Chapter 5
Current Status of Hantavirus Vaccines Development

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Abstract Hantaviruses are associated with two human diseases: hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in the Americas. These viruses are carried by persistently infected rodents and are transmitted to humans by aerosolized rodent excreta. The number of reported cases of hantavirus infection is growing in many countries. New hantavirus strains have been increasingly isolated worldwide raising public-health concerns. There is still no effective antiviral treatment against hantavirus infections. Prevention can be partially achieved by rodent avoidance, but it is not realistic in many endemic areas. The realistic preventive program has to be based on safe and effective multivalent vaccines specific for local epidemiological environment. This chapter summarizes the current status of hantavirus epidemiology and development of preventive strategy to control hantavirus infections. The current and novel hantavirus vaccines are discussed in terms of the demand, population at risk, and the potential market size for specific endemic areas.

5.1 Introduction

Hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are enveloped, single-stranded, negative-sense RNA viruses, carried primarily by rodents or insectivores of specific host species (Krüger et al. 2011). In humans hantaviruses cause two diseases, hemorrhagic fever with renal syndrome (HFRS) in Eurasia (Yanagihara and Gajdusek 1988) and hantavirus pulmonary syndrome (HPS) in the New World

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(Nichol et al. 1993; Lopez et al. 1996). Four HFRS human pathogens are *Hantaan* (HTNV) and *Seoul* (SEOV) viruses in Asia (where approximately 90 % of worldwide incidences occur) and *Puumala* (PUUV) and *Dobrava*/*Belgrade* (DOBV) viruses in Europe. Two hantaviruses, *Sin Nombre* (SNV) and *Andes* (ANDV), cause most HPS cases in North and South America, respectively.

The clinical features of HFRS were first described in 1930s in north-central Sweden (Myhrman 1934; Zetterholm 1934) and in Russia Far East (Targanskaia 1935; Smorodintsev et al. 1959; Sirotin and Keiser 2001). Approximately at the same time, a similar disease was described in Manchuria, China (Ishii et al. 1942; Johnson 2001). The Swedes called the disease as epidemic nephropathy, while the Russians and Japanese as Far Eastern nephrosonephritis and Songo fever, respectively. During the Korean War (1951–1953), a disease, known as Korean hemorrhagic fever, appeared among several thousand United Nations personnel (Johnson 2001), leading to a quarter century of efforts to identify the causative agent (Schmaljohn 2009). In 1976 HTNV was finally isolated from the lungs of Korean field mice (Lee et al. 1978) and in 1981 the virus was cultivated in cell culture (French et al. 1981). Several diseases that were clinically similar were soon shown to be caused by viruses related to HTNV. In 1983 the term "HFRS" was adopted by the World Health Organization to consolidate the nomenclature of the diseases (Bull WHO 1983). In 1994, the clinical features of HPS were first described in the southwestern part of the United States (Duchin et al. 1994).

Each year approximately 60,000–100,000 HFRS cases are reported worldwide, mostly in China and Russia (Zhang et al. 2010; Tkachenko et al. 2013). The most severe forms of HFRS are caused by DOBV and HTNV, with 5–12 % mortality. PUUV and SEOV cause less severe infections with mortality rate less than 1 % (Vapalahti et al. 2003). Although HPS is much smaller in numbers with about 3,000 cases throughout North and South America during the 1993–2012 period, SNV, ANDV, and related viruses can cause HPS in the Americas with much higher fatality rate, ~35 % (Macneil et al. 2011). Humans get mainly infected from aerosolized rodent excreta, but HPS may be also transmitted from person to person (Enria et al. 1996).

There is still no effective antiviral treatment against hantavirus infections. The main treatment of severe HPS or HFRS cases is purely supportive, often in intensive care unit surroundings. This means mechanical ventilation or even extracorporeal membrane oxygenation for HPS and all forms of extracorporeal blood purification (mostly hemodialysis) for HFRS (Maes et al. 2009). Ribavirin is not widely available and should only be given intravenously at early stage of the disease. In practical terms, the drug is applicable only during outbreaks caused by highly pathogenic Hantaan virus in Korea. In China encouraging results have been obtained only when ribavirin was given during the first 5 days after onset (Huggins et al. 1991). In a limited field study of HPS in the United States, no convincing beneficial effect could be demonstrated with ribavirin (Mertz et al. 2004).

The reported cases of hantavirus infections are increasing in many countries, and new hantavirus strains have been increasingly identified worldwide, which constitutes a public-health problem of increasing global concern. Hantavirus infection

might be underestimated even in countries were the disease is known due to its clinically asymptomatic and nonspecific mild manifestations. The lack of simple and validated diagnostics complicated diagnosis in hospitals (Bi et al. 2008). In addition, the increasing domestic and international travel exacerbates the risk of infection. Nevertheless, hantavirus-induced diseases are easily preventable as far as safe and efficacious vaccines are available.

5.2 Epidemiology of Hantavirus Infections and Rational for Vaccine Development

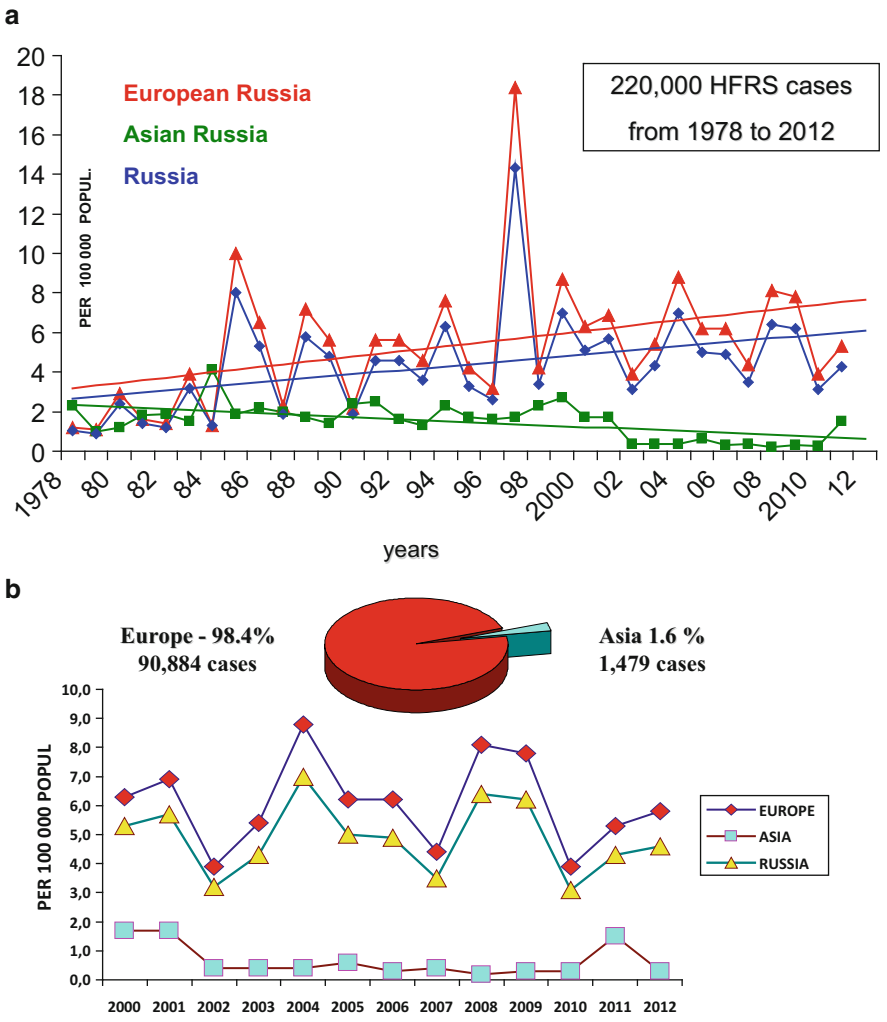
5.2.1 Hemorrhagic Fever with Renal Syndrome in Russia

The clinical features of the first HFRS cases in Russian Far East were described by Targanskaia in 1935 (Targanskaia 1935). The disease was called "hemorrhagic nephrosonephritis" (Churilov 1941). It was long believed that the area of distribution of this infection was limited to Far Eastern part (Amur River basin) of Russian. Therefore, retrospectively so-called Tula fever, known since 1930, can be considered as the first discovery of HFRS in Russia. The disease attracted attention of physicians in 1930 in Tula region, 120 km from Moscow, where during 5 years (1930–1934) 95 cases of "Tula fever," including 5 fatal cases, were reported in 1936 (Terskikh 1936). For a long time, "Tula fever" was considered, without sufficient evidence, as a peculiar leptospirosis and then as a rickettsiosis. In 1958–1959, during a large outbreak (850 cases), a quite conclusive clinical and pathoanatomical evidence of the identity of "Tula fever" with Far Eastern "hemorrhagic nephrosonephritis" was obtained.

The perception exists that in the 1950s–1960s, the disease was considered as a major medical problem in the European Russia and the end of the 1960s the disease was registered in 18 administrative regions under different names (Tula, Yaroslavl, Ural fevers, etc.). In 1954 M. Chumakov proposed the name "hemorrhagic fever with renal syndrome." In 1983 this name was recommended by the WHO Working Group to unify a nomenclature of very similar clinical diseases in Europe and Asia (Chumakov 1963; Bull WHO 1983).

Since 1978 (when HFRS has been included in the official reporting system of the Russian Ministry of Public Health) to 2012, a total of 220,177 cases had been registered in 57 from 83 administrative regions of Russia with annual average morbidity rate ~6.5 per 100,000 population. Among these cases, 214,744 cases were reported from 46 out of 58 administrative regions of the European Russia (97.5 % of total HFRS cases) and 5,433 cases from 11 out of 25 regions of the Asian Russia (2.5 %). Human epidemics have had cycles with a frequency of 3–4 years (Fig. 5.1).

The analysis of the dynamics of morbidity due to HFRS in the twenty-first century has not allowed to reveal the tendency in reduction of HFRS morbidity in



YEARS	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	TOTAL
EUROPE	7,246	8,203	4,480	6,133	10,126	7,152	7,107	5,005	9,306	8,954	4,484	5,982	6,706	90,884
ASIA	129	134	123	111	111	196	90	122	69	109	88	109	88	1,479
RUSSIA	7,375	8,337	4,603	6,244	10,237	7,348	7,197	5,127	9,375	9,063	4,572	6,091	6,794	92,363

Fig. 5.1 HFRS morbidity in Russia. (**a**) 1978–2012, (**b**) 2000–2012

Russia (annual average morbidity of more than 7,000 cases) (Fig. 5.1). The distribution of HFRS in Russia was found to be scattered throughout the country. However, different geographical regions are distinguished by the morbidity rates due to HFRS that vary considerably. In the Asian Russia, 93 % HFRS cases were

registered in four Far Eastern regions (Primorsk, Khabarovsk, Amur, and Jewish regions) and significantly less in the Western Siberia with the lack of reported cases in Eastern Siberia (Tkachenko et al. 2013). In the European Russia, most high rates of annual HFRS incidence occur in the eastern area. Here there are 11 administrative regions with high HFRS morbidity (20 per 100,000 population) including Bashkiria region with the highest morbidity in Europe. Practically 40 % of all HFRS cases in Russia are registered on the territory of Bashkiria with annual average morbidity rates of more than 50 per 100,000 (Tkachenko et al. 1999, 2013). During the last years, in addition to the primary factor, massive reproduction of bank voles infected by PUUV, very extensive construction activity of people coming from cities to rural endemic areas to build country houses resulted in significant increase of human contacts with infected rodents and in increase of HFRS morbidity in European Russia.

In general, in Russia morbidity is higher in rural areas as compared to urban. However, in Eastern European endemic area morbidity rate in large cities is approximately three times higher than those in rural areas. Most HFRS cases in the European Russia occurred during the summer and fall, while cases in the Far Eastern regions of Asian Russia occurred in fall and winter. About 70 % of the total HFRS patients were in the 20–49-year age group; children under the age of 14 years represented approximately 5 % of the cases. Males outnumbered females by a ratio of 4:1. The analysis of risk factors showed that the major risk was associated with occasional activities in the forest, gardening, and farming activities (Tkachenko et al. 1999, 2013).

Results of serological prospective studies of convalescents who were diagnosed with HFRS more than 25 years ago showed a long-term persistence of hantavirus-specific antibodies (Myasnikov et al. 1986). In Russian endemic areas hantavirus antibody prevalence rate was found to be different. The highest seroprevalence was observed in highly endemic HFRS areas with the highest rates of natural infection (up to 30 % in Bashkiria). Among the random population without clinical manifestations of HFRS, the seropositive men-women ratio is 2:1. However, among the HFRS patients, this ratio is 4:1. The difference may be explained primarily by the fact that HFRS in women is frequently diagnosed as pyelonephritis and other diseases with mild and even asymptomatic manifestations. More frequent antibody findings in subjects of older ages may be explained by increasing number of human contacts with sources of infection later in life. The fact of detection of hantavirus antibody in healthy individuals may be explained by milder, even asymptomatic nature of the infection, as well as by misdiagnosis.

Evidence for the mode of transmission of hantavirus to humans derives principally from epidemiological observations. Experimental evidence of hantavirus transmission within rodent population provided additional view on the way how the virus is transmitted to humans. The natural hantavirus infection in rodents indicates that the virus persists in rodent reservoir and causes chronic, apparently asymptomatic infection and shedding over a long period of time with urine, feces, and respiratory secretions (Gavrilovskaya et al. 1990). Aerosolized droplets containing the virus are sufficient to transmit hantavirus horizontally among

rodents. Evidence for respiratory route of hantavirus infection was demonstrated during two laboratory outbreaks involving 126 HFRS cases (Kulagin et al. 1962; Tkachenko et al. 1999). The source of unforeseen airborne infected dust was identified in large shipment cages containing forest mouselike rodents brought to the research institute's animal facilities from natural foci of infection and kept in large cages for 1–3 months. Bank voles (*M. glareolus*) were predominant among the trapped forest rodents. In a number of cases, the airborne transmission could be the only possible way of human infection.

Thus, numerous epidemiological studies of infections acquired in natural conditions suggest that close human contact with rodents should be a risk factor for hantavirus infection. The victims are primarily persons who are working permanently in accordance with their occupational duties in active natural HFRS foci or those who only visit endemic areas periodically but frequently enough to be infected by virus from wild animals. There is no evidence of human-to-human, secondary transmission or nosocomial HFRS outbreaks in Russia.

Analysis of results of hantavirus antigen detection in lung tissues of about 70 species of small mammals showed that practically each landscape zone has natural foci of the infection with different levels of virus circulation. Hantaviruses were hosted by different rodent species in all analyzed areas as it was shown by antigen detection in mammals belonging to different species (Tkachenko et al. 1987; Slonova et al. 1985; Gavrilovskaya et al. 1983a; Ivanov et al. 1989). Hantavirus antigen was also detected in tissues of 13 species of birds, trapped in the Russia Far East (Tkachenko and Lee 1991).

The first hantavirus strains were isolated in Russia at the end of the 1970s by using bank vole laboratory colonies (Gavrilovskaya et al. 1983b) and since 1983 in Vero-E6 cell cultures (Tkachenko et al. 1984). Using tissue cultures, more than 100 hantavirus strains were isolated from HFRS patients and necropsy materials, rodent lung tissues from 8 different species, and from 1 species of birds (Tkachenko et al. 1984, 2005a; Ivanidze et al. 1989; Slonova et al. 1992, 1996; Dzagurova et al. 1995; Klempa et al. 2008). Immunological studies and genotyping of hantavirus strains revealed at least eight hantavirus species circulated in Russia: HTNV, PUUV, SEOV, DOB/BELV, TULV, KHBV, TOPV, and HTNV-like (Amur/Soochong virus) (Slonova et al. 1990; Niklasson et al. 1991; Plyusnin et al. 1994, 1996; Tkachenko, 1995; Horling et al. 1996; Dekonenko et al. 1996; Yashina et al. 2001). The vast majority of rodents and insectivore species as well as other mammal and bird orders harboring hantavirus are probably ancillary hosts. Currently the epidemiological significance of certain rodents is established in different regions of Russia. In Russia Far East, HFRS cases are etiologically associated mainly with HTNV, with HTNV-like (Amur/Soochong virus), and, in the less extent, with SEOV. The principal hosts of these viruses are *A. agrarius*, *A. peninsulae,* and *R. norvegicus*. HFRS cases registered in European regions are caused mainly by PUUV associated with bank vole, *M. glareolus,* and less by DOB/BELV associated with two species, *A. agrarius* (central European regions) and *A. ponticus* (southern regions). The principal hosts of TULV, KHBV, and TOPV are *Microtus arvalis*, *Microtus fortis,* and *Lemmus sibiricus,* respectively.

Recently, novel hantaviruses have been discovered in the Black Sea coast area of European Russia, and major's pine vole, *Microtus majori*, was identified as a novel hantavirus host (Klempa et al. 2013a). The newly discovered hantavirus, provisionally called "Adler" virus (ADRV), is closely related to TULV. Amino acid differences with TULV (5.6–8.2 % for nucleocapsid protein and 9.4–9.5 % for glycoprotein precursor) are on the border line of the current ICTV species definition criteria (7 %). Sympatric occurrence of ADRV and TULV in the same region suggests that ADRV is not a geographical variant of TULV but a host-specific taxon. High intracluster sequence variability indicates the long-term presence of the virus in this region. The pathogenic potential of ADRV needs to be determined.

Until recently, HFRS cases in European Russia were associated with PUUV only. However, during the last years in Central European Russia, three large HFRS outbreaks caused by DOB/BELV were detected (more than 700 cases). A detailed investigation of outbreaks had revealed the striped field mouse (*Apodemus agrarius*) as a virus reservoir. In addition, the *A. agrarius*-borne DOB/BELV lineage (DOB-Aa) or genotype Kurkino (DOB/KURV) was identified as the causative infectious agent (Klempa et al. 2008, 2013b). The results of comparative analyses of epidemiological data of PUUV-HFRS and DOB/KURV-HFRS outbreaks indicate that 97 % of total DOB/KURV-HFRS cases were diagnosed in rural and only 3 % in urban areas (Tkachenko et al. 2005b; Mutnykh et al. 2011). At the same time, 30 % of PUUV-HFRS cases in Bashkiria were diagnosed in rural areas and 70 % of cases were found in urban areas. Most PUUV-HFRS cases were diagnosed during August–December with the HFRS peak in October, while DOB/KURV-HFRS cases were diagnosed during November–March peaking in December. However, clinical symptom differences between PUUV-HFRS and DOB/KURV-HFRS diseases were not identified. Analysis of risk factors showed that in PUUV-HFRS area, the major risk factors were linked with a short-time stay in the forest (55 %), gardening, and farming activities (36 %), while those in DOB/KURV-HFRS area were connected with hibernal cattle breeding (73 %) and other agricultural activities (25 %).

In 2000, DOB/BELV hantavirus was detected in the Sochi region, southern part of European Russia. At the same area HFRS cases were diagnosed among febrile patients (Tkachenko et al. 2005a). It suggests that the *A. ponticus*-born DOB/BELV lineage (DOB-Ap) or genotype Sochi (DOB/SOCV) hantavirus associated with the Black Sea field mouse, *Apodemus ponticus* (a novel host rodent), is the causative agent of the human HFRS. *A. ponticus* is naturally spread in the southern European Russia and in regions between the Black and the Caspian Sea. The Sochi virus was isolated in Vero-E6 cell cultures from *A. ponticus* and an HFRS patient with fatal outcome (Tkachenko et al. 2005b; Dzagurova et al. 2012).

In 2000–2011, 56 HFRS cases caused by Sochi virus were diagnosed in 7 administrative regions of Krasnodar province including 38 cases in Sochi metropolitan area (Tkachenko et al. 2013; Klempa et al. 2008; Dzagurova et al. 2009). To our current knowledge, Sochi virus seems to be the most pathogenic representative of DOB/BELV lineage of hantaviruses. The case fatality rate was determined to be as high as 14 %. Nearly 60 % of clinical cases were defined as severe (including fatal

cases) and nearly 40 % were classified as clinically moderate. Four times more males than females were affected. Notably, the age average among HFRS patients was around 30, and the proportion of young individuals (7–15 years old) was relatively high, 10 % (Dzagurova et al. 2008a).

The comparative analyses of clinical manifestations of patients with HFRS caused by five different hantaviruses showed that these viruses can cause mild, moderate, and severe forms of the disease. However, severe forms were more associated with DOB/SOCV (14 %) and HTNV (5–8 %) infections than with HFRS caused by PUUV, SEOV, and DOB/KURV (up to 1 % severe forms). In Russia, 97.7 % of the total number of HFRS were caused by PUUV associated with bank vole, *Myodes glareolus*. Only 2.3 % of HFRS cases were caused by other hantaviruses, HTNV, HTNV-like Amur/Soochong virus, and SEOV (1.5 % all together), and by DOB/BELV (0.8 %). Thus, PUUV virus plays the major role in the HFRS morbidity in Russia.

Periodical and massive reproduction of rodents, with the forming epizootics among them, is the main and determinative factor that influences HFRS epidemics in humans. The prevention of the HFRS disease mainly includes measures aimed at reducing exposure to live rodents and their excreta. However, rodent control measures are expensive and difficult to maintain over a long period of time. Preventive vaccination is the only effective measure to control hantavirus infection and reduce HFRS morbidity in endemic regions. The HFRS morbidity can be used to estimate the potential population at risk and the required HFRS vaccine doses. In Russia, vaccination campaign has to cover 20 European regions (where HFRS is caused mainly by PUUV and less by DOB/BELV) with a population of ~45 million as well as four Far Eastern regions (where HFRS is caused mainly by HTNV, HTNV-like, and Amur/Soochong virus and less by SEOV) with a population of ~5 million. Approximately 50 % of the population in these regions (25 million) potentially are at risk and are potential recipients of vaccine against HFRS.

Hemorrhagic Fever with Renal Syndrome in Europe For the period of 80 years since the first description of HFRS human cases in Sweden (Myhrman 1934; Zetterholm 1934), the list of European countries with HFRS incidence reached to date 35: Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Croatia, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Luxembourg, the Netherlands, Norway, Portugal, Romania, European Russia, Slovenia, Slovakia, Sweden, Switzerland (Vapalahti et al. 2003; Heyman et al. 2009), Albania (Eltari et al. 1987; Antoniadis et al. 1996), Belarus (Zhavoronok et al. 2008), Estonia (Vasilenko et al. 1987), Georgia (Kuchuloria et al. 2009), Latvia (Lundkvist et al. 2002), Lithuania (Moteyunas et al. 1990), Macedonia (Gligic et al. 2010), Moldova (Mikhaylichenko et al. 1994), Montenegro (Papa et al. 2006), Poland (Gut et al. 2013), Serbia (Gligic et al. 2010), Turkey (Ertek and Buzgan 2009), and Ukraine (Micevich et al. 1987). HFRS is a widespread infection in Europe with clear effects on public health. Unfortunately, hantavirus infection remains to be underestimated or not recognized by the medical and public-health authorities in many countries, mainly because of the lack of diagnostics. Practically, with

exception of Finland, Russia, Sweden, Belgium, Yugoslavia, and France, in all other countries, HFRS cases became recognizable and diagnosed only after 1990 (Heyman et al. 2009). Still many cases remained undiagnosed due to subclinical manifestations and nonspecific symptoms at the early stage of the disease.

Epidemiological analysis of HFRS morbidity in Europe is complicated due to the absence of statistical data in most European countries (with Russia exception). It seems reasonable to use the European Network for Diagnostics of Imported Viral Diseases (ENIVD) information, collected and published by Paul Heyman and other authors (Heyman et al. 2008, 2009, 2011), as well as case reports and description of HFRS outbreaks. Since 2012, approximately 300,000 HFRS cases have been reported in 35 European countries. The distribution of HFRS was found to be scattered throughout Europe. HFRS morbidity varies considerably in different countries with the highest rate in the European part of Russia, where HFRS cases make up ~70–80 % of total number of HFRS cases registered in Europe (see below). In addition to Russia, there are countries with high annual HFRS morbidity. These countries include Finland (997.5 cases), Germany (544.6 cases), Sweden (276.8 cases), and Belgium (98.1 cases). In 1999, 2002, and 2005, Finland had about 2,500 serologically confirmed HFRS cases; in 2008, a record year, 3,259 cases were diagnosed. Belgium had peak years in 2007 (298 cases) and in 2008 (336 cases). In 2007, Sweden had 2,195 cases (Vaheri et al. 2011; Heyman et al. 2009, 2011; Makary et al. 2010). In 2004–2005 local outbreaks of HFRS were reported in Germany. A large outbreak with 1,688 cases was reported in 2007 and 2,017 cases were reported in 2010 (Hofmann et al. 2008; Faber et al. 2010). Climate changes seem to be responsible for the increase in the number of sporadic HFRS cases without any traceable geographical or temporal trends (Heyman 2007). With Russia exception, Norway, Sweden, and Finland account for the most of hantavirus infections in Europe.

In Europe, HFRS is caused by three hantavirus species, PUUV (carried by *M. glareolus,* bank vole); DOB/KURV (carried by *A. agrarius*, the striped field mouse); DOB/DOBV, genotype Dobrava (carried by *A. flavicollis*, yellow-necked mouse) (Klempa et al. 2013a); and by DOB/SOCV, genotype Sochi, associated with *A. ponticus* (the Black Sea field mouse). PUUV is the major cause of HFRS in Western, Central, and Northern European countries (Finland, Sweden, Norway, Germany, Belgium, France, and European Russia). DOB/KURV has been found in Germany, Slovakia, Russia and Slovenia. This virus commonly infects humans and is associated with DOBV/KUR-HFRS. DOB/DOBV is the main cause of severe HFRS in Southern Europe, including Greece, Albania, Bulgaria Slovenia, Croatia, Bosnia, Serbia, Montenegro (Vapalahti et al. 2003; Antoniadis et al. 1996; Avsic-Zupanc et al. 1999; Markotic et al. 2002; Papa et al. 2006; Lundkvist et al. 1997). The disease in the Balkans is seen primarily among adults, especially woodcutters, shepherds, military personnel and others whose occupations occasionally require them to work or sleep outdoors. Unlike HFRS in other parts of Europe, cases in the Balkans peak during the warmer months of the year, with 80 % of them registered from June to September (Avsic-Zupanc et al. 1999). In the Balkans, DOB/DOBV-HFRS patients have more severe clinical manifestations then PUUV-HFRS

patients. The mortality rate can be up to 12 % (Papa et al. 2006; Markotic et al. 2002). By contrast, DOB/KURV infections in Baltic countries, in Central Europe, and in Central European part of Russia are mainly associated with mild-to-moderate forms HFRS with very little fatalities if any (Vapalahti et al. 2003; Klempa et al. 2008; Dzagurova et al. 2009).

In January–May, 2009, 12 HFRS cases caused by PUUV were first registered in Turkey (Ertek and Buzgan 2009); 2 more cases were additionally diagnosed in August (Kaya et al. 2010).

HFRS is endemic in Austria where approximately 30 cases of HFRS are annually diagnosed. The last epidemics were observed in 2004 and 2007 with 72 and 78 documented PUUV-HFRS cases, respectively. In 2011–2013, the first DOB/DOBV and DOB/KURV-HFRS cases have been detected. Hantaviruses DOB/DOBV and DOB/KURV were also found in *A. flavicollis* and *A. agrarius*, respectively, captured at the place of residence of HFRS patients (Aberle et al. 2013).

Risk factors for HFRS include professions such as forestry, farming and military, or activities such as camping and the use of summer cottages. Humans are thought to be infected from aerosolized rodent excreta when exposed to hay and crop during harvesting, cleaning cellars, sheds, stables or summer cottages in the fall and handling wood (especially inside the dusty woodsheds). Hantaviruses are reasonable stable and can be viable (infectious) for more than 10 days at room temperature (Hardestam et al. 2007; Kallio et al. 2006). Moreover, bank voles excrete PUUV for several months, especially in saliva (Hardestam et al. 2008). The male gender is a clear risk factor with a male/female ratio of, for example, 1.67 in Finland and 1.52 in Sweden (Makary et al. 2010; Hjertqvist et al. 2010). Risk factors also include the use of rodent traps instead of poison rodent control campaign. Additional risk has been attributed also to woodcutting and house warming with firewood and spending time and working in the forest. Increased incidence or occupational risk is also associated with military activity, farming, forestry, camping, and summer cottages (Winter et al. 2009).

In summary, the HFRS disease is endemic in many European countries and hantavirus infection is a growing public-health problem. No specific therapy or vaccine is currently available. There is a need to develop advanced vaccines which should include PUUV and DOB/BELV antigens.

Hemorrhagic Fever with Renal Syndrome in Asia In Asia, clinical HFRS cases caused by HTNV, HTNV-like viruses (Amur/Soochong virus), and SEOV have been registered mainly in China, South and North Korea, and the Far Eastern regions of Russia. China is the major HFRS-endemic country in Asia and in the world. During 1950–2007, a total of 1,557,622 HFRS cases and 46,427 deaths (3 %) were reported in China with the highest annual peak in 1986, with 115,985 cases. HFRS has been reported in 29 of 31 provinces in China with annual morbidity up to 40,000–60,000 cases (Zhang et al. 2010). In 2004 the National Disease Reporting System was established by China CDC. From 2006 to 2011, a total of 64,250 HFRS cases and 762 deaths were reported with the case fatality rate of 1.18 % (Li 2013).

HFRS morbidity is associated mainly with the northeastern, eastern, central, and southwestern parts of China (humid and semi humid zones). The disease rarely occurs in the northwestern part (arid zone) with top rate of 20.3, 18.9, 8.2, 7.7, 5.0, and 4.6 cases/100,000 population in the Heilongjiang, Shandong, Zhejiang, Hunan, Hebei, and Hubei provinces, respectively (Fang et al. 2007). Rural areas account for more than 70 % of all HFRS cases; mainly peasants were infected (Chen and Qiu 1993). Poor housing conditions and high rodent density in residential areas seem to be responsible for most HFRS epidemics. The increase in HFRS morbidity from the end of the 1970s coincided with the fast socioeconomic development started in 1978 in China. During the 1980s and 1990s, China underwent large changes such as agricultural development, irrigation engineering, urban construction, mining, and highway and railway construction. These activities increase human exposure and contact with rodents. Because rats are more mobile than other hantavirus hosts (Plyusnin and Morzunov 2001), fast socioeconomic development also causes wide expansion of rats infected with SEOV. This fact might subsequently lead to the high nationwide prevalence of SEOV infections. However, improved housing conditions, improved hygiene, and human migration from rural areas to cities might contribute to the decline of HFRS cases since 2000. In general, HFRS cases are registered throughout the year with increase in winter and spring with the peak in November (Chen and Qiu 1993; Chen et al. 1986). Early epidemiological investigations found that the winter peak resulted from HTNV carried by *A. agrarius* and that the larger spring epidemic was mainly caused by SEOV carried by *R. norvegicus* (Chen et al. 1986). HFRS affects patients of any age (from infancy to >65 years), but mostly adolescents and young adults got infected (Chen and Qiu 1993; Chen et al. 1986). The incidence in males were over three times higher than females (Li 2013). Because *A. agrarius* and *R. norvegicus* rodents are the predominant carriers and distributed nationwide, HTNV and SEOV are obviously the major threat for HFRS in China. Epidemiological studies in China suggest that camping or living in huts in fields, living in a house on the periphery of a village, and cat ownership are supposed to be risk factors (Rio et al. 1994). The gradual change in the disease structure (proportions of mild and severe disease) might have contributed to the decreased mortality rates as well. In recent decades, as rats followed human activities and migration from rural to urban areas during the fast socioeconomic development in China, the proportion of mild HFRS cases caused by SEOV steadily increased while the proportion of more severe cases associated with HTNV infection decreased (Chen and Qiu 1993).

HTNV was first isolated from striped field mice in 1981 (Yan et al. 1982). Consistent with the geographical distribution of *A. agrarius*, HTNV has been found in all Chinese provinces except Xinjiang (Yan et al. 2007). In addition to *A. agrarius*, HTNV has been also found in *Apodemus peninsulae* in northeastern China (Zhang et al. 2007). Genetic analysis of the small (S) and medium (M) genome segments suggested that at least nine distinct lineages of HTNV are circulating in China (Zou et al. 2008). In general, HTNV variants display geographical clustering. Recently, reassortment between HTNV and SEOV was detected in *R. norvegicus* (Zou et al. 2008), which indicates that genetic

reassortment occurs naturally between two hantavirus species. Because reassortment is a way for segmented viruses to achieve high infectivity and adapt to new animal hosts, further studies are warranted to evaluate susceptibility of *A. agrarius* and *R. norvegicus* rodents to these unique reassortant viruses and to determine whether these reassortants can infect humans.

HFRS cases caused by SEOV were first reported in Henan and Shanxi provinces along the Yellow River in China (Hang et al. 1982). Subsequently, SEOV (strain R22) was isolated from *R. norvegicus* in Henan (Song et al. 1984), and SEOV has been found in almost all provinces of China except Qinghai, Xinjiang, and Xizang (Zhang et al. 2009). SEOV-associated HFRS seems to have recently spread to areas where it had not been reported during previous epidemics (Zhang et al. 2009). Most known SEOV variants (from lineages 1–4 and 6), including those from China, Brazil, Japan, South Korea, North America, and the United Kingdom, are genetically homogeneous. Lineages 1–4 are widely distributed and do not follow a geographical clustering pattern. Thus, the variants from lineages 1–4 and 6 are closely related and may have a more recent common ancestor. Because *R. norvegicus* is distributed nationwide and found to be more mobile than other hantavirus hosts, SEOV has become the largest threat for public health in China. It may bring even more potential threats to humans as rat species become more widespread along with globalization of the economy. Natural HFRS cases caused by SEOV have been found almost exclusively in China and other Asian countries. The lack of HFRS in other countries may result from better living conditions, low rat densities, and low rates of SEOV carried by the rats.

Hantaviruses are thought to have coevolved with their respective hosts. Each serotype and/or genotype of hantavirus appears to be primarily associated with 1 (or a few closely related) specific rodent host species. As described above, more than 100 species of rodents and several dozens of insectivores are widely distributed in HFRS-endemic areas in China (Zhang et al. 1997). Hantavirus-specific antibodies and/or antigens have been identified in, at least, 38 rodent species. Therefore, in addition to already known HTNV, SEOV, Dabieshan virus, Hokkaido virus, Khabarovsk virus, Vladivostok virus, and Yuanjiang virus, yet-unknown hantavirus species may be circulating in China. In-depth studies on hantavirus distribution in different geographical regions and hosts in China as well as genetic characterization of hantaviruses and elucidation of the relationship between these viruses and other known hantaviruses should help prevent the diseases they cause.

A comprehensive preventive strategy has been implemented to control HFRS in China. It includes public-health education and promotion, rodent control, surveillance, and vaccination (Zhang et al. 2004). Since the 1950s, on mainland China, the rat population has been controlled by using poison bait or trapping around residential areas. During the 1980s and 1990s, deratization around residential areas effectively decreased both rodent density and incidence of HFRS, especially the disease caused by SEOV (Luo and Liu 1990).

Improving general awareness and knowledge of pathogen source, transmission routes (how to avoid contact with a pathogen), diagnostics, vaccination, and general hygiene appears to be one of the most cost-effective ways to prevent infectious

diseases. Since the 1970s, public education on HFRS and other infectious diseases has been conducted by all possible means in China, especially in rural areas. After implementation of comprehensive preventive measures, including vaccination, in the past decade in China, HFRS morbidity has decreased dramatically. Only 11,248 HFRS cases were reported in 2007 (Zhang et al. 2010). Mortality rates also declined from the highest level of 14.2 % in 1969 to 1 % during 1995–2007.

Nevertheless, despite intensive measures implemented last years, HFRS remains a major public-health problem in China (Zhang et al. 2004), and during the last years, there is a trend in the increasing number of HFRS cases (Li 2013).

HFRS is one of the important acute febrile infections and a major public-health problem in South and North Korea. It was recognized for the first time in Korea in 1951 among soldiers of the United Nations (Smadel 1953). The causative pathogen Hantaan virus was discovered by Lee et al. in 1976 (Lee et al. 1978) and named after the Hantaan River crossing the endemic areas near the demilitarized zone between North and South Korea.

In South Korea, a total of 14,309 HFRS patients were hospitalized from 1951 to 1986, with one third being soldiers (Lee 1989). Hundreds of HFRS cases were registered in the 1970s and 1980s, with a sharp increase in the number of cases in the early 1990s, up to 1,200 cases per year. From 2001 to 2008, 323–450 HFRS cases were registered annually in the South Korea (South Korean Centers for Disease Control and Prevention 2008). The number of hospitalized HFRS patients has declined to 100–300 per year in recent years in South Korea (Baek et al. 2006).

Both HTNV and SEOV are known as the etiologic agents of HFRS in Korea. These viruses establish chronic infections in certain species of rodents and are transmitted to individual primarily via aerosols or fomites from feces, urine, and saliva of infected mice (Tsai 1987). HTNV, carried by *A. agrarius* and *A. peninsulae*, causes a severe form of HFRS and is mostly distributed in rural areas, whereas SEOV, carried by *Rattus norvegicus* and *Rattus rattus*, causes urban-acquired cases and may cause a milder clinical syndrome.

There are two epidemic periods of HFRS each year, the major (October–December) and minor (May–July) epidemic periods. The majority of cases (more than 75 % of patients) occur during the major epidemic period. SEOV infection is less seasonal in occurrence. There are two high-risk groups of HFRS—residents, who are mostly farmers, and Korean soldiers stationed in the field (Lee 1989). More than 500 HFRS cases were serologically confirmed and hospitalized annually in the 1980s (Lee 1989). However, the number of the reported cases has gradually decreased to approximately 300–400 cases per year since it was legally designated as a communicable disease in 2000 (Korea Center for Disease Control and Prevention 2004). Nevertheless, the associated factors have not been defined. The inactivated hantavirus vaccine (Hantavax™, Korean Green Cross, Korea) has been commercially available since October 1990. Because of its adoption into the national immunization program in 1992, it has been widely distributed to public-health centers and the Korean army (Cho et al. 2002).

The sporadic HFRS cases have been reported in India, Indonesia, Singapore, Sri Lanka, Thailand, Hong Kong, and Taiwan (Clement et al. 2006; Chandy et al. 2009;

Groen et al. 2002; Plyusnina et al. 2004; Chan et al. 1996; Vitarana et al. 1988; Tai et al. 2005; Suputthamongkol et al. 2005). Serological investigation showed evidences of hantavirus infections in humans in Israel, Kuwait, Laos, Malaysia, Philippines, and Vietnam (George et al. 1998; Pacsa et al. 2002; Rollin et al. 1986; Lam et al. 2001; Quelapio et al. 2000).

Hantavirus Pulmonary Syndrome in the New World Hantavirus pulmonary syndrome (HPS), was discovered in the southwestern United States in 1993 (Duchin et al. 1994). The causative agent was determined to be an unidentified North American member of the *Hantavirus* genus (Nichol et al. 1993). The clinical syndrome caused by this agent, ultimately named Sin Nombre virus (SNV), came to be called hantavirus pulmonary syndrome, HPS. This designation distinguished it from previously described hantavirus illnesses, which were characterized as HFRS. At the early stage of the disease, cardiac and respiratory functions are markedly impaired by virus infection. For this reason, some authors have proposed name "hantavirus cardiopulmonary syndrome." In the United States, HPS was retrospectively traced back to as early as 1975 (Wilson et al. 1994).

Until now, about 3,000 cases of HPS have been identified in small clusters and individual cases throughout North and South America, with a total of 616 cases occurring in the United States (between 11 and 48 cases annually) (Jonsson et al. 2010; CDC 2012a, b, c). More than half of the North American hantavirus cases occur in the Four Corners area of the Southwest, but infections have been reported in 34 US states. In Canada, cases of HPS are rare, fewer than eight being reported per year, with the first Canadian case of HPS identified retrospectively back to 1989 (Weir 2005). A 26 % case fatality of HPS was reported in Northern Alberta, Canada (Verity et al. 2000). Although generally occurring in rural areas, up to 25 % of cases occur in urban and suburban areas (CDC 2012b). Although reporting of the disease appears relatively sparse, the actual incidence may be somewhat higher due to asymptomatic infections. In a study performed in Baltimore (an area with very few reported cases of HPS), 44 % of mice and 0.74 % (nine patients) were serologically positive for hantavirus despite being otherwise healthy and asymptomatic (Zaki et al. 1996).

Although there appears to be a significant spectrum of disease, the case fatality rate for symptomatic HPS patients in the United States was 38 % (Zaki et al. 1996). Most cases occur during the late spring and early summer months, which may allow clinicians to distinguish the disease from influenza, which has a similar presentation (CDC MMWR 1993). Cases almost exclusively occur in people who sleep or work in areas where they may be exposed to rodents. Transmission of the virus occurs predominately through inhalation of aerosolized rodent urine, feces, or saliva; exposure may also occur through food contaminated by rodent saliva and excreta and through rodent bites (Jonsson et al. 2010; Schmaljohn and Hjelle 1997). Although human-to-human transmission has not been observed in North America, there have been a few documented cases of such transmission in South America (Jonsson et al. 2010). The largest risk factor is entering closed buildings with rodent infestations (Armstrong et al. 1995).

In the United States, the principal virus causing HPS is SNV, which chronically infects the deer mouse, *Peromyscus maniculatus*. The deer mouse habitat occupies a huge swath of the North American continent, sparing only areas nearing the Arctic Circle, a few states in the southeastern United States, and southern Mexico. Approximately 10 % of tested deer mice in this range are infected with SNV (Lonner et al. 2008). Additionally, closely related hantaviruses are hosted by other *Sigmodontinae* rodents in areas where deer mice are sparse, including Black Creek Canal virus, hosted by the cotton rat *Sigmodon hispidus* in Florida, and the Bayou virus, hosted by the swamp rat *Oligoryzomys palustris* in Louisiana and Texas.

In Argentina, the first case of HPS was confirmed by virus detection in 1995 (Lopez et al. 1996). Three clusters involving 29 cases and a severe outbreak with 18 HPS cases were later reported in 1995 and 1996, respectively (Levis et al. 1998). By the end of 2006, a total of 841 cases were reported in Argentina (Capria et al. 2007). In 1996, an outbreak of HPS was detected in the Neuquen region of southern Patagonia, and the source was traced to *Sigmodontinae* rodent, long-tailed rice rat, *Oligoryzomys longicaudatus*. The hantavirus detected in both patients and rats was named the Andes virus (ANDV) (Lopez et al. 1996). In 2002, at least 10 HPS cases were reported in Bolivia with 6 deaths (Carroll et al. 2005). By the end of 2004, 36 cases had been reported in the country. In Brazil, the first case of HPS was reported in a family cluster in 1993 (Moreli et al. 2004), and 855 HPS cases were reported between 1993 and 2006 with a 39.3 % case fatality (Da Silva 2007). In Chile, since the first identification of HPS in 1995 (Espinoza et al. 1998), 352 cases of HPS had been reported up to 2006, with a case fatality rate of 33 %. In Uruguay, the first evidence of the circulation of these viruses came from a study of serum specimens collected from blood donors between 1985 and 1987 (Weissenbacher et al. 1996). Since then, more than 60 cases of HPS have been confirmed in (Delfraro et al. 2007). The first cluster of HPS in Central America occurred from late December 1999 to February 2000 in Los Santos Province in Panama. Through 2006, there were 85 cases of HPS reported in Panama with a case fatality rate of 17.6 % (Armien et al. 2007). In Paraguay, the first outbreak of HPS occurred in 1995 (Carroll et al. 2005), and through 2004, there had been 99 cases of HPS in that country. The overall seroprevalence of hantavirus infections in the Chaco area of Panama was 43 % (Ferrer et al. 2003).

In the Caribbean region, a single case of HPS was serologically confirmed in eastern Venezuela. A low prevalence (1.7 %) but wide distribution of hantavirus infections was demonstrated in the country (Rivas et al. 2003). Human infections in Colombia (Espinoza et al. 1998) and rodent infection with Sin Nombre-like hantaviruses in Costa Rica, Mexico, and Peru were also reported (Hjelle et al. 1995; Suzan et al. 2001; Powers et al. 1999).

Transmission largely occurs through inhalation of aerosolized urine, feces, or saliva of the rodent host. Within species, the viruses are also commonly transmitted through aggressive behavior, such as biting, especially among males, and males have a higher prevalence of infection than females (Douglass et al. 2006; Calisher et al. 2001). HPS is predominantly a rural disease, with associated risk factors of

farming, land development, hunting, and camping, because each of these activities brings humans into closer contact with the natural rodent reservoirs, which are all sylvan or agrarian in their choice of habitat. However, HPS is nearly always acquired indoors or within closed spaces, such as peridomestic buildings on farms or ranches, livestock feed containers, or the cabs of abandoned pickup trucks (Armstrong et al. 1995). Several factors contribute to the propensity for indoor acquisition by humans. Animals captured in the peridomestic environment have a higher prevalence of active infection than those captured in a sylvan environment (25 % vs. 10 %), likely because of greater supplies of foodstuffs and higher murine population densities (Kuenzi et al. 2001). Higher population densities lead to more interaction among mice and higher rates of intraspecies transmission. Likewise, humans are more likely to encounter rodent excreta when population densities are higher.

In the United States, approximately two thirds of HPS cases have been among men. The average age of patients who have HPS is 38 years, with a range of 10–83 years. There has been a striking absence of severe HPS among prepubertal individuals in the United States, although disease in 11 children aged 10–16 years had clinical courses similar to those described in adults (Kuenzi et al. 2001).

The incidence of HPS in Latin America is largely unknown but cases have been reported from Central America to southern Patagonia. The ANDV was responsible for outbreaks in Argentina and Chile and is closely related to the Bayou virus. Although most North American cases have been sporadic and isolated, most South American cases have occurred in clusters. The Patagonian outbreak in 1996 was unique in that it occurred in an area with a relatively low rodent population density, and human-to-human transmission was suspected when physicians treating infected patients became ill themselves (Enria et al. 1996). Gene sequencing of virus recovered from cases with rodent exposure and from their contacts who had no possibility of rodent exposure confirmed human-to-human transmission (Padula et al. 1998; Martinez et al. 2005).

The seroprevalence of IgG antibodies to hantaviruses differs between North and South American populations. In the United States, the Four Corners area has the highest incidence of infection; however, presence of antibodies among tested individuals in that region is less than 1 % (Auwaerter et al. 1996; Vitek et al. 1996). Childhood infection in North America is also rare. In contrast, some endemic areas in South America have a much higher rate of infection, including in children, with seroprevalence as high as 42.7 % in areas of Paraguay (Ferrer et al. 2003). In all areas studied, the seroprevalence is higher in South America than in North America, suggesting the occurrence of mild and asymptomatic infections (Pini 2004).

Based on the broad distribution of *Sigmodontinae* rodents throughout the Americas, the CDC estimates that HPS infections can be potentially detected in every county of the North and South Americas (CDC MMWR 1993).

There is currently no Food and Drug Administration-approved vaccine for the New World hantaviruses. However, several vaccine candidates are in different stages of clinical development (Schmaljohn 2009, 2012). Inactivated virus vaccines

like those used in Asia are generally not being pursued for HPS because of inadequate efficacy and concerns about the risks of mass production of a high-containment virus (Jonsson et al. 2008). Given the possible use of hantaviruses as a bioterrorism agent and its endemic status across the globe, it is clear that the development of effective hantavirus countermeasures is necessary (Hartline et al. 2013).

5.3 Inactivated Hantavirus Vaccines

Inactivated virus vaccines significantly contributed to the control of infectious diseases during the twentieth century and probably will remain an attractive strategy for vaccine development for the coming decades. Inactivated vaccines are currently widely available for poliomyelitis, influenza, rabies, hepatitis A, tick-borne encephalitis, and Japanese encephalitis (Trofa et al. 2008; Falleiros Carvalho and Weckx 2006; Webby and Sandbulte 2008; Rouraiantzeff 1988; Eckels and Putnak 2003; Schioler et al. 2007).

5.3.1 Rodent Brain-Derived Hantavirus Vaccines

The high HFRS morbidity in the 1980s in Asian countries has raised an urgent need to develop vaccines against hantaviruses. Most of these vaccines were made using either formalin or β-propiolactone inactivated rodent brain-derived hantavirus, similar (Table 5.1) to those used to prepare Japanese encephalitis and rabies vaccines (Oya 1976; Gupta et al. 1991; Acha 1967).

In the South Korea, the initial vaccines were based on the brain suspension of suckling rats infected with HTNV's strain ROK 84-105 (Lee and Ahn 1988; Lee et al. 1990). The virus strain ROK 84-105 was isolated from the blood of HFRS patient through Vero-E6 cells (French et al. 1981) and passaged 7–10 times in the brains (IC inoculation) of suckling rats (titer—7 $\log_{10}$ LD 50/mL) or mice (titer—9.2 $\log_{10}$ LD 50/mL). Brains were harvested 7–8 days after virus inoculation, and phosphate-buffered saline was added to the brains to prepare virus suspension, which was then centrifuged at 10,000 g for 15 min. At the next step, protamine sulfate was added to the supernatant to precipitate cellular proteins. The mixture was centrifuged, ultrafiltrated, and ultracentrifuged at 40,000× *g* for 2 h at 4 °C, and then 0.05 % formalin was added to the supernatant to inactivate the virus. The inactivated vaccine was then mixed with alum hydroxide (adjuvant).

The concentration of viral antigen in the vaccine preparation was determined by enzyme-linked immunosorbent (ELISA) assay. The immunogenicity of vaccine was tested in inbred BALB/c mice after intraperitoneal inoculation. The mice were bled by heart puncture 2 or 4 weeks after immunization, and hantavirus antibody titers in sera were determined by immunofluorescence (IFA), by ELISA, and by a

Table 5.1 Inactivated hantavirus vaccines

Country	Hantavirus	Substrate	Inactivation	State of development
Rodent brain-derived hantavirus vaccines				
Japan	SEOV	Suckling mouse brain	Formalin	Preclinical
South Korea	HTNV	Suckling rat brain	- " -	Clinical
- " -	- " -	Suckling mouse brain	- " -	Commercial
- " -	PUUV	Suckling hamster brain	- " -	Clinical
- " -	PUUV-HTNV	- " -	- " -	- " -
North Korea	HTNV	Suckling rat brain	Formalin	Commercial
- " -	- " -	Suckling hamster brain	- " -	Preclinical
China	HTNV	Suckling mouse brain	β-propiolactone	Commercial
- " -	SEOV	- " -	- " -	Preclinical
Russia	HTNV	Suckling mouse brain	Formalin	Preclinical
Cell culture-derived hantavirus vaccines				
China	HTNV	Golden hamster kidney cells	Formalin	Clinical
- " -	SEOV	- " -	- " -	Commercial
- " -	HTNV-SEOV	- " -	- " -	- " -
- " -	HTNV	Mongolian gerbil kidney cells	β-propiolactone	Commercial
- " -	SEOV	- " -	- " -	Clinical
- " -	HTNV-SEOV	- " -	- " -	Commercial
- " -	SEOV	Striped field mouse kidney cells	- " -	Clinical
- " -	HTNV	Chicken embryo cells	Formalin	Clinical
- " -	HTNV-SEOV	Vero cells	β-propiolactone	Commercial
South Korea	HTNV	Vero-E6 cells	Formalin	Preclinical
Russia	PUUV-DOBV	Vero cells	- " -	Preclinical

plaque reduction neutralizing test (PRNT). Protective efficacy of the vaccine was tested by challenging the mice with prototype strain 76-118 of HTNV and then measuring viral antigen in the lungs. Immunogenicity and protective activity studies showed that experimental vaccine was effective against HTNV infection in mice.

In general, in other endemic countries, the method of producing of rodent brain-derived hantavirus vaccine was similar to the protocol described above. The Japanese vaccine was based on the brain of mice, infected with SEOV (Yamanishi et al. 1988); the North Korean vaccines, on the brain of suckling rats and hamsters,

infected with HTNV (Kim and Ryu 1988; Kim et al. 1989, 1991); Chinese vaccines, on the brain of suckling mice, infected with HTNV or SEOV (Sun et al. 1992; Yu et al. 1990a); and Russian vaccine, on suckling mice, infected by HTNV (Astakhova et al. 1995). In rodent brain-derived vaccines produced in China, β-propiolactone was used for virus inactivation (Yu et al. 1990b). The experimental rodent brain-derived vaccines usually elicited good immune responses in rodent models as measured by IFA, ELISA, and neutralizing test.

A commercial South Korean inactivated HTNV ICR mouse brain-derived vaccine, named Hantavax™, was shown to be effective in protecting experimental mice and humans from HFRS (Cho and Howard 1999; Cho et al. 2002). A month after vaccination of 64 human volunteers with Hantavax™ subcutaneously (s.c.), the vaccinated individuals developed hantavirus antibody measured by IFA (79 %) and ELISA (62 %) (Cho and Howard 1999). One month after a second vaccination, the seroconversion rate increased to 97 %. Neutralizing antibody titers followed this trend, with 13 % of vaccine recipients producing neutralizing antibody 1 month after the first dose and 75 % of vaccine recipients responding 1 month after boost. Antibody titers had declined during the time and at 1 year after immunization only 37 % and 43 % of sera found to be positive by IFA and ELISA, respectively. Revaccination at this time produced a vigorous immune response, with 94 and 100 % of vaccine recipients yielding positive antibody titers. Approximately 50 % primary vaccinees produced neutralizing antibodies following the booster dose 1 year later. Another study found a neutralization response in 33 % of recipients after two immunizations (Sohn et al. 2001). It was concluded that the booster vaccination is necessary at 1 year after primary vaccination for maintaining a high level of antibodies. After the boost, antibodies persisted for 2 additional years.

During 1991–1998, more than 5 million people were vaccinated with Hantavax™ in South Korea (Cho et al. 2002). Vaccination significantly decreased the total number of hospitalized HFRS patients, from 1,234 cases in 1991 to 415 cases in 1997 (Cho et al. 2002). It seems that in addition to vaccination, some additional factors contributed to this decline (Cho et al. 2002; Hjelle 2002).

In 1996–1997, a clinical trial was conducted in endemic areas of HFRS in Yugoslavia. Vaccinees received Hantavax™ twice and boosted a year later. Twenty-five HFRS patients were documented among a control group, but none were reported among 2,000 vaccine recipients (Lee et al. 1999; Bozovic et al. 2001).

After vaccination with the Chinese inactivated HTNV mouse brain-derived vaccine (i.m.), IFA antibody were detected in 84 % and 18 % vaccinees 2 weeks and 1 year after vaccination, respectively; neutralizing antibodies were detected in 51 % and 10 %, respectively (Sun et al. 1992). 2 weeks, 1 year, and 2 years after booster revaccination, the seroconversion rates were 83 %, 42 %, and 13 % in IFA and 62 %, 41 %, and 25 % in PRNT assay, respectively. In 30 volunteers immunized with Chinese inactivated SEOV mice brain-derived vaccine, vaccination resulted in the induction of high titers of specific antibodies measured by ELISA and by PRNT (Yu et al. 1990a).

Three weeks after the boost immunization with North Korean inactivated HTNV rat brain-derived vaccine, IFA antibodies and antibodies detected in reversed passive hemagglutination inhibition assay (RPHI) were found in 78.1 % and 88.8 % of vaccinees, respectively. No neutralizing antibody data were detected. Nevertheless, in clinical trial performed in North Korea where 1.2 million people were vaccinated, the high protective efficacy (88–100 %) was reported (Kim et al. 1991).

In general, the inactivated rodent brain-derived hantavirus vaccines elicited good humoral immune responses (IFA, ELISA) in rodent models. In most cases neutralizing antibody responses were detected only after boost immunization (Cho and Howard 1999). Whereas some authors describe a high protection and significant HFRS case reduction after prime-boost immunization with these vaccines (Zhang et al. 2010; Li 2010), the clinical efficacy of these vaccines is still questionable (Hammerbeck et al. 2009; Schmaljohn 2009).

In the 1990s, formalin-inactivated suckling hamster brain-derived vaccines against PUUV were developed (Lee et al. 1997, 1999). Monovalent vaccine PUUVAX contained formalin-inactivated K-27 strain of PUUV isolated from HFRS patient from Bashkiria region of Russia. One dose of PUUVAX contained 5,120 U/ELISA of virus antigen in 0.5 mL. Antibody response of hamsters after inoculation of PUUVAX vaccine showed high titers of IFA and PRNT antibodies against PUUV (Lee et al. 1999).

Blended HTNV-PUUV vaccine contained 5,120 U/ELISA of each HTNV and PUUV antigen in 1.0 mL. Immunization of hamsters with HTNV-PUUV resulted in production of IFA and PRNT antibodies. In fact, blended HTNV-PUUV vaccine produced even higher titers of PRNT antibodies than monovalent Hantavax™ or PUUVAX vaccines (Lee et al. 1999). To study immunogenicity and efficacy of the blended vaccine, hamsters were given 0.1 mL of vaccine twice at a 1-month interval. Antibody titers were measured by IFA and PRNT against five hantaviruses: HTNV, SEOV, DOBV, PUUV, and SNV or NYV. On day 30 after the first immunization, animals had IFA antibody titers of 78.4, 68.8, 68.8, 37.9, and 15.6 and PRNT titers of 65.4, 12, 6.1, 65.6, and 0.5, respectively. On day 30 after the second shot, IFA titers were 686.9, 567.5, 550.4, 516.3, and 430.9, and PRNT titers were 710.8, 41.9, 24.3, 409.9, and 1.6 against HTNV, SEOV, DOBV, PUUV, and NYV, respectively.

None of the vaccinated hamsters challenged with infectious HTNV, SEOV, DOBV, or PUUV showed either viremia or viral RNA in lung tissues (by nested RT-PCR). In contrast, vaccinated hamsters challenged with SNV or NYV became viremic and the challenged virus was detected in lung tissues. The vaccinated hamsters challenged with HTNV, SEOV, DOBV, or PUUV did not show any significant increase in IFA and PRNT antibodies. Meanwhile, the increase in PRNT antibody against NYV was observed in vaccinated hamsters challenged with SNV or NYV (Cho and Howard 1999).

In a limited study, 10 volunteers were vaccinated with blended HTNV-PUUV vaccine and 2 volunteers received PUUVAX vaccine (3 times, s.c., 1-month intervals) with various doses. All volunteers produced relatively high IFA

(1:128–1:2,048) and PRNT (1:10–1:640) antibodies against homologous hantaviruses after the second and third vaccinations (Lee et al. 1999; Cho et al. 2002).

Formalin or β-propiolactone inactivated rodent brain-derived hantavirus vaccines induced mostly local reactions including induration and swelling. There were no serious complains and these affects were self-limiting (Lee et al. 1999; Cho et al. 2002). Nevertheless, the case of toxic epidermal necrosis (TEN) with ocular involvement associated with vaccination against HFRS was reported (Hwang et al. 2012). In general, the lack of serious side effects indicates that rodent brain-derived hantavirus vaccine appears to be well tolerated in humans.

5.3.2 Cell Culture-Derived Hantavirus Vaccines

The cell culture-derived hantavirus vaccines have been developed mainly by Chinese researchers with some contributions of scientists from South Korea and Russia. Chinese vaccines were developed based on four primary cell cultures derived from golden hamster (*Thomasomys aureus*) kidney (GHKC), Mongolian gerbil (*Meriones unguiculatus*) kidney (MGKC), striped field mouse (*Alaetagulus pumillio kerr*) kidney (SFMC), and chicken embryo (CEC) and on one continuous cell line from African green monkey kidney cells, Vero cells. Korean and Russian vaccines were based on Vero-E6 and Vero cells, respectively. Eleven cell culture-derived hantavirus vaccines were developed. Four monovalent vaccines against HTNV were produced in GHKC (Song et al. 1992a), MGKC (Sun et al. 1992), CEC (Dong et al. 2001) and in Vero-E6 (Choi et al. 2003) cells. Three vaccines against SEOV were made in GHKC (Yu et al. 1990a), MGKC (Li and Dong 2001), and SFMC (Zhao et al. 1998) cells. In addition, three blended bivalent HTNV-SEOV vaccines were produced in GHKC (Song et al. 1992b), MGKC (Liu et al. 1992), and Vero (Hang et al. 2004), and one blended bivalent PUUV-DOBV vaccine was generated in Vero cells (Tkachenko et al. 2009, 2010).

All cell culture-derived hantavirus vaccines were produced using similar technology: virus harvest, "clarification" (low-speed centrifugation), ultrafiltration, formalin or β-propiolactone inactivation, purification by zonal centrifugation or by chromatography on Sepharose column, sterilizing filtration, mixing with alum hydroxide, and final lot testing. Bivalent vaccines were blended at the initial steps.

The immunogenicity of vaccines was tested in different rodent (hamsters, mice, rats) in IFA, ELISA, hemagglutination inhibition (HI), and PRNT assays. Protective efficacy was evaluated in challenge experiments in the hamsters or gerbils. Results of these experiments showed that practically all vaccines were effective against homologues virus (Hao et al. 1996; Yu et al. 1990b).

So far, only vaccines developed and manufactured in China were tested in humans. A human clinical trial demonstrated that a three-dose vaccination regimen resulted in 90–100 % seroconversion as assayed by PRNT (Ren et al. 1996; Zhu et al. 1991; Yu et al. 1992). Two weeks after primary vaccination, PRNT conversion rates were 51–82 % and 1 year later declined to 10–12 %. After boost

immunization, PRNT conversion rates were higher, 62–80 %, and declined to 36–41 % and 23–31 % after 1 and 2 years after boost. Conversion rates of antibody detected by IFA were higher than those in PRNT assay. These results showed that hantavirus-specific antibody titers declined significantly after primary vaccination. A 1-year boost significantly increased antibody titers and resulted in slower decline of antibody titers at the end of the second year. These antibodies were still effective in virus control (Chen et al. 1998).

The GHKC-derived SEOV vaccine, MGKC-derived HTNV vaccine, and a suckling mouse brain-derived HTNV vaccine were compared in a large human trial. Vaccination protocol for GHKC-derived SEOV vaccine consisted of three vaccinations at 28- and 14-day intervals (primary vaccination) followed by a boost at 1 year. The primary vaccination with MGKC-derived HTNV included three vaccinations on day 0, 7, and 14 followed by a boost at 1 year. The SMB-derived HTNV vaccine protocol included three vaccinations at 2-week intervals followed by a boost at 1 year. Among 55,000 vaccinees who received at least three doses of vaccines, side effects were in 2.6 % of vaccinees. Suckling mouse brain-derived HTNV vaccine produced the highest side-effect rate, 7.3 %; GHKC-derived vaccine had a middle rate, 3 %; and MGKC-derived vaccine had the lowest rate of side effect, 1.9 % (Chen et al. 1998).

To date, four inactivated cell-derived and one rodent-derived vaccines against hantaviruses have been approved for commercial production in China (Table 5.2). Since 1995, the vaccines have been successfully used in highly endemic regions of the country, and in 2007, a national Expanded Program on Immunization was initiated. The massive vaccination was found to be safe and effective (Li 2010). Currently, approximately 2 million doses of inactivated rodent brain- and cell culture-derived HFRS vaccines are given annually in China (Zhang et al. 2010).

Table 5.2 Immunogenicity of Vero cell-derived blended PUUV-DOBV vaccine

	Antibody titers							
	PUUV				DOB/KURV			
	ELISA		PRNT		ELISA		PRNT	
Vaccine dilution	+/*n*	Average titer	+/*n*	Average titer	+/*n*	Average titer	+/*n*	Average titer
n/d	8/8	2,389	8/8	136	8/8	1,792	8/8	120
1/2	7/7	1,152	7/7	54.8	7/7	1,060	7/7	73.1
1/4	8/8	896	8/8	26.3	8/8	416	8/8	36
1/8	8/8	352	7/8	18.75	8/8	192	7/8	50.3
1/16	6/8	170	4/8	10	5/8	90	4/8	40
1/32	4/8	96	2/8	8	4/8	36	2/8	16
1/64	1/8	64	1/8	8	1/8	16	1/8	16
1/128	1/8	16	0/8	<8	0/8	<16	0/8	<16
1/256	0/8	<16	0/8	<8	0/8	<16	0/8	<16

The HTNV experimental vaccine was also developed in Vero-E6 cells grown on microcarriers in suspension (Choi et al. 2003). In immunized mice the Vero-E6-derived HTNV vaccine induced more than five times higher levels of PRNT antibodies than the Hantavax™ vaccine. Two immunizations with 5 μg of cell culture-based vaccine induced strong PRNT antibody production, whereas no neutralizing antibody was induced after immunization with the same amount of Hantavax™ vaccine. Mice immunized with higher doses of Hantavax™, 10 or 20 μg, induced a similar level of neutralizing antibody but showed different protection efficacy suggesting possible involvement of cell-mediated immunity.

To date, there are no HFRS vaccines approved for use in European countries. Animal studies suggest that vaccines derived from HTNV or SEOV would not protect against PUUV infection (Schmaljohn 2012; Chu et al. 1995). There have been no efforts to develop HFRS vaccine based on PUUV, in part because PUUV is difficult to produce at high titers in cell cultures. Meanwhile, rodent brain-derived vaccines are not acceptable due to EU regulation requirements.

As mentioned above, circulation of both hantaviruses, DOB/BELV and PUUV, in the same endemic areas, e.g., in European part of Russia, indicates that effective hantavirus vaccine to control HFRS in Europe has to include antigens of both viruses (Schmaljohn 2009; Tkachenko et al. 2013).

During the last decade attempts were made to develop an inactivated Vero cell-derived blended bivalent PUUV-DOBV vaccine (Tkachenko et al. 2009, 2010). The PUUV-like vaccine strain "DTK/Ufa-97" was isolated in Vero-E6 cells from an HFRS patient during an HFRS outbreak in Bashkiria region of Russia in 1997 (Dzagurova et al. 2008b) and was adapted to grow at high titers in Vero cells with serum-free medium (SFM).

The DTK/Ufa-97 strain occupies the Bashkiria-Saratov lineage of PUUV. The amino acid sequences of the S, M, and L RNA segments of DTK/Ufa-97 were 99.2–100 %, 99.3–99.8 %, and 99.8 % identical to those of the Bashkirian PUUV strain and 96.9 %, 92.6 %, and 97.4 % identical, respectively, to those of the Sotkamo strain. The DTK/Ufa-97 and other PUUV strains exhibited similar binding patterns to a PUUV panel of monoclonal antibodies. In addition, antisera against three different PUUV strains neutralized both homologous and heterologous PUUV isolates. These results suggested that DTK/Ufa-97 strain is antigenically similar to distant PUUV strains but different from other hantaviruses (Abu Daude et al. 2008).

The envelope glycoproteins of hantaviruses play a major role in the induction of neutralizing antibodies and protective immunity (Lundkvist and Niklasson 1992). The cross neutralization test confirmed that neutralizing antibodies to DTK/Ufa-97 also neutralized other PUUV strains at almost the same neutralizing titers, and antibodies to the other PUUV strains neutralized DTK/Ufa-97 as well. These findings justify development of DTK/Ufa-97-based vaccine as PUUV vaccine to control HFRS in European countries (Abu Daude et al. 2008).

The second vaccine strain, "TEA/Lipetzk-06," belongs to DOB/KURV hantavirus lineage. The TEA/Lipetzk-06 was isolated from an HFRS patient during an HFRS outbreak in the Lipetsk region of Russia in 2006, was also adapted to

replicate at high titers in Vero cells in SFM (Tkachenko et al. 2009), and was analyzed to confirm their genetic and antigenic features (Klempa et al. 2008; Dzagurova et al. 2009). Both virus strains, DTK/Ufa-97 and TEA/Lipetzk-06, were used to produce Master Virus Seeds (MVS) in Vero.

Two variants of ELISA were developed to detect antigens of DTK/Ufa-97 and TEA/Lipetzk-06 hantaviruses (Dzagurova et al. 2013). First, "Hanta-PUUV" variant was designed using monoclonal antibodies to PUUV envelope glycoprotein for detecting only PUUV antigen. The second, "Hanta-N" ELISA, was designed using monoclonal antibodies to DOBV and PUUV nucleocapsid proteins for detecting PUUV, DOB/BELV, HTNV, and SEOV. Both "Hanta-PUU" and "Hanta-N" ELISA-based assays detected specific hantavirus antigens in the blended PUUV-DOBV vaccine.

Vaccine product development included the following steps: (1) infection of Vero cells with Working Virus Seeds (WVS) of DTK/Ufa-97 and TEA/Lipetzk-06 viruses; (2) harvesting culture medium from DTK/Ufa-97-infected cultures and TEA/Lipetzk-06-infected cultures; (3) low-speed centrifugation to remove cell debris; (4) concentration by tangential flow filtration; (5) purification using Sepharose 6FF chromatography; (6) inactivation with 0.04 % formalin; (7) blending; (8) mix with adjuvant, alum hydroxide; and (9) quality control tests. Experimental blended bivalent PUUV-DOBV vaccine named "Combi-HFRS-Vac" (Tkachenko et al. 2011, 2012) was successfully tested in preclinical studies. As seen in Table 5.2, immunization of BALB/c mice with Combi-HFRS-Vac induced antibody responses against PUUV and DOBV, and these antibodies were detected by IFA, ELISA, and PRNT assays.

5.4 Recombinant Hantavirus Vaccines

Several expression systems were used to express hantavirus nucleocapsid (N), and glycoproteins (G1 and G2) and to immunize experimental animals to test immunogenicity and protective efficacy in challenge experiments. Recombinant hantavirus proteins were successfully expressed in *Escherichia coli,* yeast, transgenic plants in baculovirus system, and in viral vectors including vaccinia virus and vesicular stomatitis virus (Dargeviciute et al. 2002; Lee et al. 2006; Geldmacher et al. 2004; Khattak et al. 2002; Lundkvist et al. 1993, 1996; Schmaljohn et al. 1990; Yoshimatsu et al. 1993; Maes et al. 2006, 2008; Lundkvist and Niklasson 1992; Krüger et al. 2011; de Carvalho et al. 2002). Recombinant PUUV NP expressed in yeast induced protective immunity in experimentally immunized bank voles (Dargeviciute et al. 2002). The DOBV NP expressed in the same system induced high antibody titers after immunization of BALB/c and C57BL/6 mice (Geldmacher et al. 2004). PUUV NP was successfully expressed in transgenic tobacco and potato plants but failed to induce an antibody response in mice when administered as an oral vaccine (Kehm et al. 2001; Khattak et al. 2004).

The N protein and G1 and G2 glycoproteins were shown to induce protective immune responses in experimental rodents. Whereas this effect is explained by induction of neutralizing antibodies by the glycoproteins, the protective immune response against N, which is an internal viral protein, can be best explained by triggering cellular immunity (Krüger et al. 2011). Recombinant N protein of DOBV was tested in combination with various adjuvants for immunogenicity and protective efficacy in C57/BL6 mice. This study identified Freund's adjuvant as the additive of choice because mice that were vaccinated with this adjuvant in combination with the DOBV N showed a protection rate from challenge of 75 %, whereas the usage of other adjuvants such as Alum, which induces strong Th2-type immune responses, did not result in protective immunity (Klingstrom et al. 2004). Since the N protein is more conserved among different hantaviruses, an advantage of N protein use seems to be the induction of broader cross-reactive immunity against various hantavirus species.

Virus-like particles (VLPs) are highly structured, repetitive protein complexes that have many desirable properties as immunogens. Certain viral antigens, such as the HBV core antigen, will spontaneously form such complexes, and to varying extents, small regions of foreign proteins can be incorporated into the HBV core protein and serve as an antigen. Immunogenic epitopes of PUUV, DOBV, and HTNV NP incorporated into chimeric hepatitis B virus core particles elicited high antibody titers and protective immunity in bank voles (Ulrich et al. 1999; Geldmacher et al. 2004). These responses were strong indicating that the HBV-based VLP particles can be a promising platform for the development of hantavirus vaccines (Hjelle 2002).

In a hamster model, recombinant adenovirus expressing ANDV N and G1 or G2 protein protected vaccinated animals against homologous lethal challenge (Safronetz et al. 2009). Induction of neutralizing antibodies and protection against SEOV challenge were also observed after immunization with replication-competent recombinant canine adenovirus expressing SEOV G1 or G2 (Yuan et al. 2009, 2010).

HTNV NP, G1, and G2 expressed in baculovirus and vaccinia virus vectors were shown to induce protection after a HTNV challenge in hamster and mouse models (Yoshimatsu et al. 1993; Chu et al. 1995; Schmaljohn et al. 1990). HTNV vaccinia-vectored vaccines were shown to be efficacious and to confer cross-protection against SEOV (Chu et al. 1995; Schmaljohn et al. 1990). The vaccine was tested in a phase II, double-blinded, placebo-controlled clinical trial among 142 volunteers. Neutralizing antibodies to HTNV were detected only in 72 % of the vaccinated individuals (McClain et al. 2000). Due to limited seroconversion and the potential side effects of live vaccinia virus, the trial was terminated (Schmaljohn 2009; Hammerbeck et al. 2009).

The role of anti-N and anti-glycoprotein (G1, G2) hantavirus immunity in the protection of experimental animals was studied using DNA immunization and alphavirus replicon system. While immunogenicity of DNA vaccines varied in different animal models, these studies confirmed previous observations and showed that immune responses against G1 and G2 glycoproteins were associated with

stronger protection (Hammerbeck et al. 2009). DNA vaccines expressing glycoproteins of HTNV and PUUV were tested in 28 volunteers using DNA-loaded particles and epidermal delivery device (Schmaljohn 2009; Boudreau et al. 2010). The data showed significant levels of neutralizing antibodies against both hantaviruses.

The aerosolized DNA vaccine (PEI + rDNA) containing complexes of polyethyleneimine with recombinant DNA expressing the PUUV G1 gene under CMV promoter was prepared and used to immunize BALB/c mice in aerosolized chamber with ultrasonic generator (Filatov et al. 2007). Immunization with aerosolized vaccine was accompanied with intraperitoneal injection of adjuvant (proteoglycan of natural origin). Immunization protocol included two prime immunizations and one boost. The aerosolized DNA vaccine (PEI + DNA) induced in mice PUUV-specific IgM, IgG, and IgA antibodies assayed in ELISA.

Experimental DNA vaccines expressing the envelope glycoprotein genes of HTNV or PUUV viruses were evaluated in phase 1 study in three vaccination groups, nine volunteers/group (Boudreau et al. 2012). The volunteers were vaccinated by particle-mediated epidermal delivery (PMED) three times at 4-week intervals with the HTNV DNA vaccine, the PUUV DNA vaccine, or both vaccines (from HTNV, strain 76-118, or PUUV, strain P360). At each dosing, the volunteers received 8 μg DNA/4 mg gold. There were no vaccine-related serious adverse events. Nonspecific events were fatigue, headache, malaise, myalgia, and lymphadenopathy. Blood samples were collected on days 0, 28, 56, 84, 140, and 180 and assayed for the presence of neutralizing antibodies. In the single-vaccine groups, neutralizing antibodies to HTNV or PUUV were detected in 30 % or 44 % of individuals, respectively. In the combined-vaccine group, only 56 % of the volunteers developed neutralizing antibodies to one or both viruses (Boudreau et al. 2012).

Brocato et al. reported the synthesis of a codon-optimized, full-length M-segment open reading frame and its cloning into a DNA vaccine vector to produce the plasmid pWRG/PUU-M(s2). pWRG/PUU-M(s2) delivered by gene gun produced high-titer neutralizing antibodies in hamsters and nonhuman primates. Vaccination with pWRG/PUU-M(s2) protected hamsters against challenge with PUUV but not against infection with related HFRS-associated hantaviruses, DOBV and HTNV. Unexpectedly, the DNA vaccine protected hamsters against fatal disease caused by Andes virus (ANDV). This cross-protection was not associated with induction of ANDV cross-neutralizing antibodies. This was the first evidence of efficacy of an experimental DNA vaccine against HFRS in a hamster lethal disease model (Brocato et al. 2012).

A multi-epitope chimeric DNA vaccine against of SEOV, HTNV, and PUUV was constructed by Zhao et al. (2012). This vaccine elicited strong humoral and cellular immune responses against all targets providing feasibility for multi-epitope vaccination approach. In spite of these encouraging results, low immunogenicity in humans remains the major obstacle for development of DNA vaccines against hantavirus infections (see also Chapter 6).

5.5 Conclusion

The current status and future of hantavirus vaccines varies among different countries depending on endemic areas. The population of Eurasian countries and, first of all, the population with the high morbidity rate (China, Russia, North and South Korea, Finland, Sweden, Germany) are potentially the target for vaccination against HFRS. In the Americas, the HPS-caused morbidity rate is significantly lower and vaccination against HPS is not so obvious. Nevertheless, possible target groups can include rural residents of relatively small endemic areas, such as Western New Mexico, California's Sierra Nevada Mountains, and rural regions of the Pacific Northwest. Target groups will be persons with active outdoor occupations, for example, field biologists, forestry workers, and farmers. The at-risk populations will also include members of American Indian tribes as far east as Oklahoma and residents of the Rockies, Sierras, and Cascade mountains and in the surrounding foothills. Outside of the United States, there are many areas where the demand for vaccines is even stronger. These areas include Southern Chile from Santiago to Puerto Natales with population around 4–5 million and endemic areas of Argentina, Southern Brazil, Paraguay (Gran Chaco), and Bolivia with additional million of populations at risk. These regions are likely to remain hotspots for vaccine demand for the foreseeable future.

Based on the size of predicted population at risk, the potential market for hantavirus vaccines is likely to be in the tens of millions of doses in the western hemisphere and probably exceeds 100 million doses in Eurasia (Hjelle 2002). It seems that due to relatively high cost-benefit ratio, an HPS vaccine will be not recommended for routine use. However, in HPS endemic areas where increased contact with rodents is expected, vaccination would be advisable (Schmaljohn 2012). It is quite obvious that a commercially feasible HPS vaccine would be one that could protect against both clinical forms of hantavirus infections, HFRS and HPS. The ideal hantavirus vaccine should confer long-term protection against all epidemiologically significant hantaviruses circulated in the endemic region with no more than two or three timely close applications. The side-effect profile should be acceptable, and it would be beneficial to offer the vaccine simultaneously with vaccines against other agents that produce related symptoms, such as influenza or pneumococcus (Hjelle 2002).

The hantaviruses, causative agents of HFRS and HPS, require at least a biosafety level 3 containment to handle these viruses due to the hazardous nature of the infection and a possible aerosol transmission. The high level of containment is an additional challenge for hantavirus vaccine development. Recombinant DNA vaccine technologies provide a good opportunity to overcome this roadblock. During the last decades, several research groups published promising results in preclinical studies in small animal models using different approaches based on DNA recombinant technologies. Nevertheless, much more should be done to see feasible recombinant hantavirus vaccines for human use.

Currently, only inactivated culture cell-derived hantavirus vaccines are available for human use to control hantavirus infections in endemic areas. A few million doses of these vaccines were distributed (mostly in China and North and South Korea) without serious adverse events suggesting that these vaccines are well tolerated. However, new generation of hantavirus vaccines for HFRS and/or HPS with long-lasting humoral immune responses and increased cross-protective efficacy is needed to effectively control hantavirus infections.

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