CHAPTER 32

Bunyaviridae: The Bunyaviruses

CHARLES H. CALISHER and ROBERT E. SHOPE

Diseases: Hemorrhagic fever with renal syndrome, Rift Valley fever, Crimean-Congo hemorrhagic fever, Nairobi sheep disease, Oropouche, LaCrosse encephalitis, Akabane, sandfly fever, and undifferentiated fevers.

Etiologic Agents: Hantaan, Rift Valley fever, Crimean-Congo hemorrhagic fever, Nairobi sheep disease, Oropouche, LaCrosse, Akabane, sandfly Naples, sandfly Sicilian, and other viruses (virus distribution related to distribution of arthropod vector and mammalian reservoirs.

Source: Infected mosquitoes, ticks, or mammals, depending on virus.

Clinical Manifestations: Undifferentiated fevers, fever and rash, hemorrhagic fever, hemorrhagic fever with renal syndrome, encephalitis, optic retinitis, teratogenesis.

Pathology: Dependent on etiologic agent, but rather typical of specific infection (i.e., encephalitis, fever, rash, etc.).

Laboratory Diagnosis: Virus isolation and serologic tests for antibody (IgM antibody capture ELISA, neutralization).

Epidemiology: Diseases occur when humans or animals are in contact with infected vertebrates or when fed on by infected arthropods in specific geographic areas.

Treatment: Symptomatic.

Prevention and Control: Prevention of contact (removal from area, insecticides, screening, rodent-proofing) between susceptible human or animal and source of virus (infected arthropod or vertebrate), vaccination.

Introduction

During studies of yellow fever in Uganda in 1943, Smithburn et al., (1946) isolated Bunyamwera virus from pooled *Aedes* sp. mosquitoes. Casals and Whitman (1960) subsequently demonstrated that this virus is related antigenically to Wyeomyia (Colombia), Cache Valley (Utah), and Kairi (Trinidad) viruses. Relationships among these viruses were based largely on studies of their complement-fixing (CF) antigens. Further studies of these as well as Germiston (South Africa), Batai (Malaysia), Guaroa (Colombia), and other related viruses from Brazil showed that although closely related by CF, these viruses are distinguishable by neutralization (N) tests. As additional isolations of arboviruses were made, workers at The Rockefeller Foundation detected relationships between and among many of the viruses. For each two or more viruses shown to be antigenically related (usually by hemagglutination-inhibition [HI] or CF tests), a virus serogroup was established. Within about a 10-year period, not only had the Bunyamwera serogroup been recognized, but the groups C, Guama, California, Capim, Anopheles A, Simbu, Bwamba, Patois, Koongol, Tete, and other serogroups also were distinguished (Casals and Whitman, 1961; Whitman and Casals, 1961; Whitman and Shope, 1962; Theiler and Downs, 1973).

As the number of serogroups increased, a need to provide some order became apparent and definitions were proposed for classification terms. We use these terms as defined herein. A *serogroup* is two or more viruses, distinct from each other by quantitative serologic criteria (fourfold or greater differences between homologous and heterologous titers of both sera) in one or more tests, but related to another

E. H. Lennette et al. *Laboratory Diagnosis of Infectious Diseases Principles and Practice*

virus of the serogroup by some serologic method. Arboviruses that are very closely related, but distinct from each other, constitute an antigenic *complex*. Individual agents, antigenically related but easily separable (fourfold or greater differences between homologous and heterologous titers of both sera) by the N test, are considered *viruses* or *types*. *Subtypes* are virus isolates separable from the type virus by at least a fourfold difference between the homologous and heterologous titers of one, but not both, of the two sera tested. *Varieties* are those isolates differentiable only by the application of special tests or reagents (kinetic HI, monoclonal antibody assays, and the like). In present practice, the first discovered virus of a newly recognized serogroup lends its name to the antigenic cluster.

Through the efforts of Casals and others of The Rockefeller Foundation, low-level, often one-way relationships between individual viruses in two or more serogroups were demonstrated. For example, as determined by CF tests, Guaroa virus is a member of the Bunyamwera serogroup. However, it is related to certain members of the California serogroup by both HI and N tests. Because of the intergroup cross-reactivity among members of Group C, Guama, Capim, California, and Bunyamwera serogroups, Casals suggested the establishment of what he called the Bunyamwera supergroup (Casals, 1963). Subsequent electron microscopic studies supported and extended Casals' concept, in that the viruses of the supergroup could not be distinguished by size, morphology, or morphogenesis in infected cells (Murphy et al., 1973). Other viruses, belonging to other serogroups, such as the phlebotomus fever and Uukuniemi serogroup viruses, morphologically resembled the supergroup members, but antigen sharing could not be demonstrated among members of these serogroups.

The term "arbovirus" denotes a virus maintained in nature by a biologic (propagative) transmission cycle between susceptible vertebrate hosts and hematophagous arthropods. The International Catalogue of Arboviruses lists more than 500 viruses, most with antigenic relationships to others, but some that are antigenically, taxonomically, and/or ecologically distinct (Karabatsos, 1985). Many of the viruses registered in this catalogue do not meet the definition of an arbovirus, some because information is lacking regarding transmission by arthropods and other catalogued viruses because they certainly are not arthropod-borne.

The term "arbovirus" is used here to denote an ecologic description, but in keeping with newer classification, these viruses will be referred to by universal taxonomic descriptions. Differences in antigenic, morphologic, biochemical, and genetic characteristics are used to separate the arboviruses into families, genera, serogroups, complexes, viruses, subtypes, and varieties in an increasing order of relatedness. Most recently, molecular analyses have substantiated the previous antigenic classification schemes, and a clear view of the taxonomy of the arboviruses has emerged.

The Viruses

Members of the family *Bunyaviridae* have certain characteristics in common: single-stranded RNA, three RNA segments whose ends are hydrogen-bonded so that the molecules are circular; and spherical or oval enveloped 90 to 100-nm diameter virions. All members of the family so far tested are acid (pH 3), lipid solvents (ether, chloroform), and detergent (sodium deoxycholate) labile; heat (56° for 15 to 30 min) labile; and formalin, 70% ethanol, 5% iodine, and ultraviolet (UV) irradiation labile (Karabatsos, 1985). Molecular weight of viruses in the family is 300 to 400 $\times$ 10^6. Sedimentation coefficients of representative members are between 400 and 500 S and bouyant densities are about 1.18 to 1.20 g/ml (Obijeski and Murphy, 1977). Uukuniemi virus has been determined to contain about 2% RNA, 58% protein, 33% lipid, and 7% carbohydrate (Obijeski and Murphy, 1977), but the composition of most other members of the family is not known.

The virions consist of a unit membrane envelope with what have been termed "fuzzy" (bunyaviruses) or "ordered" (uukuviruses) projections surrounding a rather unstructured interior from which a helical, 2.5-nm wide nucleocapsid can be extracted (von Bonsdorff et al., 1969; Murphy et al., 1973). Virus particles contain a transcriptase enzyme. Constituent synthesis takes place in the cytoplasm, and morphogenesis occurs without prior core formation, by budding directly into the Golgi complex and vesicles of infected cells (Murphy et al., 1973). No enzymatic function has been associated with the envelope glycoproteins of members of the family. No evidence has been obtained for reassortment of genes between members of the different genera within the family. However, reassortment has been detected between viruses belonging to the same genus and closely related antigenically, that is restricted to RNA segment reassortment between closely related viruses. Naturally occurring reassortants have been obtained from genotype analyses of field isolates (Klimas et al., 1981; Ushijima et al., 1981).

At least four genera of viruses have been distinguished within the family Bunyaviridae: *Bunyavirus, Phlebovirus, Nairovirus,* and *Uukuvirus* (Bishop et al., 1980). Recently, a fifth genus, *Hantavirus,* has been proposed for this family of biologically diverse viruses (Schmaljohn and Dalrymple, 1983). The

prominent role of The Rockefeller Foundation workers and the remarkably perceptive predictions of Casals should not be understated. All subsequent genetic studies have borne out Casals' hypothesis that viruses shown to be interrelated by studies of their antigens are related genetically. Thus, the *Bunyavirus* genus is composed of members of the former Bunyamwera supergroup as well as more recently isolated viruses shown to be antigenically related to one or more viruses within the supergroup (Table 1); the *Phlebovirus* genus includes all members of the Phlebotomus fever serogroup (Table 2); the *Nairovirus* genus is composed of viruses belonging to at least six serogroups (Table 3); the *Uukuvirus*

TABLE 1. Viruses of the family *Bunyaviridae*, genus *Bunyavirus*

Complex	*Virus (subtype) ⟨variety⟩*
	(Bunyamwera Serogroup)
Bunyamwera	Bunyamwera, Batai, Calovo, Birao, Bozo, Cache Valley (Cache Valley) ⟨Cache Valley⟩ ⟨Tlacotalpan⟩ (Maguari) ⟨Maguari⟩ ⟨CbaAr426⟩ ⟨AG83-1746⟩ (Playas) (Xingu), Germiston, Ilesha, Lokern, Mboke, Ngari, Northway, Santa Rosa, Shokwe, Tensaw
Kairi	Kairi
Main Drain	Main Drain
Wyeomyia	Wyeomyia, Anhembi (Anhembi) (Iaco) (Macaua) (Sororoca) (Taiassui) (BeAr328208)
	(Anopheles A Serogroup)
Anopheles A	Anopheles A, Las Maloyas, Lukuni, Trombetas, CoAr3624, ColAn57389
Tacaiuma	Tacaiuma (Tacaiuma) (SPAr2317) ⟨SPAr2317⟩ ⟨Virgin River⟩ (H-32580), CoAr1071 (CoAr1071) ⟨CoAr1071⟩ ⟨CoAr3627⟩
	(Anopheles B Serogroup)
Anopheles B	Anopheles B, Boraceia
	(Bwamba Serogroup)
Bwamba	Bwamba (Bwamba) (Pongola)
	(Group C Serogroup)
Caraparu	Caraparu (Caraparu) ⟨2⟩ (Ossa), Apeu, Bruconha, Vinces
Madrid	Madrid
Marituba	Marituba (Marituba) (Murutucu) (Restan), Nepuyo (Nepuyo) ⟨Nepuyo⟩ ⟨63U11⟩ (Gumbo Limbo)
Oriboca	Oriboca (Oriboca) (Itaqui)
	(California Serogroup)
California enc.	California enc. (California enc.) (Inkoo) (LaCrosse) ⟨snowshoe hare⟩ (San Angelo) (Tahyna) ⟨Tahyna⟩ ⟨Lumbo⟩
Melao	Melao (Melao) ⟨Melao⟩ ⟨AG83-497⟩ (Jamestown Canyon) ⟨Jamestown Canyon⟩ ⟨Jerry Slough⟩ ⟨South River⟩ (Keystone) (Serra do Navio)
trivitattus	trivitattus
Guaroa	Guaroa
	(Capim Serogroup)
Capim	Capim
Guajara	Guajara (Guajara) ⟨Guajara⟩ ⟨GU71U350⟩
BushBush	BushBush (BushBush) (Benfica) (GU71U344), Juan Diaz
Acara	Acara, Moriche
Benevides	Benevides
	(Gamboa Serogroup)
Gamboa	Gamboa, Pueblo Viejo (75V-2621)
Alajuela	Alajuela, San Juan (San Juan) (78V-2441) (75V-2374)
	(Guama Serogroup)
Guama	Guama, Ananindeua, Mahogany Hammock, Moju
Bertioga	Bertioga, Cananeia, Guaratuba, Itimirim, Mirim
Bimiti	Bimiti
Catu	Catu
Timboteua	Timboteua
	(Koongol Serogroup)
Koongol	Koongol, Wongal
	(Minatitlan Serogroup)
Minatitlan	Minatitlan, Palestina

TABLE 1. *Continued*

Complex	*Virus (subtype) ⟨variety⟩*
	(Olifantsvlei Serogroup)
Olifantsvlei	Olifantsvlei (Olifantsvlei) ⟨Olifantsvlei⟩ ⟨Bobia⟩
Botambi	Botambi
	(Patois Serogroup)
Patois	Patois, Babahoyo, Shark River, Abras
Zegla	Zegla, Pahayokee
	(Simbu Serogroup)
Simbu	Simbu
Akabane	Akabane, Yaba-7
Manzanilla	Manzanilla (Manzanilla) (Ingwavuma) (Inini) (Mermet), Buttonwillow, Nola, Oropouche (Oropouche) (Facey's Paddock) (Utinga) (Utive), Sabo (Sabo) (Tinaroo)
Sathuperi	Sathuperi (Sathuperi) (Douglas)
Shamonda	Shamonda, Sango (Sango) (Peaton)
Shuni	Shuni, Aino (Aino) ⟨Aino⟩ ⟨Kaikalur⟩
Thimiri	Thimiri
	(Tete Serogroup)
Tete	Tete (Tete) (Bahig) (Matruh) (Tsuruse), Batama
	(Turlock Serogroup)
Turlock	Turlock, Umbre, Lednice
M'Poko	M'Poko (M'Poko) (Yaba-1)
	(No Serogroup Assigned)
	Kaeng Khoi

TABLE 2. Viruses of the family *Bunyaviridae*, genus *Phlebovirus*

Complex	*Virus (subtype)*
	(Phlebotomus Fever Serogroup)
Sandfly fever Naples	Sandfly fever Naples, Karimabad, Tehran, Toscana
Bujaru	Bujaru, Munguba
Rift Valley fever	Rift Valley fever (Rift Valley fever) (Belterra), Icoaraci
Candiru	Candiru, Alenquer, Itaituba (Itaituba) (Oriximina), Nique, Turuna
Punta Toro	Punta Toro (Punta Toro) (Buenaventura)
Frijoles	Frijoles, Joa
Chilibre	Chilibre, Cacao
Salehabad	Salehabad, Arbia
(no complex assigned)	Sandfly fever Sicilian, Aguacate, Anhanga, Arumowot, Caimito, Chagres, Corfou, Gabek Forest, Gordil, Itaporanga, Pacui, Rio Grande, Saint-Floris, Urucuri

TABLE 3. Viruses of the family *Bunyaviridae*, genus *Nairovirus*

Complex	*Virus (subtype)*
	(Crimean-Congo Hemorrhagic Fever Serogroup)
Crimean-Congo hemorrhagic fever	Crimean-Congo hemorrhagic fever, Hazara, Khasan
	(Dera Ghazi Khan Serogroup)
Dera Ghazi Khan	Dera Ghazi Khan, Abu Hammad, Abu Mina, Kao Shuan, Pathum Thani, Pretoria
	(Hughes Serogroup)
Hughes	Hughes, Farallon, Fraser Point, Punta Salinas, Raza, Sapphire II, Soldado, Zirqa
	(Nairobi Sheep Disease Serogroup)
Nairobi sheep disease	Nairobi sheep disease, Dugbe
	(Qalyub Serogroup)
Qalyub	Qalyub, Bandia, Omo
	(Sakhalin Serogroup)
Sakhalin	Sakhalin (Tillamook), Kachemak Bay, Clo Mor, Avalon, Taggert

TABLE 4. Viruses of the family *Bunyaviridae*, genus *Uukuvirus*

Complex	*Virus (subtype)*
	(Uukuniemi Serogroup)
Uukuniemi	Uukuniemi (Uukuniemi) (Oceanside), Grand Arbaud, Manawa, Murre, Ponteves, Precarious Point, Zaliv Terpeniya, EgAn-1825-61, Fin V-707, UK FT/254

TABLE 5. Viruses of the family *Bunyaviridae*, genus *Hantavirus*

Complex	*Virus (subtype) ⟨variety⟩*
	(Hantaan Serogroup)
Hantaan	Hantaan, Seoul (Seoul) (Tchoupitoulas) (Girard Point) (Sapporo Rat), Prospect Hill
Puumala	Puumala

genus includes all members of the Uukuniemi serogroup (Table 4), and the *Hantavirus* genus includes all members of the Hantaan serogroup (Table 5). Thirty-five other viruses possess morphologic, morphogenetic, or other properties in common with viruses of the family *Bunyaviridae*. However, these 35 have not been characterized sufficiently to warrant placement in one of the recognized genera; they are listed in Table 6.

Among the nearly 300 viruses that have been assigned to the family *Bunyaviridae* are those causing Rift Valley fever (Daubney et al., 1931), phlebotomus fever (Taussig, 1905), Crimean-Congo hemorrhagic fever (Hoogstraal, 1979), hemorrhagic fever with renal syndrome (Gajdusek, 1953), and Nairobi sheep disease (Montgomery, 1917). Other members of the family have been associated primarily with encephalitis (*Bunyavirus*, California serogroup) or with febrile diseases (*Bunyavirus*, serogroups C, Guama, Bunyamwera, Bwamba, Tataguine, Simbu). Akabane virus is teratogenic in sheep and cattle, causing arthrogryposis and anencephaly (Kurogi et al., 1976). Of course, most of the viruses in the family have not been associated with any illness of humans or livestock. Those that do cause disease, specifically Rift Valley fever, the phlebotomus fevers,

TABLE 6. Viruses of the family *Bunyaviridae*, genus unassigned

Complex	*Virus (subtype)*
	(Bakau Serogroup)
Bakau	Bakau, Ketapang
	(Bhanja Serogroup)
Bhanja	Bhanja, Kismayo
	(Kaisodi Serogroup)
Kaisodi	Kaisodi, Silverwater, Lanjan
	(Mapputta Serogroup)
Mapputta	Mapputta, Maprik (Maprik) (GanGan), Trubanaman
	(Matariya Serogroup)
Matariya	Matariya, Burg el Arab, Garba
	(Nyando Serogroup)
Nyando	Nyando, Eretmapodites-147
	(Resistencia Serogroup)
Resistencia	Resistencia, Barranqueras, Antequera
	(Upolu Serogroup)
Upolu	Upolu, Aransas Bay
	(No Serogroup Assigned)
	Bangui, Belmont, Bobaya, Caddo Canyons, Enseada, Kowanyama, Lone Star, Pacora, Razdan, Sunday Canyon, Tamdy, Tataguine, Wanowrie, Witwatersrand

Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndrome and similar diseases caused by related viruses, arthrogryposis, and Nairobi sheep disease, are pathogens of great epidemiologic and economic significance.

A case in point is Rift Valley fever (genus *Phlebovirus*). Until 1977 this disease was limited geographically to sub-Saharan Africa where it circulated in an enzootic-epizootic cycle. Before 1977 only four human fatalities had been ascribed to this disease, although human infections were observed during some epizootics, and the disease was described as a self-limited dengue-like illness when it occurred in laboratory workers, field investigators, or animal handlers. In 1977 an explosive epidemic of the disease was demonstrated in the Nile Valley and the Nile Delta areas of Egypt, involving more than 200,000 humans, with 600 deaths (Meegan, 1979). Widespread and severe morbidity and excessive mortality attributed to Rift Valley fever virus was seen in a variety of domestic animals and livestock (Ali and Kamel, 1978).

Another example is a tick-borne hemorrhagic fever, which has been recognized in Central Asia since the 12th Century and later in the Balkans (Gajdusek, 1953). This disease was seen in the Crimea shortly after World War II when farmers and soldiers clearing and cultivating land in this war-devastated region became sick. The disease was called Crimean hemorrhagic fever, and more than 200 cases were documented in that outbreak. It is known to occur from southern Africa, throughout sub-Saharan Africa (Congo hemorrhagic fever), eastern Europe, the Middle East, and Asia. Its geographic distribution parallels roughly the distribution of the principal arthropod vector of Crimean-Congo hemorrhagic fever virus (genus *Nairovirus*), ticks of the genus *Hyalomma*. Although the total number of recognized cases is not large and the widespread geographic distribution of cases is continuous but focal, the significance of this disease in regard to agricultural workers, nosocomial infections, and military personnel makes it noteworthy.

Hemorrhagic fever with renal syndrome (HFRS), caused by members of the Hantaan serogroup (genus *Hantavirus*), actually is a complex of diseases. Occurring in Europe and Asia, it is known by the names nephropathia epidemica, epidemic hemorrhagic fever, Korean hemorrhagic fever, or simply "mild" or "severe" HFRS. The disease was known in ancient China and thousands of cases occurred in military personnel in Korea during the early 1950s. However, it is only recently that the etiologic agents have been isolated and specific diagnoses could be made. The essentially worldwide distribution of the hantaviruses is now causing a flurry of scientific activity.

The classification of diseases caused by viruses belonging to the family *Bunyaviridae* roughly parallels, with a few exceptions, the classification of the viruses causing these diseases (Table 7). Bunya-

TABLE 7. Some clinical syndromes and their associated viruses in the family *Bunyaviridae*

Undifferentiated fever (human)	
Africa	Germiston, Shuni, Nairobi sheep disease, Bhanja, Dugbe, Kasokero, sandfly fevers
Europe	Bhanja, sandfly fevers
Asia	Shuni, Nairobi Sheep disease, Bhanja
Americas	Wyeomyia, Guaroa, Apeu, Caraparu, Itaqui, Madrid, Marituba, Murutucu, Oriboca, Ossa, Guama, Catu, Oropouche
Fever and rash (human)	
Africa	Bunyamwera, Ilesha, Bwamba, Tataguine, Bangui
Hemorrhagic fever (human)	
Africa	Rift Valley fever (also causes hepatitis and abortion in sheep and cattle), Crimean-Congo hemorrhagic fever
Europe	Crimean-Congo hemorrhagic fever
Asia	Crimean-Congo hemorrhagic fever
Hemorrhagic fever with renal syndrome (human)	
Europe	Puumala
Asia	Hantaan, Seoul
Encephalitis (human)	
Africa	Rift Valley fever
North America	LaCrosse, California encephalitis, snowshoe hare, Jamestown Canyon
Optic retinitis (human)	
Africa	Rift Valley fever
Teratogenesis (sheep and cattle)	
Australia, Asia, Israel	Akabane

viruses generally cause either encephalitis with fevers or fevers with rash in people; phleboviruses cause fevers, hemorrhagic fevers, or encephalitis; nairoviruses cause a variety of clinical illnesses ranging from undifferentiated fevers to hemorrhagic fevers; hantaviruses cause mild to severe hemorrhagic fever with renal involvement; and uukuviruses are not known to cause human illness.

Family *Bunyaviridae*, Genus *Bunyavirus*

Members of the genus *Bunyavirus* (Table 1) possess negative-sense RNA replication strategy. Total molecular weight of the RNA segments is 4.78 to 5.9 $\times$ 10^6. The 3′ terminal sequence is UCAUCACAUG. Two of the four structural proteins are glycosylated (G1 and G2) with molecular weights of 108 to 120 and 29 to 41 $\times$ 10^3, respectively. Another is a nucleocapsid protein (N), molecular weight 19 to 25 $\times$ 10^3. A minor large protein (L) with a molecular weight of 145 to 200 $\times$ 10^3 also has been recognized. Within this genus, 16 serogroups, containing more than 150 viruses, are known. The bunyaviruses are found worldwide; are transmitted by mosquitoes and culicoids; have as their principal vertebrate hosts rodents and other small mammals, primates, birds, or ungulates; and usually exist in silent sylvatic transmission cycles. At least 30 of these viruses or their subtypes and varieties have been reported to cause disease in humans or animals of veterinary importance and three (Oropouche, LaCrosse, and Bwamba viruses) have caused epidemics in humans.

Bunyamwera serogroup viruses have not been reported from Australia, and only Batai virus has been isolated in Asia and Europe, probably because birds and migrating mammals are not involved in the mosquito–rodent cycles of these viruses in nature. Bunyamwera serogroup viruses are commonly isolated in the Americas and Africa (Karabatsos, 1985). The relative insularity of the North American Bunyamwera serogroup viruses may in some way be related to the distributions of their principal vertebrate hosts and the competence of their vectors. It is interesting to speculate that Batai and Northway viruses may represent phylogenetic links between Bunyamwera serogroup viruses in Africa and in North America. Rabbits, for example, are viremic or produce antibody after inoculation with Batai, Northway, Tensaw, or Cache Valley viruses, whereas horses and perhaps hares are not susceptible to these viruses (Karabatsos, 1985). Two other Bunyamwera serogroup viruses from North America, Lokern, and Main Drain viruses, replicate well in hares and have been isolated most frequently from *Culicoides* sp., not mosquitoes.

Restriction of a virus to one specific vertebrate-vector pairing with defined geographic distribution may lead to natural isolation and genetic stability in divergent evolution. African Bunyamwera serogroup viruses have been found in distinct or overlapping geographic areas and ecosystems, but the South American members appear to coexist in horizontally or vertically contiguous, but not identical, ecosystems. One factor influencing the separate maintenance of sympatric, closely related serotypes may be differences in vector susceptibility. Woodall (1979) has suggested that the group C bunyavirus Itaqui is transmitted mainly by *Culex vomerifer*, a species apparently resistant to infection by Oriboca virus. Apeu and Marituba, also group C viruses, have been isolated from marsupials but not rodents, whereas Caraparu and Murutucu viruses, present in the same area of Brazil, have been isolated frequently from rodents. Cross-protection tests in monkeys indicate that immunity to one group C virus confers protection against another. Thus, coexistence of group C viruses is probably explained by their separate mosquito–small mammal cycles. The application of more sophisticated analyses will be necessary if we are to confirm the reasons for the coexistence of such closely related viruses in certain areas.

Like the Bunyamwera serogroup members, bunyaviruses of the California serogroup are transmitted between small mammals by mosquitoes, principally of the genus *Aedes*. The vector and host relationships of each virus appear to be quite restricted, possibly as a consequence of transovarial transmission in the mosquito (Watts et al., 1974). Thus, California serogroup viruses are geographically distributed in relation to the range of their vectors and hosts (Sudia et al., 1971). For example, Keystone virus has been isolated from rabbits and from *Sigmodon hispidus* (cotton rats) from Georgia and Florida. Because *S. hispidus* is only infrequently found outside the southeastern United States, association of these natural hosts with the implicated principal mosquito vectors *Aedes atlanticus* and *Aedes infirmatus* limits the distribution of Keystone virus. With other mosquitoes and other mammalian hosts, similar associations could be shown for other members of the California serogroup. Such postulations have been made for all of the North American members of this serogroup (Sudia et al., 1971).

The California and group C viruses are, in many respects, quite similar biologically to those of the Bunyamwera serogroup; mammal-feeding mosquitoes transmit virus to small mammal hosts within geographic foci determined largely by the distribution and limited movements of the vertebrate hosts. Potentially competitive serotypes are excluded by natural, selective disadvantages imposed by vector-host restriction and ability to induce cross-protec-

tion. RNA segment reassortment, when it occurs, takes place only between closely related serotypes (Bishop and Shope, 1979). Shope and Causey (1962) have shown that six of the group C bunyaviruses form three indistinguishable antigen pairs in CF tests. Karabatsos and Shope (1979) extended these studies and suggested that, because the CF antigen common to the members of the pairs is not an antigen shared by all the members of the serogroup, "pairing" might have resulted from natural genetic recombination.

The absence from Australia of known Bunyamwera and California serogroup viruses, for which placental mammals are the principal vertebrate hosts, may indicate that these viruses arose somewhat later than the drift of the Australian continent, perhaps fewer than 10 million years ago.

Simbu, Tete, and Turlock serogroup viruses have been isolated from resident and migrating birds, which may account for their relatively worldwide, hemisphere-wide, or continent-wide distributions. Other bunyaviruses are confined to single continents because they replicate in mosquitoes and rodents, marsupials, bats, and other mammals, but not in birds. Gamboa serogroup viruses are an example of a third type. They replicate in and are transovarially transmitted by *Aedeomyia squamipennis* mosquitoes, which feed principally on birds. However, the geographic distribution of these viruses appears to be limited to the distribution of the arthropod vector, not the vertebrate host.

Family *Bunyaviridae*, Genus *Phlebovirus*

Members of the genus *Phlebovirus* possess an ambisense RNA replication strategy. Total molecular weight of the RNA segments is 5.1 to 5.8 $\times$ 10^6. The 3′ terminal sequence is UGUGUUUCG. Two of the four structural proteins are glycosylated (G1 and G2) with molecular weights of 55 to 70 and 50 to 60 $\times$ 10^3, respectively. Another is a nucleocapsid protein (N), molecular weight 20 to 30 $\times$ 10^3. A minor large protein (L) with molecular weight of 145 to 200 $\times$ 10^3 also has been recognized.

A single serogroup, the Phlebotomus fever serogroup, constitutes this genus. Of the 37 members, none occur in Australia, one has been found in the United States, three occur only in Europe, three only in Asia, five only in Africa, and two in Africa, Asia, and Europe (Table 2). The last, sandfly fever Sicilian and sandfly fever Naples viruses, have been responsible for epidemics in these areas. Rift Valley fever virus, thus far limited to the African continent, is widespread there and, as mentioned, has caused extensive and serious epidemics and epizootics. Many phleboviruses occur focally in South America or Central America, suggesting a relationship between arboreal or ground-dwelling mammalian hosts and virus distribution, or simple restriction of distribution in parallel to the restricted distribution of the arthropod vector.

Family *Bunyaviridae*, Genus *Nairovirus*

RNA replication strategy of viruses belonging to the genus *Nairovirus* is unknown. However, total molecular weight of the three RNA segments is 6.2 to 7.5 $\times$ 10^6. The 3′ terminal sequence is AGAGUUUCU. Two of the four structural proteins are glycosylated (G1 and G2) with molecular weights of 72 to 84 and 30 to 40 $\times$ 10^3, respectively. Another is a nucleocapsid protein (N), molecular weight 48 to 54 $\times$ 10^3. A minor large protein (L) with a molecular weight of 145 to 200 $\times$ 10^3 also has been recognized.

Six serogroups, containing 28 viruses and subtypes, comprise this genus (Table 3). All the nairoviruses have been isolated from ticks; Dugbe virus and the Ganjam strain of Nairobi sheep disease viruses of the Nairobi sheep disease serogroup also have been isolated from culicine mosquitoes; strains of Dugbe, Nairobi sheep disease, and Crimean-Congo hemorrhagic fever viruses have been obtained from *Culicoides* sp. as well. With certain notable exceptions (Nairobi sheep disease and Crimean-Congo hemorrhagic fever viruses), little is known of the vertebrate hosts of the nairoviruses; however, at least one member each of the Sakhalin and Hughes serogroups have been isolated from seabirds, probably accounting for the relatively widespread distributions of these viruses.

Family *Bunyaviridae*, Genus *Uukuvirus*

RNA replication strategy of viruses belonging to the genus *Uukuvirus* is unknown. However, total molecular weight of the three RNA segments is 3.4 to 4.4 $\times$ 10^6. The 3′ terminal sequence is UGUGUUUCUGGAG. Two of the four structural proteins are glycosylated (G1 and G2) with molecular weights of 70 to 75 and 65 to 70 $\times$ 10^3, respectively. Another is a nucleocapsid protein (N), molecular weight 20 to 25 $\times$ 10^3. A minor large protein (L) with a molecular weight of 180 to 200 $\times$ 10^3 also has been recognized.

The uukuviruses have been recovered primarily from ticks and use birds as their principal vertebrate hosts. None of these viruses is known to cause dis-

ease in humans or livestock. The genus contains one serogroup with 11 members (Table 4). With the exception of Uukuniemi virus, which is distributed throughout Europe, viruses belonging to this genus (serogroup) appear to be focally distributed in areas of Europe, Asia, Africa, North America, and Australia (Macquarie Island), where shorebirds and sea birds nest and otherwise congregate.

Family *Bunyaviridae*, Genus *Hantavirus*

Members of the proposed genus *Hantavirus* possess negative-sense RNA replication strategy. Total molecular weight of the RNA segments is about 4.5×10^6. The 3′ terminal sequence is AUCAUCAUCUG. Two of the four structural proteins are glycosylated (G1 and G2) with molecular weights of 68 to 72 and 54 to 60 $\times 10^3$, respectively. Another is a nucleocapsid protein (N), molecular weight 50 to 53 $\times 10^3$. A minor large protein with a molecular weight of about 200×10^3 also has been recognized.

At present, only one serogroup comprises this proposed genus. The seven Hantaan serogroup viruses, relatively recently described, have been found in Asia, North America, South America, or Europe (Table 5). It is possible that one or more members of this serogroup will be found in Australia and that many more members of the serogroup will be isolated in the near future. Information currently available suggests that the host associations of the hantaviruses are species specific. Hantaan virus has been recovered from *Apodemus* sp. field mice; Seoul, Tchoupitoulas, Girard Point, and Sapporo Rat viruses from rats; Prospect Hill virus from *Microtus* sp. meadow voles; and Puumala virus from *Clethrionomys* sp. bank voles. Several hantavirus isolates have not been definitively typed, so these apparent host and geographic associations may or may not be borne out.

Bunyavirus-Like Viruses

Thirty-five viruses have morphologic or molecular characteristics in common with members of the family *Bunyaviridae;* usually the common denominator is morphology. In the absence of adequate molecular and genetic studies or antigenic relatedness with a recognized member of any of the serogroups within the family, these bunyavirus-like viruses have been placed provisionally within the family but are denoted only as "possible members of the family." Included among these are 21 viruses belonging to 8 serogroups and 14 ungrouped viruses (Table 6). Viruses of the Bhanja, Kaisodi, and Upolu serogroups are principally tick-borne, whereas those of the Bakau, Mapputta, Nyando, and Resistencia serogroups appear to be principally mosquito-borne. Matariya serogroup viruses have been isolated only from birds. Knowledge of the geographic distributions of all these viruses is limited, as is that of the ungrouped mosquito-borne and ungrouped tick-borne bunyavirus-like viruses. Certain of these viruses are known to be pathogenic to humans and have widespread geographic distributions, thus studies of the taxonomy and classifications of these viruses have potential medical importance.

Pathogenesis and Pathology

The pathogenetic mechanisms and accompanying pathology of viruses belonging to the family *Bunyaviridae* can best be discussed by clinical classification (febrile illness with or without rash, encephalitides, and hemorrhagic fevers), rather than by virus classification (genera). Most viruses in the family cause febrile illnesses or no disease at all in humans. It is only the rare or exotic member of the family that causes hemorrhagic fever or other life-threatening syndromes; however, it is precisely this potential severity that creates medical and political concern and prompts efforts toward prevention and treatment.

LaCrosse virus (genus *Bunyavirus,* California serogroup) may be the most well-studied member of the family, insofar as virus ecology, disease etiology, and pathogenesis are concerned (Calisher and Thompson, 1983). In the two fatal human cases studied, neuronal and glial damage, perivascular cuffing of capillaries and venules, and cerebral edema were noted on pathologic examination. Although not quantitatively different from pathologic findings in other viral encephalitides, their distribution may be distinctive, being most focal in cortical gray matter of frontal, temporal, and parietal lobes, basal nuclei, midbrain, and pons, with other regions spared (Kalfayan, 1983). Brain biopsy in a single case showed congestion, margination of polymorphonuclear leukocytes, focal neuronal necrosis, and endothelial cell swelling (Balfour et al., 1973).

The pathogenesis of encephalitis, that is viremia followed by antibody production and encephalitis, is significant to the success of early diagnosis where antibody (IgM) is detected in a single specimen at admission to hospital. Rift Valley fever is usually an uncomplicated, temporarily prostrating febrile illness in humans, producing hemorrhagic manifestations, encephalitis, or eye lesions in a small proportion of persons. The most prominent pathologic finding in fatal cases of Rift Valley fever with hemorrhagic manifestations is hepatic necrosis, the probable cause of hepatic failure often seen in patients who are dying in shock, with jaundice, gastrointestinal

and mucous membrane bleeding, and disseminated vascular damage.

Crimean-Congo hemorrhagic fever virus has not been shown to cause hemorrhagic fever in laboratory animals. In humans the primary pathophysiologic event appears to be leakage of plasma and erythrocytes through the vascular endothelium (Karmysheva et al., 1973). At autopsy, histopathologic findings include edema, focal necrosis and hemorrhage, and vascular congestion of the heart, brain, and liver.

In hemorrhagic fever with renal syndrome, vascular instability is the basic lesion. Capillary leakage occurs without inflammation but with significant loss of protein colloid. Autopsy findings include gross hemorrhagic necrosis of the kidney, pituitary, and right auricle. Microscopic changes found in the kidneys of patients who die with this disease depend on the stage of illness at death (Oliver and MacDowell, 1957). Those who die in the late febrile or early hypotensive stages show congestion in the subcortical medullary vessels without obvious damage to the renal tubules and without obstructive changes in the tubules. In the hypotensive stage, findings include intertubular congestion in the corticomedulla and swelling of the proximal convolutions with hydration and vacuolation of the epithelial cells. Later in the course of disease, progressive damage to the tubules is observed. The lumens are compressed and filled with desquamated cells and hyaline material. During the oliguric stage, hemorrhage develops in the congested zones and tubular necrosis occurs with coagulated proteins in the lumens of tubules. In patients who die during the diuretic stage, fibrosis and epithelial proliferation have occurred (Shope, 1985).

Clinical Features

The mechanism of transmission of viruses of the family *Bunyaviridae* is intimately related to the arthropod or vertebrate from which the virus is acquired. That is, the individual becomes infected by the bite of an infected arthropod or by direct or aerosol contact with virus excreted by a reservoir vertebrate. Depending on how successful the arthropod vector has been in feeding (subcutaneous introduction of virus while probing for a capillary or capillary introduction when feeding successfully), or the viremic titer in the vertebrate serving as the source of virus, virus replication is initiated as soon as the virus comes in contact with susceptible cells in the host. After an incubation period of 3 to 6 days in bunyavirus, phlebovirus, and probably nairovirus infections, to as long as 5 weeks in hantavirus infections.

Many bunyaviruses and phleboviruses cause undifferentiated febrile diseases with or without rash (Table 7). The illness typically has an abrupt onset with accompanying chills and fever. Headache, with or without photophobia; retroorbital pain; myalgia; arthralgia; asthenia; nausea; and other signs and symptoms, including vomiting, diarrhea, or constipation, abdominal pain, upper respiratory distress, or pulmonary infiltrates with or without sore throat and cough, may occur. Over the duration of the typical acute illness, lymphadenopathy, conjunctival injection, and abdominal tenderness also may occur. Biphasic febrile episodes may be observed and leukopenia, leukocytosis, or normal leukocyte counts may be seen. Although the normal course of illness is 2 to 4 days to a week, convalescence may require several days but residua do not occur. Rash, occurring in infections with certain Bunyamwera, Bwamba, Simbu, and Nairobi sheep disease serogroup viruses and in infections due to Tataguine virus is typically maculopapular, appearing after the onset of illness, lasting 1 to 3 days and occurring most commonly on the trunk.

Persons infected with most of the bunyaviruses, phleboviruses, and nairoviruses can be considered infective only as a source of virus for a subsequently feeding, uninfected arthropod; as mentioned, Rift Valley fever virus, Crimean-Congo hemorrhagic fever virus, and probably other members of the family *Bunyaviridae* as well, can be transmitted by aerosol. The viremic period is about 2 or 3 days in individuals infected with bunyaviruses, phleboviruses, and probably most nairoviruses, but viremia persists for as long as 7 to 10 days in subjects with Crimean-Congo hemorrhagic fever. Infection with hemorrhagic fever with renal syndrome virus, acquired by aerosol or by direct contact with fomites or excreta from infected animals, may lead to viremia, but the long incubation period precludes detection.

The serious nature of these diseases and the evidence that body fluids from patients suffering from them may be highly infectious, suggests that immediate supportive care must be given them and that patient isolation measures are indicated, particularly with Crimean-Congo hemorrhagic fever patients. This is not the case with mild 3-day fevers, but until and unless a confirmed laboratory diagnosis is available, such persons should be watched carefully to see that they do not develop more serious complications.

Diagnosis

Dynamics of Viremia and Antibody Responses

In geographic areas where febrile illnesses are common and laboratory support for clinical observations is poor, it may be that only in special cases or in

epidemics are laboratories called on to isolate viruses or to provide serodiagnostic support. Bunyaviruses and phleboviruses are isolated most often from blood samples collected from febrile humans. LaCrosse virus (California serogroup) has been isolated only twice from humans (brain), although serologic evidence suggests that this is the most common arboviral cause of pediatric encephalitis in the United States. Thus, viruses of the family *Bunyaviridae* are isolated from encephalitic humans rarely or only with great effort. One reason for this is that the viremic stage in bunyavirus infections is brief, generally no more than 1 to 3 days after onset. Often the viremic stage has passed by the time CNS symptoms have appeared. Virus is readily isolated from patients with fever and with fever and rash. During the first 3 days of illness, bunyavirus viremia titers may be very high in these patients. Figure 1 presents a hypothetical model of viremia and antibody responses in a person infected with a typical bunyavirus. The initial antibody response appears at the end of the viremic period; this may be coincidental or may serve to quench the viremia. In either case, virus (antigen)-antibody complexing may make both virus isolation and antibody determinations more difficult. The IgM fraction of the serum contains both neutralizing and HI antibodies early after infection, and its presence can serve as an indicator of recent infection (Niklasson et al., 1984; Calisher et al., 1986a, b, c). Later, IgG antibody appears, containing both neutralizing and HI as well as CF antibodies; IgG antibody to the bunyaviruses appears to persist for years, perhaps for the life of the individual (Calisher et al., 1986a). In summary, the initial response to infection is by production of IgM antibody, then IgG antibody. As the titer and presence of antiviral IgM antibody wanes, antiviral IgG antibody predominates, until antibody can be detected only in the IgG fraction.

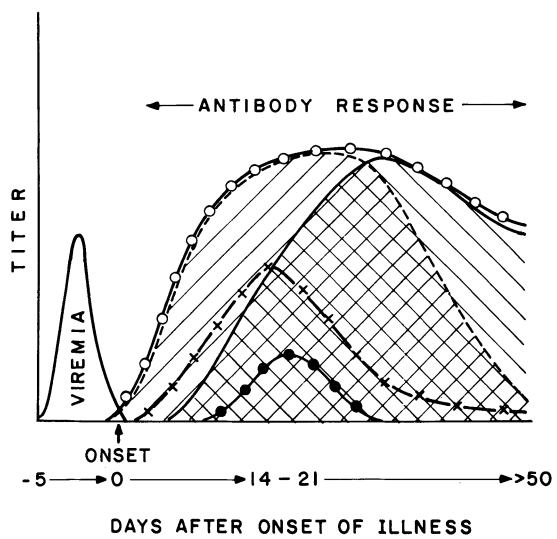

FIG. 1. A hypothetical model of viremia and antibody responses in a person infected with a typical bunyavirus. O—O—O = neutralizing antibody; ×—×—× = hemagglutination-inhibiting antibody; ●—●—● = complement-fixing antibody; ∠∠∠∠∠∠ = IgM class antibody; ⊓⊓⊓⊓⊓ = IgG class antibody.

Antiviral antibody contained in the IgM fraction of serum appears to be antigenic complex-specific but not virus-specific in alphavirus (Calisher et al., 1986b) and flavivirus (Monath et al., 1984) infections but may be only serogroup-specific in infections caused by bunyaviruses (Calisher et al., 1986a). Nevertheless, assays for IgM class antibodies are valuable because their presence provides evidence that the patient had been infected recently.

Laboratory Safety

The dynamics of both the viremia and antibody responses provide opportunities for virus isolation and for serodiagnosis. When attempting virus isolations, prime consideration should be given to safety. When handling specimens assumed to contain viruses such as Rift Valley fever, Crimean-Congo hemorrhagic fever, or other class 4 and 5 pathogens, the possibility of laboratory-acquired infection should be of concern. Transmission to humans by aerosol was demonstrated in six Egyptian laboratory workers attending the slaughter of a sheep with Rift Valley fever virus infection (Hoogstraal et al., 1979) and in agricultural workers slaughtering Rift Valley fever virus-infected sheep (Shope, 1985). Hospital-based outbreaks of Crimean-Congo hemorrhagic fever have been reported from the Soviet Union (Kulagin et al., 1962), and infection with hemorrhagic fever with renal syndrome (Hantaan) virus was recorded in more than 100 laboratory workers in Moscow, workers who apparently were infected by aerosols generated by field-collected rodents (Shope at al., 1985). Workers handling laboratory rats as well have been infected with Hantaan or a related hantavirus (Umenai et al., 1979).

Most other members of the family *Bunyaviridae* may be handled with less stringent precautions, yet with relative safety, but it should be kept in mind that under certain circumstances, many are potential pathogens.

Wearing rubber or plastic gloves and a laboratory coat and using a laminar flow hood and aseptic technique should be sufficient for handling class 1 or 2 viruses. For viruses that pose special hazards to laboratory workers (class 3), special conditions are required for containment. For viruses that pose ex-

treme hazards to laboratory workers or that may cause epidemic disease (class 4), conditions of maximum containment are needed. It is always best to direct questions regarding problem or potentially hazardous specimens to a specialty laboratory. In all instances, exposure to aerosols should be minimized or eliminated by using closed systems for grinding tissues, using blunt-ended needles for collecting and transferring liquids, and providing appropriate ventilation systems for filtering or otherwise scrubbing or cleaning exiting air. Personnel showers, disinfecting for individual decontamination, and the use of germ-free-type isolators (for class 4 viruses) are recommended. Specimens in both the field and the laboratory should be handled under the assumption that virus is present and potentially dangerous.

Specimen Collection

After collection, whole blood, serum, or tissue samples should be processed immediately or placed on dry ice (−70°C) or another suitable deep-freezing agent if virus isolation is to be attempted with the specimen. Although for antigen detection, this may not be such a critical issue, it appears sensible to ship and store specimens for this purpose at low temperatures to prevent further degradation of proteins. When sera are to be tested for antibody only, they can be shipped and stored at ambient temperatures, unless they are contaminated with microorganisms or will be in transit for long periods.

Virus Isolation and Identification

Because there is such a wide variety of individual characteristics of viruses within the family *Bunyaviridae,* no single virus isolation system suffices and suitable isolation systems have not yet been developed for all viruses of the family. In general, viruses of the genus *Bunyavirus* may be isolated in suckling mice inoculated intracranially with clarified suspensions of clinical specimens or with macerated and clarified arthropod pools. However, because essentially all the bunyaviruses were first found by using a suckling mouse isolation system, it follows that attempted isolation in other systems, including cell cultures derived from arthropods or mammals, may yield viruses not yet discovered. Because suckling mice are available to essentially all laboratories, particularly those that perform rabies virus isolations, this system holds certain advantages over others. Nevertheless, little loss in sensitivity and perhaps some increase in sensitivity may result from using mosquito cells grown in vitro, such as C6/36 (*Aedes albopictus*) or AP-61 (*Aedes pseudoscutellaris*) cell lines, for virus isolation. These hold the additional advantage of ease of containment and reduction of aerosols. Inoculation of *Toxorhynchites amboinensis* mosquitoes, which do not take blood meals but in which dengue and other viruses replicate, also have been used with sensitivity and safety (Rosen, 1981).

For more than 30 years the classical procedure for identifying an arbovirus was to: (1) isolate the virus by inoculation of suckling mice; (2) perform a modicum of characterization (filterability, sensitivity to lipid solvents (Theiler, 1957), determination of replication and titers in various laboratory hosts such as animals and cell cultures, and so on); (3) prepare a sucrose-acetone extracted antigen (Clarke and Casals, 1958); (4) test that antigen for its ability to agglutinate the erythrocyte of male, domestic geese (*Anser cinereus*) and its ability to react in CF tests (Clarke and Casals, 1958; Casey, 1965) with homologous antibody preparations; and then (5) test by either HI or CF with a battery of antibody preparations. Although we now have a greater understanding of the structure and function of arbovirus virions and newer methods of testing have been introduced, the basic techniques used to identify and to antigenically characterize arboviruses have not and probably will not change in the foreseeable future. Because the G1 and G2 glycoproteins of most members of the family *Bunyaviridae* function in virus attachment to cells and play a role in HI and neutralization of these viruses and because the nucleocapsid protein participates in CF tests, much can be learned about a particular virus by studying the participation of these antigens in relatively simple laboratory tests. Also, because most of the antigenic data already accumulated have been generated using these tests they will, therefore, remain the standards by which newly isolated viruses are judged. This does not mean that newly devised tests are to be minimized. On the contrary, any additional tests that expand our ability to characterize fully the epitopes and other antigenic moieties of viruses are of great use. In this chapter, we limit the discussion of virus identification procedures to those recently developed and shown useful and the tried and tested techniques that have been so reliable and functional.

As with other arboviruses, hemagglutination by arboviruses is pH dependent. That is, virus adsorbs to indicator erythrocytes only over a narrow, usually low (pH 5.9 to 6.3, but sometimes as high as 6.8 to 7.4) pH range. Hemagglutinins for some viruses of the family *Bunyaviridae* have been prepared with difficulty or not at all, so this procedure is not as useful for their identification as for arboviruses of other families. Nevertheless for certain viruses, such as for the California and Phlebotomus fever serogroup viruses, they are quite useful. Using a battery of polyclonal hyperimmune mouse ascitic fluids (Tikasingh et al., 1966), the hemagglutinin is then tested for re-

activity; a resulting positive reaction (HI) is then taken to indicate that the virus is antigenically related to one or more of the viruses used to prepare the polyvalent antibody. This allows one to place an isolate in a serogroup and to perform additional tests that provide more discrimination, that is, identification to antigenic complex or to type.

Complement-fixing antigens of viruses belonging to the family *Bunyaviridae* (except for some phleboviruses) are more serogroup-, or at least, complex-specific than are those belonging to other families of arboviruses because sharing of nucleocapsid protein is more extensive among the *Bunyaviridae*. This holds both advantages and disadvantages for virus identification because the CF test can be used to place an isolate in a serogroup, but specific identification of the isolate still remains to be done (Calisher et al., 1981). Nevertheless, because the known and registered number of viruses in the Catalogue of Arboviruses has surpassed 500 (Karabatsos, 1985), it is of considerable benefit to have techniques such as HI and CF available to reduce the possibilities.

Neutralization tests, whether performed in cell cultures by serum dilution-plaque reduction (Lindsey et al., 1976; Hunt and Calisher, 1979) or in mice by virus or serum dilution are the definitive tests for identification of virus types and subtypes. Virus dilution-constant serum neutralization test results provide a quantitative indication of the power of a serum sample to neutralize a virus, whereas constant virus-serum dilution tests provide a quantitative indication of the potency of that serum. The virus dilution-constant serum technique may be closer to the natural situation (that is, how much virus will this serum sample neutralize?); however, the serum dilution neutralization technique is more sensitive diagnostically (i.e., how much can the serum be diluted and still neutralize a given quantity of virus?) and the latter is most often used to determine significant differences in neutralizing antibody titers between paired sera. When a virus isolate is tested for its ability to be neutralized by antibody prepared against a reference virus, the titer of that antibody preparation is compared with its homologous titer; differences between the two titers are taken to indicate differences between the viruses. Monoclonal antibodies are increasingly being produced and used for virus typing and subtyping (Gonzalez-Scarano et al., 1983). As hybridomas are selected for epitope specificity, they are being used to prepare reagents useful not only for virus characterization but for practical and immediate applications, such as virus identifications during epidemic situations.

In summation, a virus isolate is tested for its ability to agglutinate the erythrocytes from male domestic geese. If found positive, the hemagglutinin is tested at its pH optimum for inhibition by one or more of a battery of immune reagents. Whether or not the antigen hemagglutinates, it is tested by CF against a battery of immune reagents; fewer reagents are necessary if preliminary (HI) tests give indication of the serogroup to which the isolate belongs. Serum dilution-plaque reduction neutralization tests are then performed to identify the virus to type, and epitope-specific monoclonal antibodies are then used for further, subtypic identification. A combination of monoclonal antibodies and oligonucleotide fingerprint mapping studies (Klimas et al., 1981) can then be applied to the isolate for determining genetic and epidemiologic origins.

Direct Antigen Detection

The relatively recent wide acceptance of enzyme-linked immunosorbent assays (ELISA) for detecting many viruses, including arboviruses, has proven a boon to clinical virologists, not to speak of the patient. Treatment of patients infected with a variety of viruses can be instituted as soon as laboratory tests indicate that such treatment would be useful. Judicious nontreatment, with supportive care only, can also be indicated by such tests. Therefore, it is in the interests of the patient, the attending physician, and the laboratorian to be able to determine with some certainty the etiologic agent of infection caused by members of the family *Bunyaviridae*. The more rapid, sensitive, and specific the test, the better for all concerned. For many years, respiratory viruses have been identified directly in nasopharyngeal aspirates. More recently, enteric viruses, including rotaviruses, have served as models for developing rapid identification techniques. Immunofluorescence has proved to be a relatively rapid, sensitive, and specific tool for direct identification of virus antigens in clinical specimens, without the need for an intervening amplification in a laboratory host (Gardner and McQuillin, 1980). Although offering such an advantage, immunofluorescence tests require highly skilled, precise, and accurate technicians, and expensive microscopes. These needs and the subjectivity of the results are drawbacks in addition to the absence of appropriate clinical specimens containing large quantities of virus in infections caused by viruses of the family *Bunyaviridae*.

Therefore, the ELISA is increasingly used for direct detection of viral antigens in clinical specimens as well as in tissues from wild vertebrates and in mosquitoes. ELISA systems have been developed for detecting *Bunyaviridae* members LaCrosse virus in mosquitoes (Hildreth et al., 1982) and in mice (Beaty et al., 1982) and Rift Valley fever virus in experimental animals, vaccines, and infected mosquitoes (Niklasson et al., 1983). In brief, material

suspected to contain whole virus or viral antigen is diluted in pH 9.6 carbonate-bicarbonate coating buffer and introduced to wells of a microtiter plate and incubated at room temperature overnight or for 3 hours at 37°C. Incubation of all subsequent reagents is usually done at either 37°C for 1 h or at 4°C for 18 h. In direct ELISA's specific immune serum, or another antibody-containing reagent, conjugated to an enzyme such as peroxidase or alkaline phosphatase, and then substrate for this enzyme, are added sequentially. Diluent for all antibodies and antigens except the coating buffer is phosphate-buffered saline (pH 7.4) containing 5% heat-inactivated fetal bovine serum, 0.5% Tween 20, and 40 μg of merthiolate per ml. Sufficient washing of the wells after adding each reagent ensures that substances not attaching to specific binding sites on the previously added reagents will be flushed out. An adequate washing solution is phosphate-buffered saline containing 0.1% Tween 20. If antigen is present to react with enzyme-conjugated antibody, the substrate will be reduced (change from a colorless to a translucent material in solution), and the optical density of the resulting colored solution can be read either mechanically (spectrophotometer) or estimated by eye.

Indirect (sandwich) ELISAs use an antigen-capturing antibody for coating the wells. This antibody should be sufficiently avid and potent to capture the very small quantities of antigen that are in tissues, viremic sera, or arthropod tissues; either polyclonal (Halonen et al., 1980) or monoclonal antibodies may be used. To the antiviral antibody-coated wells are added, sequentially, the specimen to be tested for viral antigen, a secondary (detecting) antibody conjugated with enzyme, and a substrate for that enzyme. As an alternative to the sandwich ELISA, a double sandwich ELISA may be used. This is simply a variant of the sandwich ELISA in that the secondary antibody is not enzyme-conjugated, necessitating a third (detecting) antibody that is enzyme conjugated. This detecting antibody is directed against the antiviral secondary antibody (antispecies). An advantage of this method is the obviation of a need for enzyme-conjugated antibodies for all the viruses tested; principal disadvantages are the extra step (time) required and the possible increase in attendant nonspecific reactivity.

Viral antigens may be detected directly in cells by use of antibodies conjugated to fluorescing dyes, such as fluorescein isothiocyanate. When viral antigens are exposed to these antibodies, the fluorescent dye is immobilized at the site of antigen-antibody complexing and may be seen through a fluorescence microscope (Gardner and McQuillin, 1980). Because clinical specimens from patients infected with most members of the family *Bunyaviridae,* are, with notable exceptions, either devoid of virus or contain insufficient quantities of virus to be detected by this method, the direct fluorescent antibody technique, and its counterpart, the indirect fluorescent antibody technique (virus-infected cells, to which antiviral antibody, fluorescein isothiocyanate-conjugated antispecies antibody are added, sequentially) are not routinely used for identifying arboviral antigens in clinical specimens. The exceptions alluded to above are Rift Valley fever, hemorrhagic fever with renal syndrome, and Crimean-Congo hemorrhagic fever. In these and possibly other infections caused by members of the family *Bunyaviridae,* virus often is detected directly in the tissues of patients, domestic animals, or wild rodents infected with the virus. Because the hantaviruses cause no cytopathology in cells infected with them, fluorescent antibody techniques have become invaluable for making an early diagnosis relevant to the course of illness of patients infected with these viruses.

Direct Detection of Viral Nucleic Acid

Recent advances in recombinant DNA technology have made it possible to use nucleic acid probes for diagnosing viral infections and for detecting viral genetic material in tissue from other sources. In situ hybridization permits detection of viral nucleic acid in infected cells. In these assays, virus-specific nucleic acids are bound to a solid phase and then detected with radiolabeled DNA probes (Hyypia et al., 1984) or RNA probes. This technique could provide a method for rapid and exquisitely specific identification of viral RNA in tissues from patients, reservoir hosts, and arthropod vectors of viruses in the family *Bunyaviridae;* however, it is not yet refined or widely accepted for use with arboviruses due to low sensitivity and the need for radiolabeled probes. It has the further potential advantage of identifying specific sequences in viral genetic material and will undoubtedly not only function in the future as a rapid diagnostic method but will also serve as an epidemiologic tool.

Antibody Assays

In the past, fourfold or greater increases or decreases in HI, CF, neutralizing, or other antibody titers have been the basis for serodiagnostic confirmation of arboviral infections. However, these tests require multiple specimens, collected days to weeks apart. When rapidity of diagnosis is not a critical issue, or when infection with closely related viruses confounds interpretation of results, these tests are often invaluable. However, their lack of specificity also may create a variety of diagnostic problems. For example, in areas hyperendemic for flaviviruses, vacci-

nation with yellow fever virus, coincidental with the circulation of the related West Nile, Zika, Uganda S, Spondweni, dengue-1, Ntaya, and wild-type yellow fever viruses limits the usefulness of both the ordinarily broadly cross-reactive HI tests as well as the usually more specific CF test (Monath et al., 1980). Viruses of the same serogroups in the family *Bunyaviridae* (California, C, Patois, Guama, Capim, phlebotomus fever, Uukuniemi, Hantaan, to name a few) may occur sympatrically. Therefore, antibody to one virus in a serogroup does not provide definitive evidence for infection with that virus. When rapidity of serodiagnosis is needed, other techniques must be used. The relatively recent adaptation of antibody capture ELISAs for IgM antibody to arboviruses has provided such a tool (Heinz et al., 1981; Jamnback et al., 1982; Burke et al., 1982; Niklasson et al., 1984; Monath et al., 1984; Calisher et al., 1985a; Calisher et al., 1985b; Calisher et al., 1986a; Calisher et al., 1986b; Calisher et al., 1986c). Unlike IgG antibody, which is produced later in infections, IgM antibody is produced early in arboviral infections and does not persist in high titer; its presence, therefore, is indicative of recent infection.

Briefly, the IgM antibody capture ELISA is performed as follows: Wells of a microtiter plate are coated with antibody to human IgM. Patient serum or cerebrospinal fluid is then introduced, usually at a dilution of 1:100 for serum or 1:10 for cerebrospinal fluid. Density gradient-purified virus, supernatant fluid from virus-infected cell cultures, or other viral antigen such as sucrose-acetone extracted antigen prepared from the brains of virus-infected suckling mice, and antiviral (secondary) antibody are then added. If the secondary antibody has been conjugated to an enzyme, substrate is then introduced and the intensity of the color change recorded as optical density, using a spectrophotometer. If incubations and washings between the addition of each reagent were sufficient, the results can be read as the ratio of test to control; usually ratios greater than or equal to 2.0 are considered positive for IgM antibody. Sera can be tested at single dilutions or diluted serially twofold and each dilution tested.

A double sandwich ELISA, using an antispecies IgG third (detecting) antibody conjugated to an enzyme that reacts with the secondary (antiviral) antibody also can be used. As was mentioned in the section on antigen detection, such a system has advantages and disadvantages. However, when testing only a few or a large number of sera for IgM antibody to many viruses the double sandwich ELISA is preferred, because enzyme-conjugated antibody preparations are not needed for each of the antiviral reagents.

The presence of IgM antibody in a single serum specimen or spinal fluid specimen can be taken as essential evidence for recent infection by the virus with which the test was done. Not enough is known yet about the reactivity of IgM antibody produced during infections with viruses of the family *Bunyaviridae*. It may be that, as in infections with alphaviruses and flaviviruses, IgM class antibody produced after infection with viruses of the family *Bunyaviridae* are complex-specific but are not type (virus)-specific. Furthermore, recent evidence suggests that IgM antibody persists in cerebrospinal fluid and serum for months, years, and perhaps for life after central nervous system involvement in Japanese encephalitis virus infections of humans (Burke et al., 1985); should this prove to be the case for members of the family *Bunyaviridae* as well (IgM to LaCrosse virus has been shown to persist [Jamnback et al., 1982]), the IgM antibody capture ELISA, as applied to these viruses must be considered somewhat less confirmatory than it now appears.

IgG antibody produced after infections with arboviruses persists for months or years. Therefore, its presence does not necessarily denote an active or recent viral infection, contrary to the significance of the presence of IgM antibody. The fetus or newborn produces IgM but not IgG in response to infections in utero or shortly after birth. Thus, the presence of IgG in the fetus or newborn indicates passive reception of IgG across the placenta; IgM cannot cross the placenta. IgM and IgG are produced after vaccination with live, including attenuated, viruses.

For estimating antibody prevalence in populations, HI, indirect fluorescent antibody, and ELISA IgG, or neutralization assays are the most useful; CF and IgM assays are often the least useful, although CF is quite useful in specific instances, such as for determining antibody to the dengue viruses.

IgG antibody in sera or spinal fluids may be determined as follows: (1) coat wells of a microtiter plate with about 350 to 500 ng of gradient-purified virus; an alternative is to coat the wells with antibody (prepared in species A) and then add sucrose-acetone extracted antigen or supernatant fluid from virus infected cells; (2) introduce the clinical specimen suspected to contain IgG antibody, testing only a single dilution of serum or the first of a series of dilutions beginning at 1:40; (3) add antihuman IgG (which does not contain antibody to species A conjugated with an enzyme; and (4) add the substrate and record the optical density of the test and control sera. Again, ratios of test/control greater than or equal to 2.0 are considered positive. This IgG antibody assay is performed relatively quickly and has the distinct advantages of allowing precoating and storage of plates as well as requiring few steps for performance. It is somewhat less sensitive and specific than neu-

tralization tests but, because it is relatively easy to perform and is a binding assay, it can be used to rapidly detect antibody to a variety of viruses. A distinct disadvantage of this test is the need for either purified virus for every virus used for determining antibody and the need for separate coating antibodies to each of these viruses.

In summary, the IgM antibody capture ELISA is suggested for use in rapidly detecting antibody, and IgG assays for use in determining antibody in paired sera and for serosurveys. HI and CF tests can be used to supplement these tests and neutralization tests used for definitive and confirmatory determinations of the infecting agent.

Interpretation of Serologic Data

Viruses of the family *Bunyaviridae* differ in their antigenic relationships within and between the various serogroups, that is, certain viruses are very closely related and others are very distantly related. This is reflected not only in cross-tests between viruses but in antibody responses in humans and other animals infected with these viruses. As mentioned, infection with more than one member of a serogroup may or may not confer protection to heterologous members of that serogroup but almost assuredly will create serodiagnostic problems. Therefore, the combined use of IgM and IgG antibody assays as well as HI, CF, and neutralization tests may be required for final, presumptive determination of the etiologic agent of infection. Table 8 presents test results with sera from three persons naturally infected with viruses of the California, C, and Phlebotomus fever serogroup bunyaviruses. Note that a combination of tests was needed to make a presumptive determination of the infecting virus. Although inhibition of hemagglutinin of a virus by patient serum is indicative of past infection with a member of the serogroup to which the virus belongs, close antigenic relationships between viruses prevents specific determination of the infecting agent by HI tests (Table 8, case A, La Crosse and snowshoe hare viruses). Where viruses share CF antigen but not hemagglutinins (Oriboca and Murutucu viruses, case B), HI and neutralization tests are valuable. Where HI and CF titers are low, neutralization tests are valuable. Increasingly, it will likely be found that IgM assays are a valuable addition to the present diagnostic armamentarium and may very well replace the more classical tests in many instances.

In summary, the best method for serodiagnosis is the one that works best for the individual laboratory performing the tests. Labor intensiveness and overall costs are always considerations, but if testing of a serum sample is worth doing at all, it is worth doing with the best available tests. For serodiagnosis of arbovirus infections, using an inadequate test is worse than not testing at all, because false-negative results will bias the diagnostic perception and perhaps allow an invasive diagnostic procedure that is unwarranted (Calisher and Bailey, 1981). Routine use of an insensitive CF test, an IgG assay on a single serum, an HI test in a single serum from a vaccinated person, tests with poor antigens, or otherwise uncontrolled assays and inappropriate tests are misleading and are to be avoided. The World Health Organization Centres for Arbovirus Research and Reference at the Centers for Disease Control, Fort Collins, Colorado and at Yale University, New Haven, Connecticut, are available to provide advice and reagents to laboratories establishing diagnostic procedures for arboviruses.

Epidemiology and Natural History

The terms "arthropod-borne" and "rodent-borne" refer to the major biologic phenomena involved in the cyclical transmission of certain viruses. In the natural setting, these viruses are maintained in invertebrate-to-vertebrate-to-invertebrate or vertebrate-to-vertebrate cycles, usually not involving humans. Although Rift Valley fever and Crimean-Congo hemorrhagic fever viruses have been known to be transmitted by aerosols and infected body fluids, in most instances transmission is a complex interaction of virus, vector, and host. The bunyaviruses, nairoviruses, phleboviruses, and uukuviruses are transmitted from infected arthropods (mosquitoes, ticks, phlebotomine flies, and ticks, respectively) to susceptible vertebrates. After an incubation period, the vertebrate becomes viremic and is capable of infecting an uninfected arthropod that happens to feed on it. If the vector is genetically competent both to support replication and to transmit virus, and if the now-infected arthropod feeds on a susceptible vertebrate host, the natural cycle is continued. This general concept holds true where biologic, not simply mechanical, transmission occurs. The following factors come into play as regards vector competence: virus and vector genetics, virus concentration in the infected vertebrate host that is the source of virus, ambient temperature, autogeny, salivary gland and gut barriers, and other less clearly understood factors. Certain arthropods may serve as maintenance or amplification vectors. Vectors that could maintain the virus would have low infection thresholds, minimum evidence of barriers to disseminated infection of the arthropod, and high transmission efficiency. Vectors that could amplify the virus would have high

TABLE 8. Results of antibody determination with sera from humans presumably infected with bunyaviruses LaCrosse (California serogroup), Oriboca (Group C), or Toscana (Phlebotomus fever serogroup) viruses

Case	Serum no.	Days after onset		Antigen[a] used in test						
				LAC	*SSH*	*CE*	*SAN*	*KEY*	*JC*	*TVT*
A	1	2	(HI)	–[b]	–	–	–	–	–	–
			(CF)	–	–	–	–	–	–	–
			(IgM)	2.1	2.2	2.4	2.0	–	2.0	–
			(IgG)	–	–	–	–	–	–	–
			(N)	10	–	–	–	–	–	–
	2	15	(HI)	160	80	40	40	20	–	–
			(CF)	8	8	–	–	–	–	–
			(IgM)	4.2	4.5	3.9	3.2	2.7	3.3	–
			(IgG)	+[c]	+	+	+	+	+	+
			(N)	640	160	40	80	40	10	–
	3	130	(HI)	20	20	20	10	–	10	–
			(CF)	8	16	8	–	–	–	–
			(IgM)	–	–	–	–	–	–	–
			(IgG)	+	+	+	+	+	+	+
			(N)	160	320	40	40	20	–	–
				ORI	*MUR*	*ITQ*	*APEU*	*CAR*	*MTB*	*NEP*
B	1	0	(HI)	–	–	–	–	–	–	–
			(CF)	–	–	–	–	–	–	–
			(IgM)	2.8	–	–	–	–	–	–
			(IgG)	–	–	–	–	–	–	–
			(N)	40	–	–	–	–	–	–
	2	23	(HI)	160	–	–	–	–	–	–
			(CF)	64	128	8	8	–	–	–
			(IgM)	4.7	–	–	–	–	–	–
			(IgG)	+	+	+	+	–	–	–
			(N)	320	20	–	–	10	–	–
				TOS	*SFN*	*SFS*				
C	1	2	(HI)	–	–	–				
			(CF)	8	–	–				
			(IgM)	12.1	11.2	4.2				
			(IgG)	–	–	–				
			(N)	–	–	–				
	2	87	(HI)	20	–	–				
			(CF)	8	8	–				
			(IgM)	7.6	3.6	2.8				
			(IgG)	+	+	+				
			(N)	640	–	–				

[a] Abbreviations of virus names are: La Crosse (LAC), snowshoe hare (SSH), California encephalitis (CE), San Angelo (SAN), Keystone (KEY), Jamestown Canyon (JC), Trivitattus (TVT); Oriboca (ORI), Murutucu (MUR), Itaqui (ITQ), Apeu (Apeu), Caraparu (CAR), Marituba (MTB), Nepuyo (NEP), Toscana (TOS), sandfly fever Naples (SFN), and sandfly fever Sicilian (SFS).

[b] Signifies <10 HI or neutralization; <8 CF; ratio of optical densities of test : control sera <2.0 at the lowest dilution tested by ELISA for IgM (1 : 100) or IgG (1 : 40) antibodies.

[c] Signifies ratio of optical densities of test : control sera equal to or greater than 2.0 at the lowest dilution (1 : 40) tested by ELISA for IgG antibodies.

infection thresholds and poor rates of bite transmission (often compensated for by extremely high seasonal population densities and feeding on a variety of vertebrate host species). Many members of the family *Bunyaviridae* that are arthropod transmitted have been shown to be transovarially transmitted in the arthropod. Mosquito-borne, tick-borne, and phlebotomine fly-borne members are among those that have this capacity. Such a mechanism allows the virus to be maintained without intervening feedings of the arthropod on viremic vertebrates.

Vertebrate hosts of these viruses also play a critical role in their maintenance and amplification. Replication of the virus in the vertebrate must attain high enough titer to serve as a source of virus for the host-feeding arthropod. In addition, the size of the population of susceptible vertebrates must be sufficient to provide relative assurance of contact with the arthropod, and population turnover must be adequate to provide a continual number of susceptible hosts. The vertebrate host becomes another source of amplification in epidemic situations, because one infected vertebrate can serve to infect many arthropods. Except for the Bunyamwera, Simbu, certain of the California, and Nairobi sheep disease serogroup viruses, most of the viruses of the family *Bunyaviridae* are transmitted to small mammals. Among the vertebrate hosts of the viruses of this family are rodents, camels, and birds, attesting to the remarkable diversity and adaptability of the viruses.

The hantaviruses are exceptions to the rule of the family, in that they appear to be transmitted only directly from mammal to mammal. Thus, maintenance and transmission of these viruses occur in the same host. Chronic infection of the vertebrate host, usually rodents, is characterized by urinary and salivary excretion and persistence of virus in noncytolytic, immunocompetent infection. The epidemiology of the hantaviruses is, therefore, limited by the geographic and ecologic distributions of the rodent host. Rodent genetics undoubtedly play a role in the induction of chronic infection, but this is, as yet, not understood.

When humans impinge upon the natural ecosystem of the virus, infection can result. Viruses causing mild febrile illnesses, or no illnesses at all in humans, may go unnoticed. But when epidemics or epizootics of life-threatening proportions occur in agricultural workers or livestock, in forest workers or forest animals of esthetic importance, or in military personnel, it is only with disastrous consequences that such occurrences are ignored. Human social and behavioral patterns impact upon the epidemiology of all the viruses of the family *Bunyaviridae,* so understanding such patterns can assist in controlling the spread of the viruses, or at least in lessening the probability of acquiring them.

Prevention and Control

The basic tenet of controlling any disease is to prevent its occurrence. To control the prevalence of viruses of the family *Bunyaviridae,* it is necessary first to understand the ecology of the disease: persistence in interepidemic periods, mechanisms of amplification of the virus, and mechanisms of transmission to the affected host. For mosquito-borne, tick-borne, and other arthropod-borne viruses, a thorough understanding of the maintenance and amplification cycles of the viruses is the sine qua non of both prevention and control.

First and foremost, contact between the susceptible human or animal and the vector must be reduced. This can be done by either removing the target vertebrate from contact with the maintenance and amplification vectors or by removing the arthropods from contact with the target vertebrate. An alternative is to remove the intermediate vertebrate host from contact with the arthropod vector. As this is more easily said than done, a more realistic target is to reduce contacts between arthropod, intermediate vertebrate host, and susceptible human or other animal hosts. This can be effected by mosquito- or tick-control operations, using larvacide or adulticide techniques, either on a large (spraying of insecticides from airplanes) or a small (spraying of insecticides from backpack units) scale. Public education projects with adequate, on-going publicity and legal means are also often used alternatives to direct intervention. On-going clinical, virologic, serologic, and histologic arthropod and rodent surveillance techniques should be used to ascertain the presence and prevalence of these viruses.

For preventing contact of mosquito, culicoid, and phlebotomine fly vectors with humans, the use of netting and window and door screening is recommended. Removing and subsequently destroying mosquito breeding sites (source reduction), providing piped water, maintaining liquid waste systems, and managing irrigation systems have been successful ways of reducing populations of both maintenance and amplification vectors.

Protection from tick infestations can be effected by personal protection (wearing clothes impregnated with suitable repellents), dipping cattle, sheep, dogs, and other vertebrate hosts, and treating camp sites with acaricides. These measures have all been used with success.

Preventive measures taken against commensal rodents include rodent-proofing habitations and food-storage areas, proper disposal of domestic and public wastes, and using permanent anticoagulant baiting stations to depress rodent populations.

If measures taken to control arthropod vectors and rodent hosts of viruses are not effective or are

not possible, given economic, political, or geographic considerations, vaccines hold great potential for disease prevention. A Rift Valley fever vaccine for use in humans at risk and vaccines for sheep and cattle have been developed. Given that viruses of the family *Bunyaviridae* possess segmented genomes, it may be possible to construct live attenuated vaccines for any of these viruses. The G1 and G2 glycoproteins, appear to be responsible for major virulence characteristics, as well as for inducing neutralizing antibodies. If the middle-sized RNA segment of all *Bunyaviridae* codes for the G1 and G2 glycoproteins, then such live attenuated vaccines can be produced for Rift Valley fever, Crimean-Congo hemorrhagic fever, hemorrhagic fever with renal syndrome, or any other virus affecting humans.

Literature Cited

Ali, A. M. M., and S. Kamel. 1978. Epidemiology of Rift Valley fever in domestic animals in Egypt. J. Egypt. Publ. Hlth. Assoc. **53**:255–263.

Balfour, Jr., H. H., R. A. Siem, H. Bauer, and P. G. Quie. 1973. California arbovirus (La Crosse) infections: clinical and laboratory findings in 66 children with meningoencephalitis. Pediatrics **52**:680–691.

Beaty, B. J., S. W. Hildreth, D. C. Blenden, and J. Casals. 1982. Detection of La Crosse (California encephalitis) virus antigen in mouse skin samples. Am. J. Vet. Res. **43**:684–687.

Bishop, D. H. L., and R. E. Shope. 1979. Bunyaviridae, p. 1–156. *In* H. Fraenkel-Conrat, R. A. Wagner, (ed.), Comprehensive virology, Plenum Press, New York.

Bishop, D. H. L., C. H. Calisher, J. Casals, M. P. Chumakov, S. Ya. Gaidamovich, C. Hannoun, D. K. Lvov, I. D. Marshall, N. Oker-Blom, R. F. Pettersson, J. S. Porterfield, P. K. Russell, R. E. Shope, and E. G. Westaway. 1980. Bunyaviridae, Intervirology **14**:125–143.

Burke, D. S., A. Nisalak, and M. A. Ussery. 1982. Antibody capture immunoassay detection of Japanese encephalitis virus immunoglobulin M and G antibodies in cerebrospinal fluid. J. Clin. Microbiol. **16**:1034–1042.

Burke, D. S., W. Lorsomrudee, C. J. Leake, C. H. Hoke, A. Nisalak, V. Chongswasdi, and T. Laorakpongse. 1985. Fatal outcome in Japanese encephalitis. Am. J. Trop. Med. Hyg. **34**:1203–1209.

Calisher, C. H., and R. E. Bailey. 1981. Serodiagnosis of La Crosse virus infections in humans. J. Clin. Microbiol. **13**:344–350.

Calisher, C. H., T. P. Monath, N. Karabatsos, and D. W. Trent. 1981. Arbovirus subtyping: applications to epidemiologic studies, availability of reagents, and testing services. Am. J. Epidemiol. **114**:619–631.

Calisher, C. H., and W. H. Thompson (ed.). 1983. California serogroup viruses. Prog. Clin. Biol. Res. **123**:1–399.

Calisher, C. H., J. D. Poland, S. B. Calisher, and L. Warmoth. 1985a. Diagnosis of Colorado tick fever virus infection by enzyme immunoassays for immunoglobulin M and G antibodies. J. Clin. Microbiol. **22**:84–88.

Calisher, C. H., O. Meurman, M. Brummer-Korvenkontio, P. E. Halonen, and D. J. Muth. 1985b. Sensitive enzyme immunoassay for detecting immunoglobulin M antibodies to Sindbis virus and further evidence that Pogosta disease is caused by a western equine encephalitis complex virus. J. Clin. Microbiol. **22**:566–571.

Calisher, C. H., C. I. Pretzman, D. J. Muth, M. A. Parsons, and E. D. Peterson. 1986a. Serodiagnosis of La Crosse virus infections in humans by detection of immunoglobulin M class antibodies. J. Clin. Microbiol. **23**:667–671.

Calisher, C. H., A. O. El-Kafrawi, M. I. Mahmud, Al-D. Travassos, A. P. A. da Rosa, C. R. Bartz, M. Brummer-Korvenkontio, S. Haksohusodo, and W. Suharyono. 1986b. Complex-specific immunoglobulin M antibody patterns in humans infected with alphaviruses. J. Clin. Microbiol. **23**:155–159.

Calisher, C. H., V. P. Berardi, D. J. Muth, and E. E. Buff. 1986c. Specificity of immunoglobulin M and G antibody responses in humans infected with eastern and western equine encephalitis viruses: application to rapid serodiagnosis. J. Clin. Microbiol. **23**:369–372.

Casals, J., and L. Whitman. 1960. A new antigenic group of arthropod-borne viruses. The Bunyamwera group. Am. J. Trop. Med. Hyg. **9**:73–77.

Casals, J., and L. Whitman. 1961. Group C. A new serological group of hitherto undescribed arthropod-borne viruses. Immunological studies. Am. J. Trop. Med. Hyg. **10**:250–258.

Casals, J. 1963. New developments in the classification of arthropod-borne animal viruses. Anais Microbiol. **11**:13–34.

Casey, H. L. 1965. Part II. Adaptation of LBCF method to micro technique. Standardized diagnostic complement fixation method and adaptation to micro test. Publ. Hlth. Monogr. **74**:1–34.

Clarke, D. H., and J. Casals. 1958. Techniques for hemagglutination and hemagglutination-inhibition wth arthropod-borne viruses. Am. J. Trop. Med. Hyg. **7**:561–573.

Daubney, R., J. R. Hudson, and P. C. Garnham. 1931. Enzootic hepatitis or Rift Valley fever: an undescribed disease of sheep, cattle, and man from East Africa. J. Pathol. Bacteriol. **34**:545–579.

Gajdusek, D. C. Acute infectious hemorrhagic fevers and mycotoxicoses in the Union of Soviet Socialist Republics. Med. and Sci. Publ. No. 2, Army Med. Serv. Grad. Sch.; Walter Reed Army Medical Center, Washington, D. C.

Gardner, P. S., and J. McQuillin. 1980. Applications of immunofluorescence (2nd ed.), Butterworth, London.

Gonzalez-Scarano, F., R. E. Shope, C. H. Calisher, and N. Nathanson. 1983. Monoclonal antibodies against the G1 and nucleocapsid proteins of LaCrosse and Tahyna viruses, p. 145–156. *In* C. H. Calisher and W. H. Thompson (ed.), California serogroup viruses. Prog. Clin. Biol. Res., Vol. 123, Alan R. Liss, Inc., New York.

Halonen, P. E., H. Sarkkinen, P. Arstila, E. Hjertsson, and E. Torfason. 1980. Four-layer radioimmunoassay for detection of adenovirus in stool. J. Clin. Microbiol. **11**:614–617.

Heinz, F. X., M. Roggendorf, H. Hormann, C. Kunz, and F. Deinhardt. 1981. Comparison of two different enzyme immunoassays for detection of immunoglobulin M antibodies against tick-borne encephalitis virus in serum and cerebrospinal fluid. J. Clin. Microbiol. **14**:141–146.

Hildreth, S. W., B. J. Beaty, J. M. Meegan, C. L. Frazier, and R. E. Shope. 1982. Detection of La Crosse arbovirus antigen in mosquito pools: application of chromogenic and fluorogenic enzyme immunoassay systems. J. Clin. Microbiol. **15**:879–884.

Hoogstraal, H. 1979. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J. Med. Entomol. **15**:307–417.

Hoogstraal, H., J. M. Meegan, G. M. Khalil, and F. K.

Adham, 1979. The Rift Valley fever epizootic in Egypt 1977–1978. 2. Ecological and entomological studies. Trans. Royal Soc. Trop. Med. Hyg. **73:**624–629.

Hunt, A. R., and C. H. Calisher. 1979. Relationships of Bunyamwera group viruses by neutralization. Am. J. Trop. Med. Hyg. **28:**740–749.

Hyypia, T., P. Stalhandske, R. Väinionpää, and U. Pettersson. 1984. Detection of enteroviruses by spot hybridization. J. Clin. Microbiol. **19:**436–438.

Jamnback, T. L., B. J. Beaty, S. W. Hildreth, K. L. Brown, and C. B. Gunderson. 1982. Capture immunoglobulin M system for rapid diagnosis of La Crosse (California encephalitis) virus infections. J. Clin. Microbiol. **16:**577–580.

Kalfayan, B. 1983. Pathology of La Crosse virus infections in humans, p. 179–186. *In* C. H. Calisher, W. H. Thompson, (ed.), California serogroup viruses. Prog. Clin. Biol. Res., Vol. 123, Alan R. Liss, Inc., New York.

Karabatsos, N., and R. E. Shope. 1979. Cross-reactive and type-specific complement-fixing structures of Oriboca virions. J. Med. Virol. **3:**167–176.

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

Karmysheva, V. Ya, E. V. Leshchinskaya, A. M. Butenko, A. P. Savinov, and A. F. Gusarev. 1973. Results of some laboratory and clinical–morphological investigations of Crimean hemorrhagic fever. Arkh. Patol. **35:**17–22.

Klimas, R. A., W. H. Thompson, C. H. Calisher, G. G. Clark, P. R. Grimstad, and D. H. L. Bishop. 1981. Genotypic varieties of La Crosse virus isolated from different geographic regions of the continental United States and evidence for a naturally occurring intertypic recombinant La Crosse virus. Am. J. Epidemiol. **114:**112–131.

Kulagin, S. M., N. I. Fedorova, and E. S. Ketiladze. 1962. Laboratornaia vspyshka gemorragicheskoi likhoradki s pochechnym sindromom (kilinko-epidemiologicheskaia charakteristika) [A laboratory outbreak of hemorrhagic fever with renal syndrome (clinical-epidemiological characteristics)]. Zh. Mikrobiol. Epidemiol. Immunobiol. **33:**121–126.

Kurogi, H., Y. Inaba, E. Takahashi, K. Sato, T. Omori, Y. Miura, Y. Goto, Y. Fujiwara, Y. Hatano, K. Kodama, S. Fukuyama, N. Sasaki, and M. Matumoto. 1976. Epizootic congenital arthrogryposis-hydranencephaly syndrome in cattle: isolation of Akabane virus from affected fetuses. Arch. Virol. **51:**67–74.

Lindsey, H. S., C. H. Calisher, and J. H. Mathews. 1976. Serum dilution neutralization test for California group virus identification and serology. J. Clin. Microbiol. **4:**503–510.

Meegan, J. M. 1979. The Rift Valley fever epizootic in Egypt 1977–1978. 1. Description of the epizootic and virological studies. Trans. Royal Soc. Trop. Med. Hyg. **73:**618–623.

Monath, T. P., R. B. Craven, D. J. Muth, C. J. Trautt, C. H. Calisher, and S. A. Fitzgerald. 1980. Limitations of the complement-fixation test for distinguishing naturally acquired from vaccine-induced yellow fever infection in flavivirus-hyperendemic areas. Am. J. Trop. Med. Hyg. **29:**624–634.

Monath, T. P., R. R. Nystrom, R. E. Bailey, C. H. Calisher, and D. J. Muth. 1984. Immunoglobulin M antibody capture enzyme-linked immunosorbent assay for diagnosis of St. Louis encephalitis. J. Clin. Microbiol. **20:**784–790.

Montgomery, E. 1917. On a tick-borne gastroenteritis of sheep and goats occurring in British East Africa. J. Comp. Pathol. Therap. **30:**28–57.

Murphy, F. A., A. K. Harrison, and S. G. Whitfield. 1973. Bunyaviridae: morphologic and morphogenetic similarities of Bunyamwera supergroup viruses and several other arthropod-borne viruses. Intervirology **1:**297–316.

Niklasson, B., M. Grandien, C. J. Peters, and T. P. Gargan II. 1983. Detection of Rift Valley fever virus antigen by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **17:**1026–1031.

Niklasson, B., C. J. Peters, M. Grandien, and O. Wood. 1984. Detection of human immunoglobulins G and M antibodies to Rift Valley fever virus by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **19:**225–229.

Obijeski, J. F., and F. A. Murphy. 1977. Bunyaviridae: recent biochemical developments. J. Gen. Virol. **37:**1–14.

Oliver, J., and M. MacDowell. 1957. The renal lesion in epidemic hemorrhagic fever. J. Clin. Invest. **36:**99–222.

Rosen, L. 1981. The use of *Toxorhynchites* mosquitoes to detect and propagate dengue and other arboviruses. Am. J. Trop. Med. Hyg. **30:**177–183.

Schmaljohn, C. S., and J. M. Dalrymple. 1983. Analysis of Hantaan virus RNA: evidence for a new genus of Bunyaviridae. Virology **131:**482–491.

Shope, R. E., and O. R. Causey. 1962. Further studies on the serological relationships of group C arthropod-borne viruses and the application of these relationships to rapid identification of types. Am. J. Trop. Med. Hyg. **11:**283–290.

Shope, R. E. 1985. Bunyaviruses, p. 1055–1082. *In* B. N. Fields (ed.), Virology. Raven Press, New York.

Smithburn, K. C., A. J. Haddow, and A. F. Mahaffy. 1946. Neurotropic virus isolated from *Aedes* mosquitoes caught in Semliki Forest. Am. J. Trop. Med. Hyg. **26:**189–208.

Sudia, W. D., V. F. Newhouse, C. H. Calisher, and R. W. Chamberlain. 1971. California group arboviruses: isolation from mosquitoes in North America. Mosq. News. **31:**576–600.

Taussig, S. 1905. Die Hundskrankheit, endemischer Magankatarrh in der Herzegowina. Wien Klin. Wochenschr. **18:**129–136, 163–169.

Theiler, M. 1957. Action of sodium desoxycholate on arthropod-borne viruses. Proc. Soc. Exper. Biol. Med. **96:**380–382.

Theiler, M., and W. G. Downs. (compilers and editors). 1973. The arthropod-borne viruses of vertebrates. Yale University Press, New Haven, CT.

Tikasingh, E. S., L. Spence, and W. G. Downs. 1966. The use of adjuvant and sarcoma 180 cells in the production of mouse hyperimmune ascitic fluids to arboviruses. Am. J. Trop. Med. Hyg. **15:**219–226.

Umenai, T., H. W. Lee, P. W. Lee, T. Saito, T. Toyoda, M. Hongo, K. Hoshinaga, T. Nobunaga, T. Horiuchi, and N. Ishida. 1979. Koren hemorrhagic fever in staff in an animal laboratory. Lancet **1:**1314–1316.

Ushijima, H., C. M. Clerx-van Haaster, and D. H. L. Bishop. 1981. Analyses of the Patois group bunyaviruses: evidence for naturally occurring recombinant bunyaviruses and existence of viral coded nonstructural proteins induced in bunyavirus infected cells. Virology **110:**318–332.

von Bonsdorff, C. H., P. Saikku, and N. Oker-Blom. 1969. The inner structure of Uukuniemi and two Bunyamwere supergroup arboviruses. Virology **39:**342–344.

Watts, D. M., W. H. Thompson, T. M. Yuill, G. R. DeFoliart, and R. P. Hanson. 1974. Overwintering of La

Crosse virus in *Aedes triseriatus*. Am. J. Trop. Med. Hyg. **23**:694–700.

Whitman, L., and J. Casals. 1961. The Guama group: a new serological group of hitherto undescribed viruses. Immunological studies. Am. J. Trop. Med. Hyg. **10**:259–263.

Whitman, L., and R. E. Shope. 1962. The California complex of arthropod-borne viruses and its relationship to the Bunyamwera group through Guaroa virus. Am. J. Trop. Med. Hyg. **11**:691–696.

Woodall, J. P. 1979. Transmission of Group C arboviruses (Bunyaviridae), p. 123–128. *In* E. Kurstak, (ed.), Academic Press, New York. Proc. Second Intl. Symp. on Arctic Arboviruses.