durch Mücken und Zecken. Fortbildungsseminar für Technische Assistentinnen und Assistenten am Max-Planck-Institut für Infektionsbiologie, Berlin, 21 April 2009.

20. Donoso Mantke O. Surrogate markers for tick-borne encephalitis virus in Germany. 18th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD)/1st Meeting of the ENIVD Collaborative Laboratory Response Network (ENIVD-CLRN), Prague (Czech Republic), 2009.

21. Donoso Mantke O. WP4: Preparedness activities. 18th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD)/1st Meeting of the ENIVD Collaborative Laboratory Response Network (ENIVD-CLRN), Prague (Czech Republic), 2009.

22. Donoso Mantke O. Tick-borne diseases. ZIBI International Summer School 2009 "Pathogen-Host Interplay", Berlin, 22 July 2009.

23. Donoso Mantke O. The role of the laboratory in surveillance and outbreak investigations. 15th EPIET Introductory Course on Intervention Epidemiology, Lazareto (Menorca/ Spain), 28 September – 16 October 2009.

24. Donoso Mantke O. TBE Surveillance in Europe. 12th ISW-TBE Meeting, Vienna (Austria), 28-29 January 2010.

25. Donoso Mantke O. WP4: Preparedness activities. 19th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD)/2nd Meeting of the ENIVD Collaborative Laboratory Response Network (ENIVD-CLRN), Stockholm (Sweden), 2010.

26. Donoso Mantke O. Preliminary results of the TBE survey in Europe II. 19th Meeting of the European Network for Diagnostics of 'Imported' Viral Diseases (ENIVD)/2nd Meeting of the ENIVD Collaborative Laboratory Response Network (ENIVD-CLRN), Stockholm (Sweden), 2010.

27. Donoso Mantke O. Tick-borne diseases. Göttinger Infektiologisches Kolloquium, Göttingen, 16 June2010.

28. Donoso Mantke O. Tick-borne diseases. ZIBI International Summer School 2010 "Pathogen-Host Interplay", Berlin, 14 July 2010.

29. Donoso Mantke O, Escadafal C, Pfeffer M. Tick-borne encephalitis in the EU: epidemiology, clinics, and prevention. Expert consultation on Tick-borne diseases with emphasis on Lyme borreliosis and Tick-borne encephalitis, Stockholm, 23-24 November 2010.

List of posters at scientific conferences

1. Donoso Mantke O, Meyer R, Pregla R, Prösch S, Hetzer R, Niedrig M. Prevalence of cardiotropic viruses in cardiac transplant recipients and donor hearts – Relevance for heart valve banking? Jahrestagung der Gesellschaft für Virologie, Erlangen, 2002: Abstracts S. 94.

2. Donoso Mantke O, Meyer R, Pregla R, Prösch S, Hetzer R, Niedrig M. Prevalence of cardiotropic viruses in cardiac transplant recipients and donor hearts – Relevance for heart valve banking? XIIth International Congress of Virology, Paris (France), 2002: Abstracts 90-V-579, S. 199.

3. Donoso Mantke O, Biel SS, Lemmer K, Niedrig M and members of ENIVD. Results of the first and second external quality assurance on the sero-diagnostics of Hantavirus infections. XIIth International Congress of Virology, Paris (France), 2002: Abstracts 115-V-951, S. 319.

4. Donoso Mantke O, Meyer R, Prösch S, Hetzer R, Niedrig M. Detection of viral genome sequences in myocardial tissue of heart valve donors – A risk to the health of homograft recipients? Winter Meeting/Joint Meeting of the Sociedade Portuguesa de Virologia and the European Society for Clinical Virology, Estoril (Portugal), 2003: Abstracts #25.

5. Lemmer K, Donoso Mantke O, Bae HG, Niedrig M and members of the ENIVD. Quality control measures in PCR diagnostics of Dengue virus infections. Winter Meeting/Joint Meeting of the Sociedade Portuguesa de Virologia and the European Society for Clinical Virology, Estoril (Portugal), 2003: Abstracts #26.

6. Donoso Mantke O, Meyer R, Prösch S, Hetzer R, Niedrig M. Detection of viral genome sequences in myocardial tissue of heart valve donors – A risk to the health of homograft recipients? Jahrestagung der Gesellschaft für Virologie, Berlin, 2003: Abstracts CLI 07, S. 169.

7. Donoso Mantke O, Nitsche A, Meyer R, Niedrig M. A new quantitative real-time PCR assay for the detection of Parvovirus B19 DNA in myocardial tissue of heart transplant recipients and multi-organ donors. Jahrestagung der Gesellschaft für Virologie, Berlin, 2003: Abstracts CLI 08, S. 170.

8. Lemmer K, Donoso Mantke O, Bae HG, Niedrig M for the members of ENIVD. Quality control measures in PCR diagnostics of Dengue virus infections. Jahrestagung der Gesellschaft für Virologie, Berlin, 2003: Abstracts EMV 06, S. 398.

9. Donoso Mantke O, Lemmer K, Niedrig M for the members of ENIVD. Quality control measures for the sero-diagnosis of Dengue virus infections. Jahrestagung der Gesellschaft für Virologie, Berlin, 2003: Abstracts EMV 07, S. 399.

10. Donoso Mantke O, Meyer R, Prösch S, Hetzer R, Niedrig M. Detection of viral genome sequences in myocardial tissue of heart valve donors – A risk to the health of homograft recipients? Pathol Res Pract 2003; 199:186. Tagungsband der Deutschen Gesellschaft für Pathologie und der Deutschen Gesellschaft für Zytologie. Jahrestagung, 11 - 14 June 2003, Bamberg.

11. Niedrig M, Donoso Mantke O, Meyer H, Becker S, ter Meulen J, Nitsche A, Ellerbrok H, Schmitz H, Drosten C. Quality control measures for viral haemorrhagic fever viruses (Filo-, Lassa-) and Orthopox viruses. 2nd European Meeting on Viral Zoonoses, St. Raphaël (France), 2003: Abstracts 50, S. 62.

12. Donoso Mantke O, Meyer R, Prösch S, Niedrig M. Screening for cardiotropic viruses in myocardial tissue from explanted hearts of heart transplant recipients and multi-organ donors – Results from a three-year retrospective study. 2nd European Congress of Virology/Eurovirology, Madrid (Spain), 2004: Abstracts P7-3, S. 171.

13. Kallies R, Donoso Mantke O, Niklasson B, Niedrig M. Process of Ljungan virus infection in animals. Jahrestagung der Gesellschaft für Virologie, Hannover, 2005: Abstracts PSV 10, S. 320.

14. Donoso Mantke O, Niklasson B, Kallies R, Nitsche A, Meyer R, Niedrig M. First quantitative real-time reverse transcriptase PCR assay for detection of Ljungan virus (LV). Infection 2005; 33:220. Tagungsband der Deutschen Gesellschaft für Infektionskrankheiten und der Paul Ehrlich Gesellschaft für Chemotherapie. 8. Kongress für Infektionskrankheiten und Tropenmedizin (KIT 2005), 9 - 11 June 2005, Hamburg.

15. Donoso Mantke O, Aberle S, Avsic-Zupanc T, Labuda M, Niedrig M. Quality control assessment for the PCR diagnosis of TBEV infections. J Clin Virol 2006; 36 (Suppl 3): S25. Abstracts of the Joint Meeting of the European Society for Clinical Virology and the Clinical Virology Group of the Society for General Microbiology, Birmingham (UK), 3 - 6 September 2006.

16. Donoso Mantke O, Niedrig M & members of ENIVD. External quality assurance programmes for the diagnostics of viral diseases to enhance the emergency preparedness in Europe. International Meeting on Emerging Diseases and Surveillance, Vienna (Austria), February 23-25, 2007: Abstracts 13.016, S. 97.

17. Achazi K, Donoso Mantke O, Nitsche A, Niedrig M. A new quantitative RT-PCR able to detect all tick-borne encephalitis virus subtypes. IX International Jena Symposium on Tick-borne Diseases 2007: Abstracts 40, S. 139.

18. Donoso Mantke O, Aberle SW, Avsic-Zupanc T, Labuda M, Ferenczi E, Rozentale B, Niedrig M. External quality assurance studies for the serological and PCR diagnostics of tick-borne encephalitis virus infections. IX International Jena Symposium on Tick-borne Diseases 2007: Abstracts 41, S. 140.

19. Donoso Mantke O, Niedrig M & members of ENIVD. External quality assurance programmes for the diagnostics of viral diseases to enhance the emergency preparedness in Europe. 17th ECCMID/ 25th ICC 2007, Network Corner.

20. Donoso Mantke O, Aberle SW, Avsic-Zupanc T, Labuda M, Ferenczi E, Rozentale B, Niedrig M. External quality assurance studies for the serological and PCR diagnostics of tick-borne encephalitis virus infections. 4th European Meeting on Viral Zoonoses, St. Andrews (Scotland), 2007: Abstracts 38, S. 49.

21. Donoso Mantke O, Linke S, Zeller H, Drosten C, Altmann D, Niedrig M. First international diagnostic accuracy studies for the serological and molecular detection of West Nile virus infection. 4th European Meeting on Viral Zoonoses, St. Andrews (Scotland), 2007: Abstracts 39, S. 50.

22. Achazi K, Donoso Mantke O, Nitsche A, Niedrig M. A new quantitative RT-PCR able to detect all tick-borne encephalitis virus subtypes. 4th European Meeting on Viral Zoonoses, St. Andrews (Scotland), 2007: Abstracts 45, S. 56.

23. Achazi K, Donoso Mantke O, Nitsche A, Patel P, Paliwal R, Niedrig M. Surrogate marker for tick-borne encephalitis virus in Germany. X International Jena Symposium on Tick-borne Diseases 2009: Abstracts P69, S. 147.

24. Niedrig M, Brown D, Koopmans M, Kurkela S, Dittrich S, Bremer V, Leitmeyer K, Donoso-Mantke O. The European Public Health Microbiology Training Program (EUPHEM), 2008-2011. 19th ECCMID 2009, European Network Corner.

25. Domingo C, Donoso Mantke O, Jarman R, Niedrig M. 2nd proficiency test for molecular detection of dengue virus. Emerging Infectious Diseases 2009, Singapore: Abstracts, p. 159.

26. Hagedorn P, Rumer L, Donoso Mantke O, Niedrig M. Prevalence of Anaplasma, Babesia, Borrelia burgdorferi and Rickettsia spec. in different hard ticks species in the area of Berlin, Germany. EDEN 2010, Emerging vector-borne diseases in a changing European environment, Montpellier (France): Abstracts, p. 112.

27. Escadafal C, Pfeffer M, Donoso Mantke O. Survey on cases of tick-borne encephalitis in Europe. ESCAIDE, Lisbon (Portugal), 11-13 November 2010: Abstracts, p. 172.