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Viral zoonoses in Europe

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Abstract

A number of new virus infections have emerged or re-emerged during the past 15 years. Some viruses are spreading to new areas along with climate and environmental changes. The majority of these infections are transmitted from animals to humans, and thus called zoonoses. Zoonotic viruses are, as compared to human-only viruses, much more difficult to eradicate. Infections by several of these viruses may lead to high mortality and also attract attention because they are potential bioweapons. This review will focus on zoonotic virus infections occurring in Europe.

Keywords: Viral zoonoses; Europe; Emerging infections; Arbovirus; Vertebrate-borne viruses; Vector-borne viruses

Contents

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1. Introduction

During the past 15 years a number of new virus infections have emerged or re-emerged. Most of them, such as Sin Nombre and Andes hantaviruses, SARS coronavirus, avian influenza, Nipah and Hendra viruses, have appeared in subtropical or tropical regions. Dengue is spreading to new areas and West Nile virus has reached the New World. Infections by several of these viruses may lead to high mortality and also attract attention because they are potential bioweapons. Some viruses such as tick-borne encephalitis virus are spreading to new areas along with climate and environmental changes. Most of these infections are zoonoses and clearly viruses shared by animals and humans are, unlike human-only viruses, much more difficult to eradicate. Here, we review zoonotic virus infections occurring in Europe. The infections like Lassa fever and dengue that are imported to Europe but are not indigenous to European nature will not be discussed in detail in the review. We have divided the virus infections into two categories, those that are transmitted to humans directly from vertebrate animals (like rodents, foxes, bats and birds) and those that are primarily transmitted by arthropods (mosquitoes, ticks, sandflies). The latter class is formed by arboviruses but notably they have vertebrate hosts in nature.

2. Vertebrate-borne viruses

2.1. Hantaviruses: a prime example of emerging and re-emerging infections

2.1.1. Virology

Hantaviruses are enveloped viruses with a tri-segmented negative-stranded genome and belong to the family *Bunyaviridae* [1,2]. The 6.4 kb L (large) segment RNA encodes the ~250 kDa RNA polymerase, the ~3.6 kb M (medium) segment the two glycoproteins 68–76 kDa Gn and 52–58 kDa Gc, – formerly known as G1 and G2; and the ~1.7 kb S (small) segment the 50–54 kDa nucleocapsid protein (N) (Table 1, Fig. 1). In addition, the S segment of some hantaviruses has another open reading frame named Ns but its product or function remains to be discovered. Viral messenger RNAs of the members of the *Bunyaviridae* are not polyadenylated and are truncated relative to the genome RNAs at the 3′ termini. Messenger RNAs have 5′-methylated caps and 10–18 nontemplated nucleotides which are derived from host cell mRNAs. The termini of all three segments are conserved and complementary to each other, a feature that has assisted in cloning and discovery of new hantaviruses. Unlike most other *Bunyaviridae*, hantaviruses are not arthropod-borne (arboviruses), but are ROdent-BOrne, roboviruses. Each hantavirus is primarily carried by a distinct rodent/insectivore species although a few host switches seem to have occurred during the tens of millions of years of their co-evolution with their carrier animals [3]. We now know that the genetic diversity of hantaviruses is generated partly by (i) genetic drift (accumulation of point mutations and insertions/deletions) leading to quasispecies [4,5], (ii) genetic shifts (reassortments of genome fragments within the same virus genotype/species), and (iii) according to recent findings [6,7], by homologous recombination, a mechanism not previously observed for negative-strand RNA viruses.

2.1.2. Ecology and epidemiology

Hantaviruses, which cause hemorrhagic fevers with renal syndrome (HFRS) in Eurasia and hantavirus cardiopulmonary syndrome (HCPS) in the Americas, are prime examples of emerging and re-emerging infectious agents. Like most of these infections hantaviral diseases are zoonoses. With the exception of the South-American Andes virus, which can be transmitted directly from human to human, hantavirus infections are thought to be transmitted to humans primarily from aerosols of rodent excreta. Only some

Table 1
Viral structure

Genus (*Family*)	Genome	Genome size (kb)	Genome segments	Lipid envelope	Virion size (nm)	Proteins
Hantavirus (*Bunyaviridae*)	ssRNA, neg	11–12	3	+	80–120	L, Gn/G1, Gc/G2, N, (Ns)
Lyssavirus (*Rhabdoviridae*)	ssRNA, neg	12	1	+	200 × 60	N, P, M, G, L
Arenavirus (*Arenaviridae*)	ssRNA, neg	10–14	2	+	50–300	N, G1, G2, L, Z
Orthopoxvirus (*Poxviridae*)	dsDNA	160–220	1	+	220–450	Appr. 200 ORF's
Orthomyxovirus (*Orthomyxoviridae*)	ssRNA, neg	10–14	6–8	+	80–120	PB1, PB2, PA, HA, NP, NA, NB, M1, M2, BM2, NS1, NS2
Alphavirus (*Togaviridae*)	ssRNA, pos	8–12	1	+	70	NSP1–4, C, E1, E2
Flavivirus (*Flaviviridae*)	ssRNA, pos	10–11	1	+	40–60	C, M, E, NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5
Nairovirus (*Bunyaviridae*)	ssRNA, neg	18–19	3	+	80–120	L, Gn/G1, Gc/G2, N
Orthobunyavirus (*Bunyaviridae*)	ssRNA, neg	11–21	3	+	80–120	N, NSs, G1, G2, NSm, L
Phlebovirus (*Bunyaviridae*)	ssRNA, neg	11–12	3	+	80–120	L, Gn/G1, Gc/G2, N, Ns

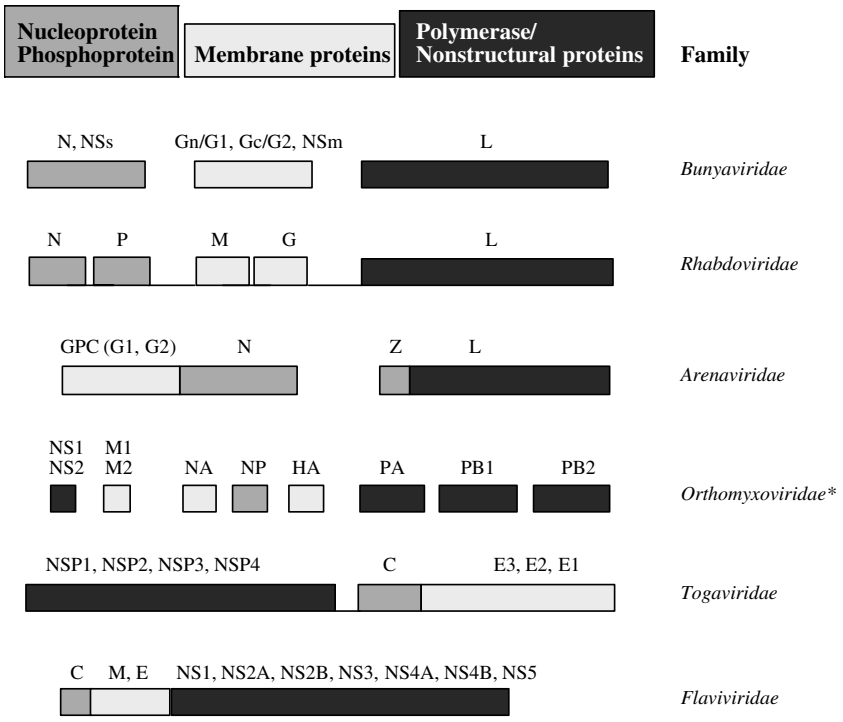

Fig. 1. Schematic representation of genome structures and expression strategies of the RNA virus families described in this review. (*) Segments and proteins according to Influenza A virus.

hantaviruses cause disease in humans. In Europe there are three major hantaviral pathogens [8] (Table 2, Fig. 2): Puumala virus carried by the bank vole (*Clethrionomys glareolus*) causes a relatively mild disease, also known as nephropathia epidemica (NE); Dobrava virus carried by the yellow necked mouse, *Apodemus flavicollis*, causes severe HFRS; and the genetically and antigenically closely related Saaremaa virus carried by the field mouse, *Apodemus agrarius*, causes mild NE-like disease. There are also reports of Seoul virus carried by rats (*Rattus norvegicus* and *Rattus rattus*) which causes HFRS of intermediate severity. In addition, European common voles (*Microtus arvalis* and *Microtus rossiaemeridionalis*) carry Tula hantavirus, which can infect humans but apparently asymptomatically [9,10]. Topografov hantavirus isolated from Siberian lemmings (*Lemmus sibiricus*) has not been detected in North European lemmings (*Lemmus lemmus*) although it can grow in them [11]. Infections in rodents are mainly asymptomatic and persistent.

Hantavirus infections are quite common in Europe [8] (Table 3), Puumala virus is common in Northern Europe, European Russia and parts of Central-Western Europe (Fig. 2). Dobrava virus is found mainly in the Balkans (Fig. 2). Saaremaa virus has been detected in Eastern and Central Europe but its epidemiology is not well defined (Fig. 2). Apart from laboratory infections [12,13] Seoul virus has been detected in wild rats, only in France [14]. It is also apparent that many parts of Europe, such as Britain, Poland and Byelorussia, remain "white" on the European hantavirus map [8]. This means either that HFRS is rare or nonexistent in these regions or is not widely recognized and diagnosed by the biomedical community.

In Northern Europe HFRS as well as the carrier rodents exhibit peaks in 3–4 year cycles [15] while in Central Europe the HFRS incidence follows the fluctuations of "mast years", i.e. the availability of beech and oak seeds for the hantavirus-carrying rodents. In Central Europe HFRS peaks in the summer while in Northern Europe most cases occur in late autumn and early winter, from November to January. Risk factors to catch hantavirus infections and HFRS include professions such as forestry, farming, and military, or activities such as camping, and the use of summer cottages. Males are more likely to be exposed than females [15,16].

2.1.3. Clinical picture and pathogenesis

Puumala and Dobrava viruses both cause HFRS but the infections differ considerably in severity [17]: both are characterized by acute-onset fever, headache, abdominal pains, backache, temporary renal insufficiency – first oliguria, proteinuria and increase in serum creatinine and then polyuria – and thrombocytopenia but the extent of hemorrhages (hematuria, petecchiae, internal hemorrhages), requirement for dialysis treatment, hypotension and mortality are much higher in

Table 2
Zoonotic viruses circulating in Europe

Genus (*Family*)	Virus	Carrier (host/vector)	Disease in humans	Mortality
Hantavirus (*Bunyaviridae*)	Puumala	*Clethrionomys glareolus* (bank vole)	HFRS (mild, NE)	0.1%
	Dobrava	*Apodemus flavicollis* (yellow-necked mouse)	HFRS (severe)	10%
	Saaremaa	*Apodemus agrarius (striped field mouse)*	HFRS (NE-like)	Low
	Seoul	*Rattus norvegicus* and *Rattus rattus* (rat)	HFRS (intermediate)	1–2%
	Tula	*Microtus arvalis (European common vole)*	Apathogenic?	
Lyssavirus (*Rhabdoviridae*)	Classical rabies	Dog, fox, raccon dog, North American bats	Rabies (encephalitis)	100%
	EBLV 1a, b	*Eptisicus sp* (insectivorous bat)	Rabies (encephalitis)	100%
	EBLV 2a, b	*Myotis sp* (insectivorous bat)	Rabies (encephalitis)	100%
Arenavirus (*Arenaviridae*)	LCMV	*Mus musculus* (house mouse)	Meningoencephalitis	Low
Orthopoxvirus (*Poxviridae*)	Cowpox	*Apodemus, Clethrionomys, Microtus* rodents	Skin eruptions	1/ca.70
Orthomyxovirus (*Orthomyxoviridae*)	Influenza A/H7N7	Wild aquatic birds	Conjunctivitis, respiratory infection	1/85
Alphavirus (*Togaviridae*)	Sindbis	Birds/*Culex, Culiseta* (mosquitoes)	Rash, arthritis/arthralgia	None reported
Flavivirus (*Flaviviridae*)	TBE	*Ixodes* spp. (ticks)	Encephalitis	0.5%
	Louping Ill	*Ixodes* spp. (ticks)	Encephalitis	Low
	West Nile	*Culex* spp. (mosquitoes)	Encephalitis	Low
	Usutu	*Culex* spp. (mosquitoes)	Rash, flu-like illness	None reported
Nairovirus (*Bunyaviridae*)	CCHF	*Hyalomma, Rhipicephalus, Dermatocento* (ticks)	HF	20–35%
Orthobunyavirus (*Bunyaviridae*)	Inkoo	*Aedes sp* (mosquitoes)	Meningitis, encephalitis	Not reported
	Tahyna	*Aedes sp* (mosquitoes)	Meningitis, encephalitis	Occasionally
Phlebovirus (*Phleboviridae*)	Toscana Fever	*Phlebotomus perniciosus* (sandfly)	Meningitis, encephalitis	Not reported
	Sandfly Fever	*Phlebotomus papatasii* (sandfly)	Meningitis, encephalitis	Not reported

Dobrava HFRS than in NE. About a third of NE patients experience temporary visual disturbances (myopia), which is a very characteristic, if not pathognomonic sign of the disease. Notably, the clinical consequences of all of the hantaviral pathogens in humans vary from asymptomatic to lethal. Severe NE is associated with a certain haplotype, HLA-B8, DR3, DQ2 alleles [18]. However, although Puumala virus infection is generally associated with mild HFRS, NE may have significant long-term consequences. A 5-year follow-up study demonstrated that 20% of the patients show an increase in systolic blood pressure and proteinuria [19]. This is important since the infection is so common in many areas of Europe [8] (Table 3, Fig. 2). In addition, in some patients Puumala virus infection may invade the pituitary gland and lead to mortality or at least hypophyseal insufficiency requiring hormone-replacement therapy [20].

The pathogenesis of HFRS is poorly understood [17,21]. However, it is known that β3 integrins can mediate the entry of pathogenic hantaviruses [22] and that hantaviruses can regulate apoptosis [23–26,28]. Also there is evidence [17,21] that increased capillary permeability is an essential component in the pathogenesis of both HFRS and HCPS, although different target tissues, kidneys and lungs are affected in the two diseases. HFRS patients show locally increased levels of TNF-α in the plasma and kidneys [27,28] and high levels of urinary secretion of the proinflammatory cytokine IL-6 [29]. Studies with a monkey model mimicking human Puumala virus infection [30] may assist in elucidating the mechanism of pathogenesis.

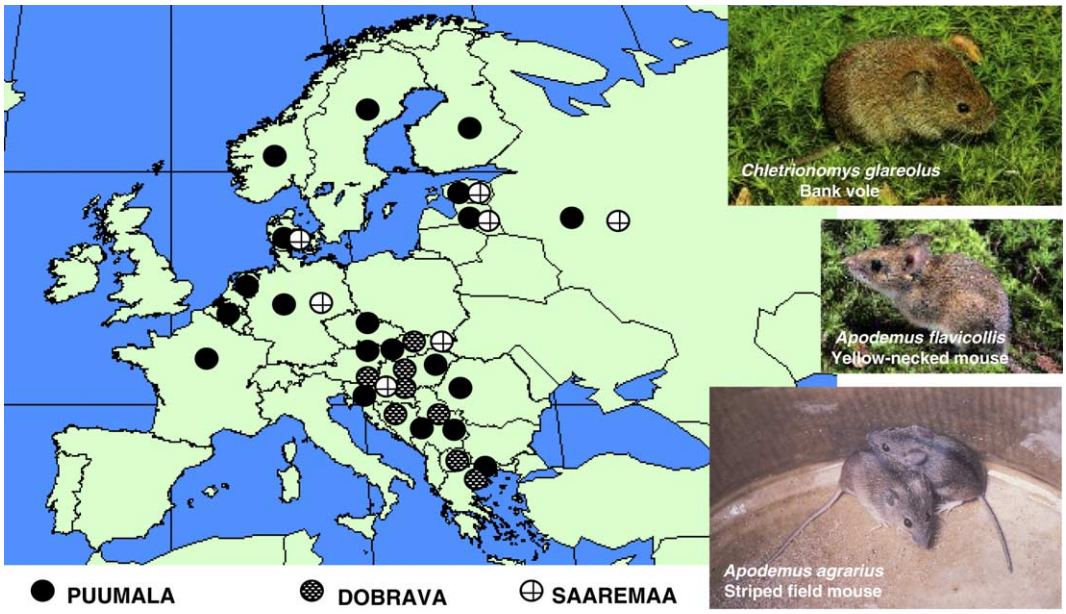

Fig. 2. Geographic distribution of hantaviruses pathogenic to humans in Europe.

Table 3
Human hantavirus infections in Europe

Region	Puumala	Dobrava	Saaremaa	Cases/year[a]	Seroprevalence
European Russia	+		+	3000	6%
Finland	+			1000	5%, 20% in areas
Sweden	+			300	8% in Northern part
Germany	+		+	100	1–3%
France	+			100	
Belgium	+			100	1.5%
Norway	+			100	
Slovenia	+	+	+	15	2%
Netherlands	+			10	1%
Denmark	+		+	10	1% in some areas
Slovakia	+		+	10	
Bosnia-Herzegovina	+	+		10	
Greece	+	+		5	4%
Estonia	+		+		9%
Latvia	+		+		4%
Austria	+				1.2%
Czech Republic					1–2%
Hungary	+	+			
Portugal					1%
Albania		+			
Yugoslavia	+	+			

[a] Number of cases diagnosed serologically. The numbers are estimations.

Of the four structural proteins, both in humoral and cellular immunity, the N protein appears to be the principal immunogen [31]. Cytotoxic T-lymphocyte (CTL) responses are seen [32] and may be important both for protective immunity and pathogenesis of hantavirus infections [21].

2.1.4. Diagnostics and prevention

The diagnosis of acute HFRS is primarily based on serology, since viral RNA cannot be regularly detected in the blood or urine of patients [33,34]. Both immunofluorescence tests and enzyme immunoassays are widely used for detection of specific IgM or low-avidity IgG antibodies, characteristic of acute infection [35–37]. In addition, immunochromatographic 5-min IgM-antibody tests [38,39] have been developed. Vaccines against hantavirus infections have been used for years in China and Korea, but not in Europe or the Americas [40]. No specific therapy is used in Europe, although both ribavirin and interferon-α have been successfully used in trials

in China [41,42]. A major problem is that at the time HFRS patients are hospitalized, virus replication is already disappearing.

2.2. Lyssaviruses

2.2.1. Virology

Members of the genus Lyssavirus within the family *Rhabdoviridae* are bullet-shaped, enveloped viruses approximately 60 nm in diameter and 200 nm in length. The ~12 kb non-segmented negative-strand genome encodes five proteins (starting from the 3′ end): the 58–62 kDa nucleoprotein (N), the 35–40 kDa phosphoprotein (P), the 22–25 kDa matrix protein (M), the trimeric 65–80 kDa glycoprotein (G) and the 190 kDa polymerase protein (L) (Table 1, Fig. 1). Proteins are separately transcribed in cascade by a special mechanism from a single 3′-end promoter which results in a decreasing transcription and expression gradient for proteins encoded from the 3′ to 5′ end.

The major antigen with neutralizing epitopes and pathogenetic determinants is the glycoprotein, which is responsible for receptor recognition and membrane fusion. After endocytosis the viral envelope fuses with endosomal membranes provoking the release of the internal viral nucleocapsid in the cytoplasm where transcription and replication takes place. The helically wound nucleocapsid results from the intimate association of the nucleocapsid protein and the RNA genome. It serves as template for the polymerase (L protein and P cofactor) for transcription and replication. It buds to intracytoplamic membranes in infected neurons, but on plasma membranes in salivary gland epithelial cells.

The lyssaviruses currently consist of 7 established genotypes, or lineages, of rabies(-like) viruses [56] of which the classical rabies virus, found throughout the world and associated with terrestrial mammalian hosts and American bats, forms genotype 1 (Table 2, Fig. 3). Other lineages, or genotypes, are found in bats, of which Mokola and Lagos bat viruses seem to be less pathogenic, and towards which the vaccines based on the classical genotype 1 rabies virus are not protective [43a,43b]. In addition to the 7 genotypes, new bat lyssa-

Fig. 3. Geographic distribution of reported rabies cases in Europe in the 2nd quarter of 2004. A total of 974 cases reported: ALB (3), AUT (1), BEL (rabies free), BIH (11), BLR (45), BUL (8), CHE (0), CZH (0), DEU (2; 2 bat cases included), DNK (0), ESP (North Africa 1), EST (58), FIN (rabies free), FRA (0), GRC (rabies free), HRV (80), HUN (34), IRE (rabies free), ITA (rabies free), LTU (125), LVA (123), MDA (no data), MKD (no data), NED (0), NOR (rabies free), POL (20; 2 bat cases included), PRT (rabies free), ROU (17), RUS (221; 2 human cases included), SCG (40), SVK (17), SVN (1), SWE (rabies free), TUR (30), UKR (131), UNK (0). Rabies free = no indigenous case reported for at least two years (Map obtained from http://www.who-rabies-bulletin.org/q2_2004/startq2_04.html).

virus genotypes have been recently found, e.g. from Russia [43c].

2.2.2. Epidemiology and ecology

During the past 100 years or more in Europe, classical rabies virus has made two major host shifts, firstly from the dog to red foxes, and then to racoon dogs brought from East Asia to be raised for fur; this species then became widely established in the wild (Fig. 3). Phylogenetic data also suggest that west- and southward spread of rabies virus occurred during the last century [44a]. Although in Europe the most important carriers of classical rabies virus are foxes and racoon dogs, the virus can be transmitted to secondary hosts such as domestic animals (dog, cat, cattle, horse, sheep) or e.g. deer – practically any mammal species could be a potential carrier. Bats are a special case; lyssaviruses are maintained in bats, even in the absence of classical rabies in carnivores; thus in countries where classical rabies has been eliminated, bat rabies has become the dominating or only source of rabies virus and retains the potential for host-switching into the carnivore reservoir which constitutes a more direct threat for public health [44b].

As many bats are protected species, the detection or "absence" of bat rabies is dependent also on the intensity of screening efforts [45,46]. As of the beginning of 2004, the following countries in Europe were declared "rabies-free" by WHO (meaning no indigenous cases occurred during the last two years): Belgium, Cyprus, Finland, Greece, Iceland, Ireland, Italy, Luxembourg, Norway, Portugal and Sweden (Fig. 3). In 2003, rabies virus was detected in Europe in 7095 wild animals (excluding bats), 3951 domestic animals; 33 bat and 6 human rabies cases were diagnosed (see Table 4). The countries where rabies was circulating include (in diminishing order of cases) Russia, Ukraine, Lithuania, Belarus, Latvia, Estonia, Croatia, Poland, Slovakia, Serbia-Montenegro and Turkey with hundred(s) to thousands of (animal) cases, Romania, Bosnia-Herzegovina, Germany, Moldova and Bulgaria with dozens of cases, and individual cases were registered (some imported and not affecting the rabies-free status) in Slovenia, the Netherlands, Denmark, France, Albania, Finland, Austria and Switzerland (Table 4, Fig. 3). All the rabies cases (2003) detected in Denmark, France and the Netherlands were bat rabies, in addition, bat rabies was found in Germany, Poland, Russia and Ukraine, most commonly EBL-1 carried by *Eptesicus serotinus* bats [47–51a], http://www.who-rabies-bulletin.org. The first case of bat rabies in a human in the UK for decades, was recorded in 2002. The etiological agent was EBL-2 virus, transmitted by an autochtonous Daubenton bat (*Myotis* sp.) [51b].

Table 4
Classical rabies virus infections in Europe (Year 2003)

Country	No. cases/wildlife	No. cases/domestic animals	No. cases/bats	No. cases/humans	No. cases/total
Albania	2	0	0	0	2
Austria	1	0	0	0	1
Belarus	761	316	0	0	1077
Bosnia Hercegovina	63	17	0	0	80
Bulgaria	15	4	0	0	19
Croatia	590	43	0	0	633
Estonia	697	117	0	0	814
Finland	0	1	0	0	1
France	0	0	2	0	2
Germany	24	0	13	0	37
Hungary	129	43	0	0	172
Latvia	828	135	0	1	964
Lithuania	796	312	0	0	1108
Moldova	13	20	0	0	33
Poland	310	72	6	0	388
Romania	67	28	0	0	95
Russian Federation	1360	1502	1	3	2866
Serbia a Montenegro	207	54	0	0	261
Slovak Republic	284	42	0	0	326
Slovenia	8	0	0	0	8
Switzerland & Liec.	0	1	0	0	1
Turkey	17	139	0	0	156
Ukraine	924	1104	1	2	2031
Total	7095	3951	33	6	2031

Rabies free European countries:
Belgium, Finland, Iceland, Ireland, Italy, Luxembourg, Norway, Sweden

Data obtained from Rabies Bulletin Europe, vol. 27, no. 4, Quarter 4 (2003).

2.2.3. Clinical picture in man and pathogenesis

Members of 6 out of the 7 lyssavirus genotypes (except Lagos bat virus, i.e. genotype 2) have caused rabies disease in man. The infection is inevitably fatal in humans or other mammals unless immune intervention (vaccination and administration of antibodies) is used. Transmission may occur though the bite of an animal delivering the virus deep in to striated muscle or connective tissue, but infection may also occur after abrasion of the skin or licking of mucous membranes. The bite of a bat with small teeth may go unnoticed; on the other hand the bat lyssaviruses may infect human skin more easily than has been recognised. The incubation period is 20–60 days, but has been shown in some cases to be months, even years. The classical picture of rabies includes prodromal illness with fever and non-specific symptoms, as well as itching and local paresthesia. This is followed by neurological signs, consisting of either encephalitic ''furious rabies'' or paralytic ''dumb rabies''. In the former, episodic hyperactivity and excitation of the CNS manifest as, e.g. hydrophobia, hypersalivation and convulsions. The patient finally develops paralysis, coma and cardiorespiratory failure. In the paralytic form, no excitation is seen, but paralytic disease develops to coma and death [45,52a,52b].

After entry of the virus to the body, the virus must gain access to peripheral nerves and to be transported towards the CNS. Rabies virus components are attached either directly or by encapsulated vesicles to the dynein motor carrying the ''cargo'' along the axonal microtubular system towards the cell stroma, approximately at a speed of 25 mm/day [45,53,54]. It is transmitted after replication on neuronal membranes transsynaptically to adjacent neurons and finally invades the CNS where it first disturbs the limbic system associated with the excitation, and later neocortex, with little histopathological changes in neurons. Centrifugal spread of the virus from the CNS to many tissues through somatic and autonomic nerves also occurs, where the salivary glands are especially important for the spread of the virus to the next victim. Immunological responses do not occur before CNS involvement.

All the viruses in phylogroup I are also pathogenic to mice by i.m. injection, and can cross-neutralize each other. This has practical implications, as fortunately the vaccine strains (of genotype 1) also appear to protect against the EBVL viruses [55]. A pathogenic determinant common to phylogroup I viruses seems to be amino acid R333 in the glycoprotein [56]; substitution of this amino acid also abolishes the retrograde transport [57].

When symptoms develop, no cure is available. However, after exposure to a bite or scratch of a potentially rabid animal, rabies must and can effectively be prevented by post-exposure prophylaxis, originally developed by Louis Pasteur. The treatment includes in a non-vaccinated person (a) washing (with water and detergent) and disinfecting the wound to minimize the amount of cell-free virus (this alone can increase survival 50%), (b) starting a post-exposure vaccine regimen which includes (in Europe) five doses of cell-culture derived vaccine intramuscularly into the deltoid muscle on days 0, 3, 7, 14, 28 and (c) in case of severe or deep injury additional passive anti-rabies immunoglobulin, which should be administered principally to the wound area. If the person was pre-vaccinated or the animal can be caught and studied for the presence of rabies, the protocol can be adjusted accordingly [45,52a].

2.2.4. Diagnosis and prevention

Clinical suspicion of rabies in a case of encephalitis of unknown origin is the starting point. *Ante mortem* diagnostics can be achieved most easily with RT-PCR from saliva. In addition, rabies antigen can be detected in brain or nuchal skin biopsies, and in some cases antibodies in serum or CSF may be found. *Post-mortem* diagnostics is most rapid with antigen detection or RT-PCR from the brain, virus isolation is also possible.

In addition to post-exposure prophylaxis, vaccines in humans can be used for pre-exposure prophylaxis (3 doses) in risk groups (veterinarians, wildlife workers, travellers to endemic areas; especially small children who may be unable to explain a potential exposure to a rabid animal) [45,52a,58].

The primary method for control of rabies is vaccination of dogs (and cats). This practice was established at the beginning of the last century in most of Europe, but has not yet been achieved in many developing countries, where annually 60 000 people still die of rabies. In most of Europe (excluding Eastern Europe) rabies has been eradicated from terrestrial wildlife species (carnivores) by aerial distribution of vaccine baits across the countryside. The vaccines used to protect wildlife species include attenuated vaccines as well as a recombinant vaccinia virus carrying the rabies virus glycoprotein (the latter has in one case caused a skin infection in man). Although rabies in terrestrial animals can be controlled efficiently, eradication from the bat reservoir is not currently feasible.

2.3. Arenaviruses

2.3.1. Virology

Arenaviruses are the only members of the RNA virus family *Arenaviridae*. These viruses are enveloped, lipid solvent-sensitive, pleomorphic particles with a mean diameter of 120 nm (ranging between 50 and 300 nm). Host cell-derived ribosomes are present in the virions, and give the virus particles a ''sandy'' appearance under the electron microscope, hence the name arenavirus (arena: sand in Latin). The virion structure of arenaviruses is quite simple, virus particles contain two RNA

segments (S and L) linked to nucleocapsid proteins and viral polymerase molecules, and these nucleocapsid-polymerase complexes are surrounded by a lipid envelope into which two glycoproteins (G1 and G2) are linked protruding on the outside of the virion. Arenaviral RNA segments have an ambisense coding arrangement, the S segment (~3.5 kb) encoding a 63-kDa nucleocapsid protein (N) in the viral complementary sequence, and in the viral-sense 5′-end sequence a 75-kDa glycoprotein precursor (GPC) which is posttranslationally cleaved to two glycosylated proteins 34–44 kDa G1 and G2; and the L segment (~7.2 kb) encoding a 180-kDa viral polymerase (L) in the viral complementary sequence, and in the viral-sense 5′-end of the sequence a 10–14 kDa RING finger Z protein, which has been shown to have a role in arenavirus budding [59a] (Table 1, Fig. 1). Initiation of transcription may involve cap-snatching, although the transcription mechanism is not yet fully elucidated.

2.3.2. Ecology and epidemiology

Arenaviruses include 23 viruses all carried by different rodent hosts (except Tacaribe virus which has been isolated only from fruit bats) [59b]. Arenaviruses are capable of causing chronic infections in their rodent hosts, and infectious virus is present in the blood and is also secreted into body fluids (saliva, urine, semen), which is presumably the route of transmission to humans. The appearance and incidence of arenaviral infections are closely associated with the distribution of the rodent host species and the rodent population dynamics.

Arenaviruses have co-evolved with their specific host species during millions of years, and have been divided into Old World and New World groups first on a serological bass, and later into evolutionary lineages (New World group) using genetic analysis [60a,60b,60c]. Both groups contain viruses that are included in the Category A pathogen list (defined by CDC, USA), which means that the propagation of these agents is allowed only in Biosafety Level 4 laboratories. These highly pathogenic arenaviruses include the South American Junin, Machupo, Guanarito, and Sabia viruses from the New World group, and the African Lassa virus from the Old World group. All these viruses can cause hemorrhagic fevers in humans, and are considered potential bioterrorism agents being thus included in biohazard preparedness programs. In Europe, these viruses occur only rarely as imported cases. The only arenavirus endemic in Europe is lymphocytic choriomeningitis virus (LCMV), shown to circulate in *Mus musculus* populations (Table 2), and associated with pet hamster derived epidemics [59b,61a]. Relatively few epidemiological data are available concerning the actual distribution of LCMV in Europe, but in addition to serological evidence from *Mus* sp. material from Spain [61b], antibodies against LCMV have been detected in rodent species other than *Mus musculus* (our unpublished data), which indicates that other yet unknown arenaviruses may circulate in Europe.

2.3.3. Clinical picture and pathogenesis

At least ten arenaviruses have been reported to be able to cause disease in humans. As mentioned above, five arenaviruses (Lassa, Junin, Machupo, Guanarito, and Sabia viruses) are even capable of causing a life-threatening viral hemorrhagic fever [62a,62b]. None of these five viruses are endemic in Europe, but a few imported Lassa virus infections have been diagnosed in Germany and Great Britain during the last few years with no secondary infections detected [63–65]. Increased travelling increases also the risk for transmission of exotic arenaviruses to non-endemic areas such as Europe.

LCMV is thus far the only known endemic arenavirus in Europe [62b]. In humans LCMV infections are mostly either asymptomatic or influenza-like diseases. In some cases aseptic meningitis or meningoencephalomyelitis is seen. LCMV is also capable of causing congenital infections manifested by hydrocephalus, microcephalus, chorioretinitis, and mental or psychomotor retardation [66,67a,67b]. LCMV infections are rarely fatal for humans.

2.3.4. Diagnostics and prevention

The diagnosis of arenaviral infections is based on serology and/or direct detection of the virus [68]. For serodiagnosis methods using immunofluorescence assay (IFA) as well as enzyme immunoassay (EIA) have been described. Either a four-fold rise in IgG antibody titers or presence of IgM antibodies is considered indicative of acute infection. The antibodies that appear first in the acute phase of infection are directed against the nucleocapsid protein; neutralizing antibodies against the glycoproteins appear later in the convalescent phase (if at all). This means that typing of the causative agent is difficult on a serological basis at the early stage of infection, and is actually possible only in the convalescent phase due to the slow rise of virus type-specific neutralizing antibodies. For direct detection of the virus, antigen detection assays are useful in the early diagnosis of Lassa fever especially. Also reverse transcriptase (RT)-PCR tests have been developed to detect arenaviral RNA in patient samples [69a,69b,70a,70b,70c]. Virus isolation attempts can also be successful.

The supportive treatment of arenaviral infections includes ensuring the fluid, electrolyte and osmotic balance. In severe hemorrhagic fever-cases early diagnosis is important because the use of ribavirin has been found effective if the treatment commences within the first six days after onset of symptoms [71]. Immune plasma containing neutralizing antibodies has also been useful in some cases.

For prevention of arenaviral diseases, several attempts to develop vaccines have been made [72a]. One vaccine, Candid 1, has been successfully used in the prevention of Argentine hemorrhagic fever caused by Junin virus with a clear reduction in the number of infections observed in humans [72b,73].

2.4. Orthopoxviruses

2.4.1. Virology

The genus Orthopoxvirus in the family *Poxviridae* consists of large 220–450 nm brick-shaped viruses, with a double-stranded DNA genome (160–220 kb) (Table 1), that are serologically cross-reactive and -protective. The middle part of the genome is very conserved among orthopoxviruses encoding structural proteins and replication machinery whereas the ends are more variable comprising genes involved with host-specificity and counteracting the immune response [74]. Replication of orthopoxviruses occurs in the cytoplasm and includes translation of early mRNAs (such as DNA polymerases and immune defense molecules), DNA replication, translation of intermediate mRNAs (for late transcription factors), and late mRNAs encoding structural proteins and late enzymes, respectively. Altogether, e.g. the cowpox virus genome encodes nearly 200 open reading frames. Intracellular non-enveloped virions are first formed, comprising the majority of the infectious viral progeny; some particles develop in ER/Golgi into enveloped, either cell-attached or extracellular viral particles, often motile due to attached actin tails [75]. Homologous recombination occurs readily between orthopoxvirus sequences which has raised some concerns about the use of vaccinia virus-based vaccines in wild animals [76].

2.4.2. Epidemiology and ecology

Some orthopoxviruses are host-specific, whereas some are more promiscuous but have a distinct reservoir. Their nomenclature may be misleading; e.g. monkeypox is not carried by monkeys, neither is cowpox carried by cows. Smallpox or variola virus, now eradicated and historically the cause of one of the most feared human diseases, was specific to man. Many other orthopoxviruses circulate in wildlife species and are often zoonotic. Examples include the vaccinia virus, the modern smallpox vaccine, the origins of which are unclear but was originally described as cowpox by Edward Jenner [77] in late 18th century England, and which later has also re-escaped to nature in other parts of the world [78]; monkeypox virus, pathogenic to primates, including humans, causing a smallpox-like disease with secondary transmission and with a likely reservoir in small rodents in Central Africa; cowpox virus, which is the main orthopoxvirus in Europe and may be transmitted to man either directly from rodents or from a secondary carrier, typically cat. Cowpox virus has been detected in Western Eurasia and in Europe, voles of *Clethrionomys* and *Microtus* species and *Apodemus* mice are the main reservoir hosts [79] (Table 2). Shedding of the virus from rodents is apparently transient. Infection of cattle is rare; domestic cats relatively frequently present with clinical disease, but infection of zoo animals, e.g. elephants, has also been reported [80]. Following the cessation of smallpox vaccination, more than 25 years ago, the number of humans susceptible to cowpox has increased. More than 60 human cowpox cases have been reported in the literature since 1969 [81,82].

2.4.3. Clinical picture and pathogenesis of cowpox

All age groups may acquire cowpox, but most cases have been in girls under 12 years of age, who have had a cat or e.g. a field mouse as a pet. Infection probably occurs through abrasions in the skin; persons with atopic eczema are more prone to the infection. [81–84]. The incubation period is 5–7 days, after which papules develop into lesions 1–3 cm in diameter which proceed through pustular, ulceral and eschar stages over a period of a about two weeks. They may be painful and vary in number, size and severity. Local lymphadenopathy, pyrexia and nausea may occur; secondary bacterial infections are common. Typically, solitary lesions are found, located mainly in fingers, hands or face (e.g. eyelid) [81,85]. In 6–8 weeks the lesions heal gradually, some residual scars may remain. In some cases severe generalized skin infection occurs [83], especially in atopic and in immunocompromised individuals and may in extreme cases lead to death [86]. Cidofovir (a phosphorylated nucleoside analog of cytosine) may have potential as an antiviral against cowpox virus [87]. Man-to-man transmission of cowpox virus (unlike for monkeypox) has not been reported.

2.4.4. Diagnostics and prevention

It is usually possible to detect orthopoxvirus particles directly from the skin lesions by electron microscopy. The virus can also be readily isolated in e.g. Vero cells or chorioallantoic membrane of chicken embryos from the lesions and subsequently characterised [80]. Several sensitive PCR approaches have been described, some related to the bioterrorism (smallpox) preparedness [88–90]; in each case further typing at the species level is needed. In addition, during acute cowpox infection, IgM antibodies and low-avidity IgG antibodies have been detected [83].

Following the cessation of smallpox vaccination approximately 30 years ago, the younger age groups are the most susceptible population, both to smallpox and to cowpox, which is more closely related to vaccinia virus. Recent estimates indicate as low as 40% protection levels among Europeans. For instance in Finland, in the age group over 50, everybody had orthopoxvirus antibodies as measured by immunofluorescence assay.

The seroprevalence decreased gradually towards younger age groups reflecting the gradual cessation of smallpox vaccination, with the last vaccinations in Finland occurring in 1977 [83]. Smallpox was finally declared to be eradicated from the world in 1980 [91] after which few people in Europe have received the vaccine. In addition to wild rodents carrying cowpox, import of exotic pets may also pose a risk for orthropoxvirus transmission, as was seen in a recent outbreak of monkeypox virus in the USA [92].

2.5. Orthomyxoviruses

2.5.1. Virology

Orthomyxoviruses are enveloped, negative-strand RNA viruses with 6–8 genome segments, of which avian influenza, (i.e. influenza A) viruses may cause severe disease in domestic poultry and cause zoonotic infections. The influenza viruses in wild aquatic birds are the source of these epidemics in chickens as well as providing a gene pool for reassortants with human influenza A viruses which then may become established in human-to-human transmission resulting in influenza pandemics. In addition, influenza A viruses are known pathogens of pigs, horses, mink, seals and whales [93,94].

Influenza A virus is 80–120 nm in diameter and has 8 genome segments varying in size from 0.89 to 2.3 kb. The three largest segments 1–3 encode the polymerase subunits PA (83 kDa), PB1 (87 kDa) and PB2 (84 kDa), respectively; whereas the segments 4–6 each encode one viral protein, namely the 63 kDa hemagglutinin (HA), the 56 kDa nucleoprotein (NP), and the 50 kDa neuraminidase (NA), respectively (Table 1, Fig. 1). The two smallest segments, 7 and 8, encode each two proteins, the 28 kDa matrix protein (M1) and the 11 kDa membrane protein (M2), and the 27 kDa NS1 and the 14 kDa NS2 proteins, respectively (Table 1, Fig. 1). In common with the *Bunyaviridae*, the genomic RNA is packed in the nucleoprotein which carries polymerase subunits, and the 5′ and 3′-ends are conserved and complementary to each other and thus able to form panhandle structures in which the promoter regions reside. ''Cap-snatching'' from cellular mRNAs and an oligo-U motif are used to create viral mRNAs starting with a cap structure and ending in a poly-A region. The virus enters the cells after binding to the sialic acid receptors by endocytosis, which is followed by acidification of the endocytic vesicle and, mediated by the ion channel forming M2 protein, of the interior of the virus, which leads then to fusion of the viral and endosomal membrane and release of the viral nucleocapsids to the cytoplasm, respectively. However, untypically for an RNA virus, the replication, transcription and nucleoprotein assembly occur in the nucleus. The envelope proteins are processed to the plasma membrane, where budding of the virions finally occurs. The envelope proteins are also the most important antigenic determinants. The homotrimeric hemagglutinin (HA), defines the ''H type'' which is responsible for the binding to the sialic-acid containing host cell receptors and membrane fusion properties. The neuraminidase (NA) defines the ''N type'', and cleaves terminal sialic acid residues from glycoconjugates enabling the virus to reach target cells in the mucin-rich epithelium and facilitating release of the virus from the cells [93,94]. The catalytic site of the neuraminidase is a target for antivirals oseltamivir and zanamivir, and the M2 protein is a target for amantadine and rimantadine.

To become active, the hemagglutinin protein needs to be cleaved by trypsin-like proteases found in respiratory and gastrointestinal epithelia. Human-adapted influenza viruses replicate in the respiratory tract, whereas in avians the virus replicates primarily in the gut. When transmitted to and within poultry, the normal hemagglutinin of influenza A virus H5 or H7 of wild aquatic birds of the ''low pathogenic type (LPAI) may be mutated. Accumulation of basic residues at the cleavage site makes the HA cleavable by most proteases of cells, such as furin, and results in ''highly-pathogenic avian influenza'' (HPAI) virus able to replicate in most tissues killing rapidly up to 100% of chickens [93–95]. HPAI, also known as ''fowl plague'' was first described in 1878 and the virus was first isolated in 1901 suggesting that humans have been directly exposed to avian influenza A viruses for centuries [96].

2.5.2. Epidemiology, clinical aspects and treatment

Influenza A virus gene pools reside in wild aquatic birds where at least 15 HA types and 9 NA types are found, as compared to 3 HA (H1–H3) and 2 NA (N1, N2) types circulating in man. Also, the genes in aquatic birds are in evolutionary stasis without undergoing changes due to selective pressures. Influenza A viruses in man are under constant selective pressure imposed by population immunity causing the hemagglutinin of influenza A virus to change its antigenic properties by accumulation of mutations (genetic drift). However, through double infection and reassortment (genetic shift), novel (e.g. hemagglutinin) genes from aquatic birds may become established in human influenza viruses giving rise to pandemics due to lack of adaptation to and immunity in, humans.

Previously, it was thought that the different receptor specificity – favoring different side chains of sialic acid – of human and avian influenza viruses would make direct transmission of avian viruses to humans unlikely, but both could be expected to infect swine, which carry receptors for both. Thus pigs are considered to be potential reservoirs for generating new influenza virus variants. It has only recently been discovered that viruses considered unique to avian species may also infect man, although this may involve change in receptor

specificities [97]. In 1996 conjunctivitis in UK caused by avian influenza viruses (and previously, by seal influenza A viruses) was reported [98,99] (Table 2). Direct zoonotic transmission of avian influenza viruses to man resulting in human respiratory illness, was not known to occur or had not been diagnosed before the outbreak of H5N1 avian influenza virus in Hong Kong in 1997 where 6/18 patients died of lower respiratory tract infection [100]. After this, in Europe, an outbreak of H7N1 HPAI avian influenza was encountered in Italy (1999–2000) without reported transmission to humans [101]. In 2003, a major H7N7 HPAI avian influenza outbreak occurred in the Netherlands [102,103]. During this epidemic, veterinarians and people who culled infected poultry were at greatest risk of infection. It was noted by active surveillance that human H7 infections (and simultaneous H3 infections) were occurring, and consequently prophylactic treatment with oseltamivir was started. The H7 virus was confirmed to be transmitted to 85 humans of which the majority had conjunctivitis, seven had an influenza-like illness; one veterinarian (who did not receive prophylactic oseltamivir) died. His symptoms started with high fever and headache two days after visiting a farm with infected chickens. One week later, he was admitted to hospital with pneumonia, where his status deteriorated to multi-organ failure, with death due to respiratory insufficiency 2 weeks after onset of symptoms. In addition, in three cases household primary contacts were shown to have the disease by human-to-human transmission [102].

Both NA inhibitors, zanamivir and oseltamivir inhibited virus obtained from humans during this outbreak and a significant difference was found in avian influenza virus detection between oseltamivir users (1/38) and those who had not taken prophylactic medication (5/52) [102].

Outbreaks of avian influenza have continued to occur in other parts of the world, especially the devastating ongoing H5N1 epidemic in South-East Asia since late 2003 with as of June 2005 a total of 54 deaths/107 cases in Viet Nam, Thailand and Cambodia. The epidemic has had a dramatic impact on poultry farming and industry with million birds dying or being destroyed to reduce virus dispersal. This ongoing epidemic clearly has "pandemic potential".

2.5.3. Diagnosis and prevention

Avian influenza viruses may be detected by virus isolation (in cell culture or embryonated eggs) or RT-PCR which may be targeted at the specific HA subtype or may be generic to influenza A viruses (e.g. targeting the matrix protein gene) [104]. Also, serological tests such as. hemagglutination inhibition may be used.

Typing may be based on serological methods (such as hemagglutination inhibition), specific primers/probes or sequencing. Many commercial influenza A antigen tests detect the nucleoprotein or NA activity and should be applicable to the avian viruses, although this is poorly studied and documented. Furthermore, it should be noted that RT-PCR from throat swabs of the lethal case in the Netherlands were negative [102].

Avian influenza, or "fowl plague" outbreaks usually arise following contact with wild aquatic birds, such as mallards and ducks, in which the virus replicates as a rule without causing symptoms. The virus that is excreted in the gut can typically be isolated (or detected by RT-PCR) from cloacal swabs. Hinshaw et al. [105] studied over nine thousand birds and detected 30% prevalence in young and 11% prevalence in adult birds. Influenza virus is readily transmitted in cold environments and is stable for 126–207 days at +17 °C, or in wet faeces (as shown for H5N1 virus) for at least 4 days at 25 °C and more than 40 days at +4 °C [106,107].

The main preventive and control measures include proofing the chicken breeding facilities against wild birds. Raising chickens and turkeys in the open is a risk, minimizing secondary spread of outbreaks by stamping out the infected poultry, followed by cleaning, disinfection and controlling movements of humans and animals, trade embargoes and reporting the outbreaks ("fowl plague" is in the top priority "list A" of the International Animal Health Code of the Office International des Epizooties). Selling poultry live, a common practice in South-East Asia, is a definite risk factor. Vaccination of poultry has been used as an additional control measure but may lead to undetected shedding and transmission of mutant virus selected under the pressure imposed by the vaccine [94].

To prevent further transmission to humans, rapid measures are needed due to the short incubation period. The Dutch experience suggests that results can be achieved by personal protection (e.g. protective eye glasses, masks) for all workers who screen and cull poultry, vaccination with regular inactivated influenza virus vaccine and prophylactic oseltamivir for those handling potentially infected poultry, to be continued for 2 days after last exposure [102]. For humans, specific vaccines containing H5 and H7 antigens would be welcome.

3. Vector-borne viruses

3.1. Alphaviruses

3.1.1. Virology

The only known zoonotic agent of the *Togaviridae* family to cause human disease in Europe is the mosquito-borne Sindbis virus (SINV), in the genus Alphavirus. SINV is distributed throughout the Old World and Australia and causes rashes and polyarthritis outbreaks in Northern Europe, similar to Chikungunya, O'nyong-nyong virus and Ross River virus in Far East

Asia, Africa and Australia, respectively, whereas other alphaviruses causing encephalitic infections in man (Venezuelan, Western and Eastern equine encephalitis virus) are found in the New World.

Alphaviruses are enveloped, positive-strand RNA viruses with an ~11 kb genome. The non-structural proteins (NSP1–4) are encoded from the 5′-terminus of the genome, and a separate subgenomic 26S RNA from the 3′-end is used as a messenger for the structural proteins: the 30–33 kDa capsid protein (C), and the 45–58 kDa envelope glycoproteins (E1 and E2) [94] (Table 1, Fig. 1).

3.1.2. Epidemiology and ecology

SINV was first isolated in the Nile Delta in the 1950s from a pool of mosquitoes without knowledge of any disease association. It is now known to be the causative agent of mosquito-borne epidemic polyarthritis with accompanying rashes and when described in Northern Europe it was given the names Ockelbo disease, Pogosta disease, or Karelian fever when found in Sweden, Finland and Russia, respectively. Most infections occur during August-September, and larger outbreaks tend to appear with a seven-year interval (e.g. in Finland, 1282 serologically verified cases occurred in 1995, 600 cases in 2002; and in Sweden 50–65 cases are reported during peak years) [108,109], with a peak incidence (56%) in 50-year old females. The association of SINV with Pogosta disease was first discovered in the early 1960s and it is believed that the virus may have been distributed throughout Northern Europe around this time. This conclusion is based on the evidence that thousands of human and bird sera collected during the early 1960s in Finland, were negative for SINV antibodies, whereas in the 1990s 2–5% of the population were SINV-antibody positive [108]. Antibodies to SINV without polyarthritis outbreaks have been recorded in Italy, Romania, Greece and the former Yugoslavia [109]. However, human disease due to SINV has been recorded in South Africa, from where it may have originated. SINV was isolated from mosquitoes in Sweden, Norway and Russia in the early 1980s [109–111] and SINV antibodies are found in wild tetraonic and migratory birds, most commonly (in Sweden) from *Passeriformes* such as redwing (*Turdus iliacus*), fieldfare (*Turdus pilaris*), blue tit (*Parus caeluleus*), chaffinch (*Frinigilla coelebs*), songthrush (*Turdus philomelos*); thrushes have been suggested to be a major candidate as an amplifying host [109,112]. In addition, antibodies are commonly found in *Galliformes* [109,108]. Phylogenetically, SINV strains are similar in Northern Europe and South Africa (in the north to south dimension), but differ considerably from strains circulating in Asia and Australia, where SINV-associated rash-arthritis is not recorded. This is consistent with the notion of a north-south dispersal of SINV strains by migratory birds [113]. Several bird species can be infected experimentally [114]. It is evident that SINV cycles between birds and ornithophilic (*Culex* and *Culiseta*) mosquitoes (Table 2). However, it may spill over to other hosts (evidence from many vertebrates from a frog to a bear) and vectors (including ticks and *Aedes* mosquitoes) [109].

Recently, during an outbreak in Finland in 2002, the causative agent of Pogosta disease was isolated for the first time in Europe from skin biopsies and a blood sample of patients [115]; the virus strains were most closely related to SINV strains isolated from mosquitoes in Sweden and Russia 20 years previously.

3.1.3. Clinical picture and pathogenesis

The incubation period for the disease is about one week and the onset is accompanied by arthritis/arthralgia and itchy rash as the dominant symptoms, and also fatigue, mild fever, headache and muscle pain. Hematological laboratory parameters are within the normal range and levels of C-reactive protein (CRP) are not elevated. The rash is usually located on the trunk and thighs and lasts for a couple of days. One third to a half of patients, suffer from joint pains for more than 12 months [116,117]. Usually several joints are affected, the most common being the ankle, wrists, knee, and finger joints (50% or more of patients), as well as hip, shoulder and elbow joints [118,119a,119b].

3.1.4. Diagnostics and prevention

Diagnosis is based on serology using enzyme immunoassay, immunofluorescence assay or hemagglutination-inhibition, and detection of seroconversion or a 4-fold rise in titre between two samples, or positive IgM in a single sample. The first sample is usually taken during the first week after onset of illness, another sample is required to diagnose or exclude SINV infection; IgM antibodies are detectable until approximately 6 days post-onset, and IgG antibodies can be detected approximately 10 days after onset of illness [119b,120]. In some cases persisting IgM antibody can be detected years after infection [117]. For research purposes, the virus can be detected by RT-PCR [119b,121] or isolated from skin biopsies [115]. No specific preventive measures are available, apart from avoiding mosquito bites.

3.2. Flaviviruses

3.2.1. Virology

Flaviviruses comprise a diverse group of pathogens that have been traditionally classified as arthropod-borne viruses. They are linear positive single-stranded RNA viruses with a monopartite genome (10–11 kb) that encodes 3 structural proteins: the 13 kDa capsid protein (C), the 51–59 kDa major envelope protein (E) and the 8.5 kDa glycoprotein M (in mature virions); and 7 non-structural proteins (NS1, NS2A, NS2B,

NS3, NS4A, NS4B and NS5) (Table 1, Fig. 1). Non-coding or untranslated regions flank the infectious RNA genome. When seen in the electron microscope flaviviruses appear as uniform spherical particles, 40–60 nm in diameter. The virus particles consist of a lipid envelope that has a surface covered by protrusions that contain envelope (E) and membrane (M) structural proteins, organized as dimers. This envelope surrounds an isometric capsid protein of approximately 30 nm in diameter [122–124].

3.2.2. Ecology and epidemiology

There are currently about 70 members in the family *Flaviviridae* [125] which have been found infecting a wide variety of organisms including mammals, arthropods, avian and amphibians. Many of these viruses are major pathogens of humans, domestic and farmed animals as well as wildlife species. With the possible exception of the dengue viruses, the flaviviruses are zoonotic, depending almost entirely for their existence on wildlife vertebrate and in many cases, invertebrate species. The type species of the genus is yellow fever virus (YFV), hence the term "flavi" from the Latin word *flavus*, which in turn describes the yellowish color of the skin in yellow fever infections [126].

The classification, and serological and phylogenetic studies of flaviviruses reflect the importance of the vector on the biology and evolution of this genus. There are essentially three groups of flaviviruses: tick-borne, mosquito-borne and non-vectored flaviviruses, although this grouping is, to some extent, arbitrary since some mosquito-borne viruses are also transmitted by ticks and vice versa [127] (Table 2). Phylogenetic analysis also shows very strong correlations between genetic relationships, epidemiology and ecology of these viruses [128,129].

Some flaviviruses are responsible for a significant proportion of the morbidity and mortality that is registered annually worldwide. They cause epidemic outbreaks that involve encephalitis and/or haemorrhagic fever, often fatal and involving millions of humans or in some case birds or mammals. Important flaviviruses affecting humans are the dengue viruses, yellow fever virus, West Nile virus (WNV), tick-borne encephalitis virus (TBEV), Japanese encephalitis virus, Saint Louis encephalitis virus, and Murray Valley encephalitis virus among others. Dengue virus alone causes more than 50–100 million cases worldwide each year and some 2500 million people are now at risk from dengue infections [130].

The most important flavivirus in Europe is TBEV, which is endemic in many European countries, and also in Russia, (Table 5, Fig. 4) Northern China and Northern Japan [126,131a]. It affects thousands of people annually and has a significant impact on public health. The virus is transmitted to humans mainly through a tick bite, however, the infection has also been reported to occur by drinking unpasteurised goat milk from viraemic animals [131b,131c]. The virus is maintained in nature in a cycle involving ticks and wild vertebrate hosts and also by transovarial and transstadial transmission in its vector [126,132]. Serological evidence and viral isolations, as well as sequence similarities have suggested that migratory birds could also play a role in the transmission of TBEV from central Europe to Scandinavian countries; moreover, endemic areas of TBEV are regions of high migratory bird activity [133]. TBEV is classified taxonomically into three subtypes: European subtype, Far Eastern subtype, and Siberian subtype. The first subtype is transmitted mainly by *Ixodes ricinus*, and the last two by *Ixodes persulcatus* [134,135]. The distribution of TBEV is well coordinated with the distribution of its vector, furthermore, different genotypes are located in distinct geographical areas and associated with specific vector hosts. Recent data have shown the co-circulation of all three subtypes of TBEV in the same geographical region, specifically in Latvia, where the two vector *Ixodes* species habitats meet [136]. However, the endemic region in Europe is patchy and covers only part of the geographical range of e.g. *Ixodes ricinus*. There has been an increase of the incidence of TBEV in many of its endemic areas but not in Austria where a countrywide successful vaccination campaign was established reducing the disease incidence to lower levels [131c,131d,137a].

Table 5
Tick-borne enchephalitis viral infections in Europe per country through time

Country	Year 1990	Year 1995	Year 2000	Year 2002
Austria	89	109	60	60
Belarus	–[a]	66	23	18
Croatia	23	59	18	30
Czech R.	193	744	719	647
Denmark	–	–	3	1
Estonia	37	175	272	90
Finland	9	23	41	38
France	2	6	0	2
Germany	–	226	133	226
Hungary	222	226	133	226
Italy	–	6	15	6
Latvia	122	134	544	153
Lithuania	9	426	419	168
Poland	8	267	170	126
Slovakia	14	89	92	62
Slovenia	235	260	190	262
Sweden	54	68	133	105
Switzerland	26	60	91	59
Ukraine	–	–	–	12
Total	1043	2944	3056	2291

[a] No data available. Source: www.tbe-info.com/reports/index.html.

3.2.3. Clinical picture and pathogenesis of TBEV

TBEV affects principally the nervous system and can cause several clinical features of different severity

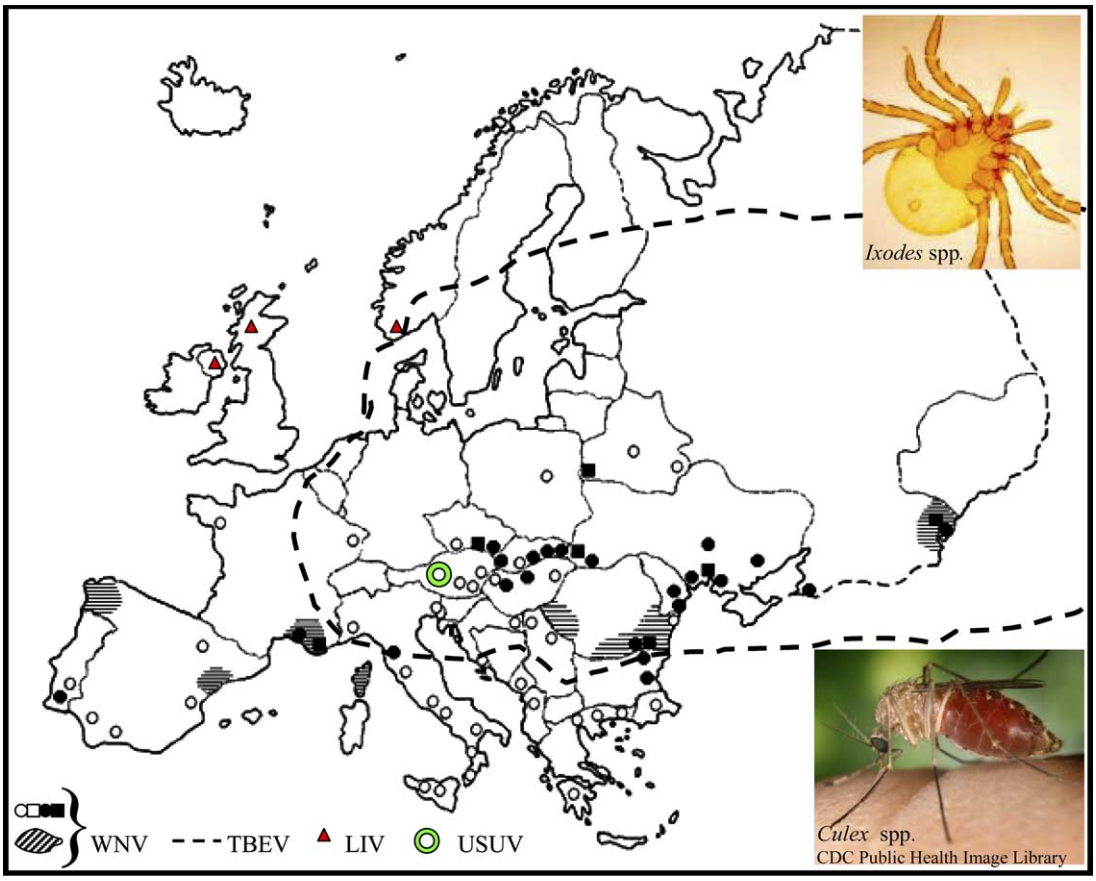

Fig. 4. Geographic distribution of flaviviruses in Europe based on the virus isolation from arthropods or vertebrates. WNV = West Nile virus, TBEV = tick borne encephalitis virus, LIV = louping ill virus, USUV = Usutu virus. WNV isolation from humans (black dots), laboratory-confirmed human or equine cases of WNV (black squares), presence of antibodies in vertebrates (circles and hatched areas). TBEV is distributed within the area enclosed by the dashed line and continues to expand to Russia, Siberia and Japan. LIV has been isolated mainly from sheep and ticks. USUV has been isolated from birds and humans in Austria (Figure adapted from Hubalek and Halouzka [145b]).

including meningitis, meningoencephalitis, meningoencephalomyelitis and meningoradiculoneuritis. Hospitalization varies from days to months and in some cases years of treatment and rehabilitation are necessary as sequelae occur in approximately 1/3 of the patients. The incubation period of TBE is between 7 and 14 days. The disease is characteristically biphasic. The first phase (day 2–4 of onset of symptoms) is viraemic and can be either asymptomatic or fever, malaise, headache, anorexia, nausea, and muscle pains may be present. The second phase occurs in up to 30% of the patients after about 8 days lag period (approximately 21 days after the tick-bite) as a neurological disease of which about 0.5% has a fatal outcome [137b,137c,137d]. The neurological symptoms and severity vary: the clinical picture includes meningitis (in about half of the patients), meningoencephalitis, meningoencephalomyelitis and meningoradiculoneuritis. Hospitalization varies between days and months and in some cases years of treatment and rehabilitation are necessary in case of e.g. paresis. Altogether, neuropsychiatric sequelae occur in approximately 1/3 of the patients.

3.2.4. Description of other important flaviviruses in Europe

Louping ill virus (LIV) is endemic in Ireland, in the northern region of Great Britain and in Norway (Fig. 4) affecting principally sheep with a disease that is known as ovine encephalomyelitis, infectious encephalomyelitis of sheep, or trembling-ill. LIV is transmitted to sheep by the tick vector *Ixodes ricinus*. The natural life cycle of LIV resembles that of TBEV, it can be sustained in the natural environment through non-systemic transmission of virus between ticks co-feeding on rodents and other wild animals, which in turn infect grazing animals such as sheep and goat as a zoonotic disease. Louping ill virus has also been observed in a bird-tick-bird cycle involving the red grouse (*Lagopus lagopus scoticus*), ptarmigan bird species and the *Ixodes* tick [138].

Infection with LIV in sheep is characterised by a biphasic fever, depression, ataxia, muscular in-coordination, tremors, posterior paralysis, coma, and death. There is evidence for infection by LIV in other domestic species and wildlife, i.e. cattle, horses, pigs, dogs, deer,

shrews, wood-mice, voles, and hares [139,140a]. It is generally believed that the majority of avian infections occur through a tick bite, however laboratory experiments and field observations have demonstrated that the red grouse can also become infected by feeding on infected ticks indicating that the vector bite is not the only route of infection [140b].

Humans are also susceptible to infection with LIV. However, the majority of the cases reported are accidentally acquired infections in laboratory workers. The second most frequent infection results from handling infected animal carcasses. Infection with LIV in humans can cause a neurological disease resembling the clinical picture observed for TBEV infections, i.e. biphasic encephalitis, influenza-type illness, fever, articular pain, meningitis, myagia and poliomyelitis-like illness [141]. Other possible transmission routes for LIV infection to humans include drinking contaminated milk from goat or sheep in an acute phase of infection [142], tick-bite, exposure to infective material, or through skin abrasions or wounds. Antigenic and phylogenetic studies have shown that LIV is most closely related to strains of the Western European subtype of TBEV and it is estimated to have emerged from this lineage approximately 800 years ago [143].

West Nile virus (WNV) was first discovered in 1937 in Uganda [144]. It occurs throughout Africa, the Middle East, Europe, Russia, India and Indonesia and was recently introduced into North America (New York) [126,145]. In the Old World WNV is primarily considered to be an African virus which annually disperses northwards out of Africa when birds migrate to Europe, the Middle East and Asia [145]. In humans, the majority of WNV infections cause a non-symptomatic or mild febrile illness; however some infections can cause encephalitis and in most severe cases can lead to death, particularly in elderly patients. The incubation period is between 3 and 15 days after a mosquito bite (http://www.cdc.gov/ncidod/dvbid/westnile/wnv_factsheet.htm). West Nile virus is an illustrative example of the human impact on the dispersal and evolution of flaviviruses. The virus appeared for the first time in the USA, in New York in 1999 causing sixty-two confirmed human infections and seven deaths [146]. The virus successfully over-wintered and during the next years dispersed widely throughout North America and now more recently also to Central America and the Caribbean. In North America the virus infects a very wide range of mosquito and animal species. The exact mechanism of introduction into North America is not known [146–148a]. To date, more than 14 000 human cases and 586 deaths from WNV have been reported in the United Sates of America (http://www.cdc.gov/ncidod/dvbid/westnile). In Europe, outbreaks caused by WNV have been recorded since the early 1950s, especially in Mediterranean countries, Romania and Southern Russia (Fig. 4). Larger outbreaks (over 800 cases) have been reported since the mid 1990s in urban settings, especially a large outbreak in Bucharest, Romania in 1996 [145a,145b,145c]. In Southern Russia, specifically in Volgograd, Astrakhan and Krasnodar regions, WNV has caused large epidemics of approximately 1000 human cases, with 4% mortality rate of reported cases. Molecular epidemiological studies have shown that the latest large outbreaks in Volgograd were caused by strains genetically similar to that of Romania-1996, Kenya-1998 and NewYork-1999 reflecting the widespread distribution capacity of these epidemic viral strains [149b,151b]. The incidence of WNV in Europe is largely unknown. Phylogenetic studies have showed that WNV has diverged into two main lineages, which form internal clusters or clades [149c,150a,150b,150c]. More recently, a novel virus (Rabensburg virus), antigenically and genetically closely related to WNV was isolated from a *Culex pipens* mosquito pool in Czech Republic. This new virus could represent a third lineage of WNV, however more studies are needed to confirm this hypothesis or to determine whether or not this could represent a new member of the *Flaviviridae* family [151a].

Usutu virus (USUV) was first isolated from a mosquito in Africa in 1959 [148c]. It was characterised serologically and classified within the Japanese encephalitis virus serocomplex. Usutu virus had rarely been isolated since then, with only one reported human case and being present only in two regions of Africa. However, in the late summer of 2001, USUV was found in Vienna in Austria during a study of an avian epidemic characterized by fatal encephalitis that was thought to be caused by WNV. The virus caused die-off among the bird population, especially blackbirds, in Vienna (Fig. 4) and when isolated from birds and mosquitoes it showed 97% genetic identity to the African USUV [148b]. This is the first time USUV has been observed outside Africa and also the first time it has been associated with fatal disease in animals. The virus re-appeared in the late summer of 2002 in the same region of Vienna [149a]. Most recently, in 2003 the virus has been isolated from humans (associated with rash in one patient) and from birds in Austria and evidence exists as to the virus being able to overwinter establishing itself in this geographical area (Nowotny, N. Second European Congress of Virology, Eurovirology 2004).

3.2.5. Imported flaviviruses in Europe

As mentioned above the distribution of flaviviruses is worldwide, and we have described the zoonotic flaviviruses endemic to Europe. However, it is important to note the increasing incidence of imported flaviviruses totaling over 500 cases mainly due to changes in

human behavior, such as increased travel to non-European destinations (http://www.eurosurveillance.org).

The dengue viruses (DENV) are a world-wide public health problem. They are transmitted to man by mosquitoes, particularly *Aedes aegypti* and cause a wide range of different clinical outcomes [151c,152a]. It is estimated that 100 million cases of dengue fever (DF) and 250 000 of dengue hemorrhagic fever (DHF) cases occur annually [126]. In a study based on the epidemiology and clinical course of 294 travellers with symptoms of dengue infection it was found that the incidence of DF among European travellers is underestimated, since diagnostic procedures, in general, for febrile patients often do not include tests for tropical arthropod-borne diseases, such as dengue virus [152b]. There have also been some imported cases of YFV to Europe mainly by tourists travelling from endemic areas. However, the incidence of YFV as an imported disease is much lower than that of dengue infections (http://www.eurosurveillance.org) presumably because of the different nature of these diseases, DENV having spread worldwide throughout the tropics and YFV occurring only in its endemic regions in Africa and South America [152c].

3.2.6. Diagnostics and prevention

Traditionally, diagnosis of flaviviruses in Europe has been restricted to detecting TBEV infections through serological assays; IgM-capture enzyme immunoassay, hemagglutination inhibition-, and immunofluorescence assay. IgM antibodies in serum, and in some cases in cerebrospinal fluid during the neurological disease usually provide the diagnosis. RT-PCR is rarely positive at the second phase of the disease [153a].

Because of the close antigenic relatedness of flaviviruses, laboratory findings can easily lead to misdiagnosis [153b]. In a study conducted in Hungary, TBEV-positive serum panels were tested retrospectively against WNV and some sera were found to show higher titres to WNV than to TBEV when compared in serological assays (E. Ferenczi, personal communication). As mentioned above, a study including travellers returning to Europe from tropical destinations proved that many cases remain undetected. There is a need to improve the diagnosis for flaviviruses in order to determine the true incidence and prevalence of these infectious diseases in Europe, and to study the ecology of imported viruses to determine which viruses have been able to establish themselves in the continent and which are still continuously being imported from Africa or Asia. Meanwhile anti-vector campaigns and vaccination for travellers to endemic areas are the only measures to prevent and control these infectious diseases [146]. There are effective vaccines for TBEV and YFV but a protective immunogen does not exist for DENV or WNV. TBEV vaccines from two commercial manufacturers (Baxter and Chiron) are available and widely used, especially in Austria and Germany, both are based on formalin-inactivated virions, three injections are needed for full protection. Booster immunizations are recommended every 3–5 years for people living or spending holidays in endemic areas.

3.3. Nairoviruses: Crimean-Congo hemorrhagic fever virus

3.3.1. Virology

The genus Nairovirus (family *Bunyaviridae*) is composed of 34 predominantly tick-borne viruses that have been divided into seven serogroups [154] including several associated with severe human and livestock diseases (especially Crimean-Congo hemorrhagic fever virus (CCHFV) and Nairobi sheep disease virus). Of the pathogenic viruses only CCHFV causes significant human morbidity and mortality and is found in Europe. Like other members of the *Bunyaviridae* family nairoviruses possess a tripartite single-stranded RNA genome of negative polarity consisting of large (L), medium (M) and small (S) segments. The ~12 kb L segment encodes an RNA-dependent RNA polymerase (deduced size ~448 kDa in CCHFV), the ~4.9 kb M segment encodes the precursor for the two envelope glycoproteins Gn (~75 kDa in CCHFV) and Gc (~37 kDa in CCHFV), and the ~1.7 kb S segment the viral nucleocapsid N (~50 kDa) (Table 1, Fig. 1). Like with other members of the *Bunyaviridae* viral messenger RNAs (mRNA) are not polyadenylated and are truncated relative to the genome RNAs at the 3′ termini. Messenger RNAs have 5′-methylated caps and 10–18 nontemplated nucleotides which are derived from host cell mRNAs. The nairovirus L segment encoding the RNA polymerase is conspicuously large, almost twice the size of many other members within the family *Bunyaviridae*, and may thus provide other functions such as viral helicase and a papain-like cysteine protease activity predicted from the nucleotide sequence [155,156]. Viral protein analysis has suggested that Gn and Gc may be derived from 85 and 140 kDa precursors, of which the latter contains an N-terminal region with a highly O-glycosylated mucin-like domain [157]. The CCHFV nucleocapsid protein colocalizes and interacts with human MxA protein; this interaction may explain the antiviral effect of interferons on CCHFV [158]. Similar to the hantavirus-rodent association, the high genetic variation of viruses of the genus Nairovirus reflects the diversity of their predominant tick hosts [159]. Within CCHFV isolates, comparison between M and S segment phylogenetic groupings suggests that reassortment events have occurred in some virus lineages [160]. Reverse genetics, recently established for CCHFV [161],

provides a unique opportunity to study the biology of nairoviruses and tailor optimal therapeutic and prophylactic measures against CCHFV infections.

3.3.2. Ecology and epidemiology

CCHFV is transmitted most efficiently by *Hyalomma* ticks, followed by *Rhipicephalus*, *Dermatocentor* spp and many other species of ixodid (hard) and some argasid (soft) ticks (Table 2). Among ticks CCHFV is capable of transmitting infection both transovarially and transstadially. The life cycle of CCHFV also includes a tick-vertebrate host cycle involving both wild and domestic animals. The virus or antibodies against it have been detected in rodents, hares, hedgehogs and some birds but human infections seem to be principally from contacts with livestock (mainly cattle, sheep, goats) including in Africa farmed ostriches and less often ticks [162,163]. Thus CCHFV is more commonly seen in persons exposed to blood and tissues of infected animals during occupational activities, such as farming, herding, veterinary examination and abattoir work. Nosocomial infection is common and often results in small outbreaks. Yet, outdoor and recreational activities also represent a risk factor. In addition, horizontal transmission of CCHFV from mother to child may occur [164].

The global distribution of CCHF follows closely that of *Hyalomma* spp. in the Middle East, Asia, Africa and Southeast Europe [162,163]. Sequence analysis of CCHFV S RNA segments gives patterns following links between different geographic locations, in some cases suggesting links originating from trade in livestock and long-distance carriage of virus or infected ticks during bird migration [165]. In Europe, CCHF occurs in the Balkan Peninsula (Albania, Bulgaria, Greece, Turkey, Yugoslavia) but also in Southern Russia, Hungary, France and Portugal [162,166,167].

3.3.3. Clinical picture and pathogenesis

The symptoms and signs of Crimean-Congo hemorrhagic fever are similar to those of other viral hemorrhagic fevers [162,163]. The incubation period is generally short ranging usually from 2 to 9 days. There is typically a very sudden onset of illness with fever, rigors, chills, intense headache and backache or leg pains, myalgia, nausea, and vomiting. Patients may also present with photophobia, somnolence and menigism with confusion or aggression. Hemorrhages in the form of petecchiae, ecchymoses, epistaxis, melena and bleeding from various organs usually begin a few days later. Tachycardia is common and lymphadenopathy is seen occasionally. The case-fatality rate is 20–35%. The pathology consists of hemorrhagic and necrotic lesions in various organs as well as fibrin deposits.

3.3.4. Diagnostics and prevention

The methods used for detection of CCHFV infection in humans or livestock include indirect immunofluorescence on virus-infected or N-expressing transfected cells. Both IgM and IgG antibodies reactive for CCHF appear within a week in patients [162,168]. Alternatively a recombinant nucleocapsid protein-based enzyme immunoassay [169–171] may be used. Rapid detection and quantification of CCHFV RNA is possible by real-time reverse transcription PCR [172].

Ribavirin is clearly effective in treatment of Crimean-Congo hemorrhagic fever [173,174] but supportive treatment (blood, thrombocytes, coagulation factors) is equally important. Immune plasma from recovered patients has also been used to treat patients but not in controlled studies with a follow-up of virus-neutralizing activities [162]. The most effective method of preventing infections is to take measure to avoid exposure to ticks (protective clothing, tick repellent, frequent body searches to remove ticks). Inactivated virus prepared in mouse brains has been used on a limited scale as a vaccine in Southeastern Europe and the former USSR but the sporadic and unpredictable occurrence of the disease renders it difficult to identify target populations and has slowed development of a safe and modern vaccine [162].

3.4. Orthobunyaviruses: Tahyna and Inkoo viruses

3.4.1. Virology

Orthobunyaviruses belong to the family *Bunyaviridae*. These enveloped viruses have a three-segmented negative-strand RNA genome. The 0.97 kb S segment encodes the 19–25 kDa nucleocapsid protein (N) and a 10–13 kDa nonstructural protein (Ns); the ~4.5 kb M segment encodes the polyprotein precursor of two glycoproteins, G1 and G2 (108–210 kDa and 29–41 kDa, respectively), and a nonstructural protein NSm (15–18 kDa); and the ~6.9 kb L segment encodes the RNA polymerase (260 kDa) (Table 1, Fig. 1). Viral messenger RNAs are not polyadenylated and are truncated relative to the genome RNAs at the 3′ termini. Messenger RNAs have 5′-methylated caps and some nontemplated nucleotides which are derived from host cell mRNAs.

3.4.2. Ecology and epidemiology

Orthobunyaviruses are transmitted to humans by a variety of mosquito species, and in Europe two known human pathogenic representatives of the genus circulate, Tahyna virus and Inkoo virus (mostly transmitted by *Aedes* spp.) [109,175,176] (Table 2). These viruses belong to the California serogroup, and their incidence is associated with the distribution of the carrier mosquitoes. The natural cycle of these viruses involve also mammal hosts. Tahyna virus circulates throughout most of

Europe, whereas Inkoo virus is mainly reported from northern Europe [109,177a].

3.4.3. Clinical picture and diagnosis

Tahyna virus causes an influenza-like disease which may also present as a meningitis. The infection caused by Inkoo virus is mostly sub-clinical or a mild disease, although encephalitis has been reported. The most typical symptoms associated with encephalitis or meningitis caused by Californian serogroup viruses include fever, headache, nausea, vomiting, convulsions and confusion. In Europe the seroprevalence for Californian serogroup viruses varies from 1% to 69%, depending on the geographic area [109]. In Finland the seroprevalence against Inkoo virus varies from 2% to 6% (Åland islands) to 61–69% (Finnish Lapland) [109, Putkuri et al, unpublished results]. In disease-endemic areas the seropositivity of the population increases with age [177b]. The diagnosis of orthobunyaviruses is based on serology, either as a rise in IgG-antibody titers, or the presence of IgM antibodies. Also RT-PCR methods are under development to detect viral RNA in cerebrospinal fluid samples of patients with encephalitis.

3.5. Phleboviruses

3.5.1. Virology

Phleboviruses are one of the five groups within the family *Bunyaviridae*. They have a negative single-stranded RNA tripartite genome, namely: L (6.4 kb), M (3.2–4.2 kb) and S (1.7–1.9 kb). The L segment encodes a single protein of 240 kDa (RNA polymerase) in the complementary sense. The M segment encodes a protein precursor of ~130 kDa in the complementary sense which is cleaved into two glycoproteins G1 (~56 kDa) and G2 (~59 kDa). The S segment has an ambisense coding arrangement, and it encodes the nucleocapsid protein (N) of ~27 kDa in the complementary sense and a non-structural protein (NSs) of ~30 kDa in the virion sense [178,179] (Table 1, Fig. 1). Viral messenger RNAs (mRNA) are not polyadenylated and are truncated relative to the genome RNAs at the 3′ termini. mRNAs have 5′-methylated caps and some nontemplated nucleotides which are derived from host cell mRNAs.

When seen under the electron microscope the virions are spherical and enveloped and approximately 80–110 nm in diameter. Glycoprotein projections of 5–10 nm are also seen embedded in the membrane. There are between 200 and 1500 spikes per virion [178,179].

3.5.2. Ecology and epidemiology

Phleboviruses are arthropod-borne viruses and are transmitted mainly by phlebotomine sandflies, hence the derivation of its name "phlebo" (Table 2). However, mosquitoes and ticks can also transmit phleboviruses. Phleboviruses are distributed throughout most of the world but have not been reported in Australia [180]. The first documented record of a disease resembling those caused by phleboviruses dates back to the 1900s in the Mediterranean countries of Europe [178]. However, the first virus isolation occurred for Rift Valley Fever virus in 1930 in Kenya [181]. Retrospective serological studies have indicated that outbreaks caused by phleboviruses have occurred since 1912 in the Sub-Saharan Africa region [182]. Phleboviruses are major causal agents of encephalitis in humans, especially among children, in Mediterranean countries. They also have an important impact in livestock especially in African endemic areas. Infection of livestock is characterised by a high rate of abortions and pathologies associated with hemorrhagic illness (leukopenia, thrombocytopenia, fibrin thrombi, intravascular coagulopathy, etc.) [183–185]. The distribution of the disease follows that of its vector. However the natural history of phleboviruses is largely unknown and for many species the amplifying host has not been identified [185–187].

3.5.3. Clinical picture and pathogenesis

In general, phlebovirus infections cause a 2–4 days illness characterised by an incubation period of 2–6 days followed by fever, general malaise, headache, photophobia, and back and joint pain [188]. The illness occurs during the summer months when the activity of phlebotomine flies is high [178].

There are three recognised members of the genus Phlebovirus, associated with disease in humans. *Rift Valley fever virus (RVFV)*, which is the type species of the genus and is transmitted by mosquitoes, causing an influenza-like disease that affects domestic animals and humans. The majority of human infections are asymptomatic; however, in 0.5% of cases it can cause severe haemorrhagic fever, encephalitis and death. The geographical distribution of RVFV includes Africa, Egypt, Saudi Arabia and Yemen and occurs as epizootics involving *Aedes* spp. mosquitoes and domestic animals [180].

Toscana virus (TSV) causes a mild influenza-like disease that can occasionally develop into an acute neurological disease, such as meningitis or meningoencephalitis in Italy and possibly other Mediterranean countries. A similar virus (Granada virus) was recently isolated in Spain [193]. TSV is the number one cause of encephalitis among children in Italy. The distribution of the virus follows that of its vector, *Phlebotomus perniciosus*, found in Italy, Spain, Portugal, and Cyprus. Human cases have also been reported from southern France and Greece. The virus replicates in the sandfly vector population; however, experimental evidence

suggests that an amplifying vertebrate host is needed to sustain the virus in the arthropod population [189–194].

Sandfly fever virus (SFV) Sicilian and Naples viruses can cause a mild-influenza like disease. The distribution of SFV also follows that of its vector, *Phlebotomus papatasii*, found in the Mediterranean basin extending to the Middle East and Arabian Peninsula, the Caucasus mountains, Pakistan and India [185–187].

3.5.4. Diagnosis and prevention

Diagnostics of Phlebovirus is performed mainly by IgM-capture EIA and IFA. The virus can be isolated using intracranial inoculation in suckling mice or using a susceptible tissue culture cell line (Vero, LLC-MK2, BHK-21) [178,195a]. Toscana virus and Naples Sandfly fever virus group together and are distinct antigenically and phylogenetically from Sicilian Sandfly fever virus [195b].

Prevention of infections by phleboviruses varies depending on the type species. Immunising livestock with the formalin-inactivated or live-attenuated vaccines can prevent RVFV by avoiding epizootics and hence human infections [178,196]. In the case of TSV and SFV, prevention is limited to controlling the vector population through insecticides and repellents [197,198].

4. Concluding remarks

What are the reasons for the appearance of all these emerging and re-emerging viruses? Have changes taken place in the viruses themselves? Apparently not. The principal reasons are changes in the environment and human activity that have created new attacks with nature. Factors such as climate change, increasing intensity of agriculture, the building of dams and introduction of other irrigation measures which generate new breeding sites for mosquitoes, crises and wars which bring rodents and arthropods closer to humans, population growth and especially in suburban slums, and global air traffic are important factors generating such new contacts, or may affect the ecological cycles of these viruses. Many epidemics may be unexpected outcomes of such changes. Moreover, the new molecular biology techniques facilitate detection of new viral agents.

Many prevention and control measures, such as the anti-*Aedes* campaigns, have been abandoned in tropical countries. The yellow-fever vaccination campaigns have been re-implemented just recently with the new outbreaks. This negligence has increased the chance of acquiring new infections from overseas and the chances of broadening the ecological niches for many diseases. The new vector of many diseases is human transportation, which introduces the artificial migration (or imports) of the vector (mosquitoes) usually as larvae.

This review could not comprehensively cover all zoonotic agents in Europe, and new viruses with zoonotic transmission are continuously suggested, such as hepatitis E virus, found in rats and connected e.g. to eating deer meat [199]. It is easy to predict that we will continue to see many more emerging and re-emerging viruses during the coming years.

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