

ARBOVIRUSES OF PUBLIC HEALTH IMPORTANCE IN PAKISTAN

Pages with reference to book, From 16 To 26

C.C. Hayes, M.I. Burney (Pakistan Medical Research Center, University of Maryland, 6-Birdwood Road, Lahore.)

Introduction

One of the main problems in developing countries is to define in quantitative terms the major health problems so that preventative and therapeutic measures can be applied. In Pakistan, as in many similar countries, acute febrile diseases account for an enormous amount of morbidity and mortality and also can severely affect the economy. Although in Pakistan some diseases such as malaria have long been recognized as well-known causes of morbidity, mortality and economic loss, other etiological agents, such as arboviruses, which may be responsible for extensive epidemics of febrile illness often go largely unrecognized and may be even frequently mis-diagnosed on a clinical basis as malaria, enteric fever, or some times labelled as P.U.O. However, proper recognition of the etiological agents responsible for a febrile disease outbreak is critical to the application of proper control measures.

Background

An arbovirus is an agent that is maintained in nature primarily "through biological transmission between susceptible vertebrate hosts by hemato-phagous (blood-feeding) arthropods" (WHO Scientific Group, 1969). Two important processes are implied in this definition. The first is that in general, before an arthropod vector can become infected it has to feed on a vertebrate host which has circulating virus in its blood. Secondly, the virus ingested by the vector must undergo a period of replication in the arthropod before transmission to another host can occur. For example, when a vector feeds on a viremic host the virus is deposited in the vector's midgut where infection of the cells lining the midgut occurs. After a period of replication, virus particles are shed into the body cavity where various tissues become infected. Eventually, the virus reaches the salivary glands, and following a phase of virus replication in these organs, the arthropod can transmit virus to a susceptible vertebrate. This period of virus replication in the vector from the time the virus is initially ingested with a blood meal until the ability to transmit is acquired is termed the extrinsic incubation period and is dependent, within limits, directly on the ambient temperature (Chamberlain and Sudia, 1961; Murphy et al., 1975).

Many different viruses fit the above criteria for definition as an arbovirus and numerous species of vertebrates and arthropods have been implicated as hosts and vectors for these agents. Arthropod vectors include ticks (both hard and soft), biting flies (mosquitoes, culicoides, and phlebotomine flies), bed bugs and possible mites. A variety of mammals such as man, lower primates, livestock, rodents, marsupials, and bats, many species of avians, and even some reptiles and amphibians have been incriminated as vertebrate hosts (Theiler and Downs, 1973). However, for any particular arbovirus the biological requirements for maintenance in nature in terms of vectors and hosts are usually very specific (Varma, 1972). Only one or a few of the many species of vertebrates and arthropods available in a given area are involved in the life cycle of a particular virus.

From the perspective of human involvement, the simplest life cycle for an arbovirus occurs when the virus can be maintained in a person to person transmission cycle by a vector. This situation occurs with urban yellow fever and dengue. In this cycle, the factors limiting intensity of transmission are the immunity rate in the human population and the abundance of arthropod vectors. A further complication making the control of this type of virus extremely difficult occurs when a sylvan transmission cycle involving control of this type of virus extremely difficult occurs when a sylvan transmission cycle

involving a reservoir host and a different species of vector also is present (Taylor, 1951).

More typically man become involved in the transmission chain of an arbovirus as a peripheral or dead-end host. In this situation, the virus is maintained in a cycle involving wild animals such as birds and is not capable of being sustained by a man-vector-man cycle. Man becomes involved only passively and does not contribute to the perpetuation of virus in nature since he fails to develop viremia sufficient enough to infect vectors. Major factors limiting infection in the human population are the intensity of transmission occurring in the wild animal cycle and the anthropophilic behaviour of the primary or secondary arthropod vectors involved. Domestic animals also can be involved in this type of cycle and may or may not contribute to a build-up of virus depending on the particular arbovirus involved. For example, with Japanese encephalitis and Venezuelan equine encephalitis, swine and horse, respectively, play an important role in amplifying the level of virus in nature prior to infection spreading to the human population (Scherer et al., 1959; Sudia and New House, 1975). With other viruses such as western equine encephalomyelitis or eastern equine encephalomyelitis, although domestic animals may be infected frequently and develop severe illness, they serve as dead-end hosts only (Reeves and Hammon, 1962; Kissling et al., 1954).

Although the above transmission cycles represent the important maintenance mechanisms of most arboviruses as far as the involvement of man is concerned, other secondary cycles with different vectors and hosts also may contribute to the survival of a virus, particularly during periods of adverse climatic conditions when active transmission may be interrupted in the main cycle. Experimental and ecological evidence indicates that some arboviruses may even be transmitted directly between vertebrate hosts without the intervention of a vector by such mechanisms as direct aerosol transmission or vertical transmission of virus from mother to offspring. The aerosol route of transmission has been demonstrated with Venezuelan equine encephalitis and West Nile virus under laboratory conditions (Slepishkin, 1959; Nir, 1959). Snakes infected with western equine encephalomyelitis have given birth to infected progeny (Gebhardt et al., 1964). Also in some instance arboviruses may be maintained for several generations solely in the vector by transovarial transmission. This probably is not an uncommon occurrence with certain tick transmitted and sandfly transmitted viruses (Burdorfer and Varma, 1967) and recently has been documented with several mosquito transmitted viruses (Aitken et al., 1979; Rosen et al., 1978; Watts et al., 1973).

Over 80 different arboviruses have been associated with natural infection of man (Intern. Cat. Arboviruses, 1975). Although the majority of infections probably result in inapparent or subclinical infections, a variety of clinical manifestations ranging from a mild febrile response to a highly fatal illness of short duration have been associated with arboviruses. A number of different arboviruses produce similar signs and symptoms such as a biphasic fever curve.

The first febrile period usually is associated with a leucopenia while the second febrile phase may be characterized by a leucocytosis, and there may or may not be an afebrile interval between the fever peaks. On the other hand, the same virus can cause a variety of disease syndromes in different individuals. Some of the more severe pathological manifestations of human arbovirus infections include a hemorrhagic syndrome associated with dengue, yellow fever, Omsk hemorrhagic fever; encephalitis associated with eastern equine encephalitis, Saint Louis encephalitis, Japanese encephalitis, West Nile and other viruses; hepatitis associated with yellow fever and Kyas-anur Forest disease; and the shock syndrome associated with dengue virus infection (Downs, 1976).

Because of the variety of overlapping disease syndromes elicited by different arboviruses in the human host, differential diagnosis is very difficult on clinical features alone except during epidemic conditions. Determination of the arbovirus responsible for a particular illness or disease outbreak depends on isolation and identification of the virus or demonstration of a significant rise in serum antibody titer (4 fold) between the acute and convalescent phases of illness.

Identification of an arbovirus is based on its antigenic relationship to other viruses as determined primarily in complement fixation hemagglutination-inhibition and neutralization tests. When published

in 1975, the International Catalogue of Arboviruses contained 359 registered viruses divided into 47 different antigenic groups with 1 to 57 members each. All of the viruses in a single group are interrelated antigenically, but unrelated to any member of the other groups. In addition, 76 of the registered viruses were ungrouped. Currently over 420 viruses are registered.

Within the last few years a number of arboviruses have been incorporated into a universal scheme of virus classification based on properties such as nucleic acid type, mode of replication of the nucleic acid, and virion morphology and morphogenesis determined in electron microscopic studies. The arboviruses studied to date have been placed in 8 different tentative family groups on the basis of such physiochemical properties (Fenner, 1974; Murphy, 1975).

Arboviruses and human diseases attributable to infection with arboviruses occur in every major zoogeographic region of the world. A total of 13 different arboviruses have been reported from Pakistan (Intern. Cat. Arboviruses, 1975; Jupp and McIntosh, 1967). This review will be concerned with 11 of these viruses, several of which are well-recognized human pathogens. Others infect man but have not been associated with any illness to date. The two viruses which will not be included, Bluetongue and African horse sickness, cause serious disease in ovines and equines, respectively, but are not known to infect man.

Human Pathogenic Arboviruses in Pakistan

West Nile Fever. West Nile (WN) virus, a mosquito-transmitted flavivirus, is distributed from Southern Europe, throughout Africa, the Middle East, Central and South Asia. The ecology of WN virus has been studied in detail only in Egypt and South Africa (Taylor et al., 1956; Anderson, 1941; Jupp and McIntosh, 1967; McIntosh et al., 1967) and in both countries, the virus was shown to be maintained in a mosquito-wild bird-mosquito cycle during the transmission season. *Culex univitalus* was implicated as the main vector in both areas. Apparently most domestic animals are dead-end hosts for this virus.

Whether or not man-mosquito-man transmission can occur has not been established; however, viremia studies indicate that some patients probably become infective from mosquitoes (Southam and Moore, 1954).

Sero-surveys and virus isolation studies have shown that man becomes infected with WN virus throughout most of its geographic range (Smithburn, 1952; Smithburn and Jacobs, 1942; Goldblum et al., 1956; Kokernot et al., 1956; Burney, 1966; Rao, 1975; Saidi et al., 1976) and clinical studies have shown that disease frequently is associated with infection. Taylor et al (1956) studied the occurrence of WN infection in children living in highly endemic area in Egypt. Their overall impression of the clinical course of illness was "fever of rapid onset, averaging 38.5°C, accompanied by gastrointestinal disturbances, malaise, profuse sweating, a fine papular rash, moderate enlargement of cervical, axillary and inguinal lymph nodes, and occasionally congestion of the eyes and throat."

Illness attributable to infection with WN virus during epidemics has been studied in Israel and South Africa (Goldblum et al., 1954; Marberg et al., 1956; McIntosh et al., 1976). In the Israeli studies, adults and adolescents were reported frequently to develop dengue-like syndrome with fever, myalgia, headache, lymphadenopathy and rash. Encephalitis has been observed as a significant complication of WN infection in Israel (Pruzanski and Altman, 1962). In one outbreak in an elderly population, 49 individuals became ill, and 12 developed severe neurologic signs. Four of these patients died (Spigland et al., 1958). In support of these observations, induced infections of WN virus in man as a treatment of advanced cancer produced signs of diffuse encephalitis in 11% of the patients (Southam and Moore, 1954). Myocarditis also has been reported as a complication of WN virus infection in Israel (Albagli and Chaimoff, 1959). In a recent epidemic of WN reported from the Cape Province area of South Africa, the clinical symptoms reported were similar to those seen in the Israeli outbreaks. Enlarged, tender liver, orchitis and encephalitis also were reported. Convalescence was frequently long with continued listlessness and weakness. In agreement with the Egyptian studies, illness in younger children usually was mild.

Based on these clinical studies, the severity of illness associated with WN virus infection appears to be

age related. In highly endemic areas, such as those in Egypt where 90% of the adult population possessed antibodies to WN virus, infection occurs early in life and the resulting illness is usually mild and may even be largely subclinical. In the epidemic situation, however, expression of disease can be significant with slow recovery, the occurrence of serious sequelae and death. In addition, outbreaks of WN fever can have a devastating short-term impact on the public health and in turn the economic productivity of a community. For example, in one of the early epidemics reported from Israel, over 60% of the population of the affected area developed overt disease (Clarke and Casals, 1965). Likewise, in the epidemic reported from South Africa in 1974, 60% of the population of one of the study areas, Uppington, were identified as having been infected with WN virus. In terms of actual population numbers, this meant that about 18,000 people in the area were infected out of a total of 30,000. As in Israel, occurrence of disease in infected individuals was significant (Mcintosh et al., 1976).

In Pakistan, WN virus has been isolated from man and from mosquitoes (Table I). From 1961-1965, 5 strains of WN virus were obtained from *Cx. tritaeniorhynchus* "complex" (*Cx. tritaeniorhynchus* and *Cx. pseudovishni*) mosquitoes-collected from Lahore and surrounding areas of the Punjab (Barnett, 1967; PMRC Ann. Rep., 1963-69). All of these isolations were made from mosquitoes collected during the months of August and September. In the Rawalpindi area during 1963-1964, Burney and Munir (1966) reported the isolation of 2 strains of WN virus from *Cules pipiens fatigans*. One isolate was made in June and the other in October. Virus isolations from man were made during the course of a 1965 study on fevers of unknown origin conducted in hospitals located in Lahore (PMRC Ann. Rep., 1963-69). From 144 plasmas collected during the first week of illness from patients with undiagnosed short-term fever, 5 recoveries of NW virus were made. In addition, 2 more cases were diagnosed by serological conversion studies on acute and convalescent serum samples collected from 136 patients. Strains of WN virus also were recovered from 2 of 178 patients examined during the course of a study on levers of unknown origin in the Rawalpindi area (Burney and Munir, 1966). All of the human isolates were obtained between the months of May to September. The clinical course of disease in the WN fever from these 2 studies was characterized by fever, severe headache, retrobulbar pain, myalgia, lymph-adenopathy and leucopenia. Rash was not a prominent clinical feature; although it was present in some of the Lahore patients.

Several sero-surveys conducted on selected population groups resident in and around Lahore from 1963-1977 have revealed an overall antibody positive rate ranging from about 40-50% for WN virus (PMRC Ann. Rep., 1963-69; Hayes, unpubl. data). A study in the Rawalpindi area found 15/54 (27.8%) human serum samples-positive for WN virus antibody (Burney and Munir, 1966). But in a 1963 survey of 93 people living in the northern mountains of Pakistan, no WN virus antibody positive samples were detected (PMRC Ann. Rep., 1963-69). More recent surveys conducted in the Changa Manga National Forest and in villages around the city of Chiniot found 45.9% and 26.8% of the population sample positive for antibodies to WN virus, respectively (Hayes, unpub. data).

Congo-Crimean Hemorrhagic Fever Virus: Congo-Crimean Fever (C-CHF) is caused by a tick transmitted virus in the Bunyaviridae family. This agent is probably maintained in nature by a combination of trans-stadial/transovarial transmission within tick populations and by horizontal transmission between tick vectors and various domestic and wild animal species (Hoogstraal, 1979). The geographic range of C-CHF virus has been shown to extend over 3 zoogeographic zones, the Palearctic, Oriental and Ethiopian, and has been reported to occur in the following countries: Senegal, Nigeria, Uganda, Ethiopia, Kenya, Egypt, France, Greece, Bulgaria, Hungary, U.S.S.R., Afghanistan, Iran, Pakistan and India (Intern. Cat. Arboviruses, 1975; Saidi et al., 1975; Horvath, 1976; Shanmugam et al., 1976; Hoogstraal, 1979; Darwish et al., 1977; Papado-poules and Koptopoulos, 1978; Wood et al., 1978).

C-CHF virus has been isolated from at least 25 different species or subspecies of ticks primarily in the genus *Hyalomma* and also including *Boophilus*, *Rhipicephalus*, *Dermacentor*, *Amblyomma* *Ixodes*,

Haemaphysalis and Argas species (Hoogstraal, 1979). In Southern Europe and South-western U.S.S.R., *Yyalomma Marginatum marginatum* (*H. plumbeum plumbeum* in the Russian literature) has been implicated as the primary vector; although *H. anatolicunt an-atolicum* also has been shown to be an important vector in Tadzhik and several areas in U.S.S.R. (Hoogstraal, 1977). In Africa, detailed ecological/ epidemiological studies on the tick vectors of C-CHF virus have not been conducted, but virus isolation data indicate that *Hyalomma* species are also important vectors in this region. In addition, in Nigeria between 1964 and 1968, 21 strains of C-CHF virus were isolated from the common one-host cattle tick, *Boophilus decoloratus* (Causey et al., 1970)

Both domestic and wild animals probably are involved in the maintenance cycle of C-CHF virus. Only a few virus isolations have been reported from domestic animals (5 from cows and 2 from goats in Africa) (Kemp et al., 1973); however, sero-epidemiological surveys have shown infection of large domestic mammals to occur in many different geographic areas (Darwish et al., 1977; Pak, 1970; Saidi et al., 1975; Shanmugam et al., 1976). Antibodies to C-CHF virus have been found in cows, buffaloes, horses, donkeys, sheep, goats and camels. These large domestic mammals also serve as the major hosts for the adult stage of most of the tick species incriminated as vectors of C-CHF virus (Hoogstraal, 1979).

A hedgehog (*Atelerix albiontries*) captured in Nigeria is the only wild animal from which C-CHF virus has been isolated (Causey et al., 1970). Evidence of past infection, however has been demonstrated in hares bats, wild rodents and a Horsfield terrapin (Casals et al., 1970; Pak, 1970; Saidi et al., 1975; Shanmugam et al., 1976). Antibodies to C-CHF virus have not been found in wild birds, even though the immature stages of many of the *Hyalomma* vector species often feed on birds. A strain of C-CHF virus has been isolated from ticks collected directly off birds (Kondratenko et al., 1974). Although wild birds apparently don't serve as intermediate hosts for C-CHF virus, they may be important in the dissemination of virus over wide geographic areas via transport of infected tick during migration flights (Hoogstraal, 1973).

Virus isolation and sero-epidemiological surveys have shown human infection with C-CHF virus to occur in Zaire (formerly Belgian Congo), Uganda, Kenya, Egypt, Bulgaria, Hungary, U.S.S.R., Iran, Pakistan and India (Hoogstraal, 1979). Detailed epidemiological studies have been conducted in foci of enzootic C-CHF virus circulation in Bulgaria and in several regions of the U.S.S.R. (Crimea, Astrakhan, Rostov, Stavropol, Turkmenia, Uzbekistan, and Kazakhstan). Several large epidemics involving hundreds of cases and many deaths have occurred in these areas since the first well-documented outbreak in the Crimea during 1944-45. As summarized by Hoogstraal (1977), enzootic foci of C-CHF virus occur mainly in "...steppe, savanna, semidesert and foothill biotopes where one or two *Hyalomma* species are the very common or predominant ticks parasitizing domestic and wild animals." A crucial factor involved in the outbreak of human disease occurring in such enzootic foci is the presence of large population of *Hyalomma* tick vectors which readily attack and feed on man. In fact, epidemics on some areas have been associated with changes in agricultural practices which favour an increase in density of the tick population. The movement of new personnel into previously uninhabited or sparsely inhabited areas which serve an enzootic foci for C-CHF circulation has also resulted in disease outbreak. A strong occupational risk has been associated with the occurrence of disease during epidemics. Agricultural workers employed in such operations as hay cutting or handling domestic animals such as cattle or sheep show the highest incidence of infection (Perelatov, 1966). Clinically, C-CHF is characterized by a sudden onset of fever (39-41°C) with frequent chills, severe headache, muscle and joint pains and malaise. Fever lasts for 7-9 days, and may be biphasic. Many patients also suffer from nausea, epigastric pains and vomiting. Both leucopenia and thrombocytopenia are common, and hepatomegaly is frequently seen. Hyperemia of the face, neck and upper throat, injected sclera, and pronounced conjunctivities are seen at the beginning or early in the disease. Hemorrhaging usually begins on day 2-6 following the onset of disease symptoms and may vary considerably in character and severity. Bleeding is manifested as hemorrhages of the skin, mucous

membranes, and different organs including stomach, uterus, intestines and lung. Neurological signs such as nuchal rigidity, excitation and coma are seen in 10-25% of the patients. The case fatality rate has been reported to vary from 9 to 40% in different epidemics. Death usually results from shock, neurological complications, or pulmonary hemorrhages. Recovery is often slow and may be characterized by severe headache, continued weakness, insomnia, irritability, and decrease in memory, sight or hearing. In some outbreaks, many patients have remained disabled for 1-2 months and some were not able to return to full work capacity for 1-2 years (Grashchenkov, 1945; Casals et al., 1966; Brumshtein and Leshchinskava, 1968; Lazarev et al., 1974).

Subclinical infections with C-CHF virus apparently are rare in human populations residing in enzootic foci. In a survey of 823 healthy donors from foci in Astrakhan, Rostov and Bulgaria, only 1 serum was found positive by complement-fixation test, and in the Southern Tadzhikistan foci, only 5/2506 inhabitants sampled possessed antibodies to C-CHF virus (Casals et al., 1970; Pak, 1970).

The results of sero-surveys in Hungary, Iran and India, where C-CHF disease in man has not been reported, revealed a low antibody prevalence rate in some populations sampled. In Hungary, a positive antibody rate of 2.9% was found among 587 persons whose work involved close contact with animals, and, in Iran, 48/351, (13%) human serum samples collected from apparently healthy donors, mostly adults living in rural areas in the Northwest, possessed antibodies to the C-CHF virus (Horvath, 1976; Saidi et al., 1975). In India, out of 643 human samples collected mainly from hospitals and medical colleges throughout the country, only 9 (1.4% antibody positive sera were found; although 7 of the positive sera apparently came from a sample of 205 human bloods collected in one area, Trivandrum Kerala (Shanmugam et al., 1976).

In Pakistan, C-CHF virus has been isolated from ticks and from man (Table I). Both of the tick isolates were made in 1965 from specimens collected off cattle in the Changa Manga Forest, which is located about 45 miles southwest of Lahore. One isolate was obtained from a mixed pool of *Boophilus microplus* and *Hyalomma anatolicum anatolicum*, and the other isolate was obtained from a pool of only *H. a. anatolicum* (Begum et al., 1970a).

The first outbreak of human disease occurred in January, 1976, when a nomad, putting up in Murree Hills near Simly Dam, 30 Km from Rawalpindi, was admitted to the Central Government Hospital, Rawalpindi in a precarious condition. He was diagnosed as a case of bleeding peptic ulcer and a surgical operation was performed. This led to a nosocomial outbreak. In all there were 13 cases; six secondary and six tertiary. There were four deaths including the index cases. Virus was isolated from six cases.

Another outbreak occurred in Quetta, Baluchistan in May, 1976. There were seven specimens brought to the laboratories for virus studies and four isolations of virus were made from human blood.

Cases of viral haemorrhagic fever were reported from the district of Muzaffarabad, Azad Kashmir in 1977, however, no virus could be isolated but there was serological evidence of the infection due to C-CHF.

In July, 1978 one case was admitted to Central Government Hospital, Rawalpindi, with haemorrhages and fever and C-CHF virus was isolated.

Seroepidemiological studies conducted in Changa Manga National Forest during 1978-79 did not reveal the presence of precipitating or complement-fixing antibodies to C-CHF virus in the wild bird, rodent or domestic animal populations. Three human sera from the 1978-79 survey and 2 samples from a 1968 survey conducted in Changa Manga National Forest had low antibody titers (1:4) in the complement-fixation test to C-CHF virus (Hayes, unpubl. data). Complement-fixing antibodies in low titer 1:8 were detected in two rodent sera trapped in Dadu district area (Burney, unpubl. data).

Sandfly Fever: Currently 25 viruses from both the Old and New World are included in the sandfly fever group of arboviruses (Intern. Cat. Arboviruses, 1975; Kissling et al., 1954). Three of these viruses, Naples sandfly fever (NSF), Sicilian sandfly fever (SSF) and Karimabad (KAR) have been isolated in Pakistan (Table I). A fourth, Salehabad (SAL), has been implicated by serological evidence

to occur in Pakistan (Barnett and Suyemoto, 1961; PMRC Annual Report, 1963-69). NSF and SSF, both of which cause clinical disease in man, also have been reported from Italy, Egypt, Iran and India (Tesh et al., 1975). KAR and SAL have been isolated from sandflies collected in Iran (Tesh et al., 1977).

The sandfly vectors of these viruses are small, dullcoloured flies that have a mouth-like appearance (Diptera: Psychodidae). Sandflies do not breed in water, but lay their eggs in cracks and crevices in homes and in the soil that are rich in organic matter. Like mosquitoes only the females take blood meals. Vertical transmission from parent to progeny is probably an important mechanism for perpetuation of these viruses, and man may serve only as a dead-end host in the transmission cycle (Tesh and Chaniotis, 1975). Recent studies in Iran have indicated that the gerbil, *Rhombomys opimus*, may be serving as vertebrate reservoir for certain sandfly fever viruses in areas away from human dwellings (Saidi et al., 1977).

Infection with SSF and NSF causes a 3-4 day fever that may exceed 104°F, but death seldom if ever results. In volunteer studies with SSF, other frequent symptoms besides fever included headache, myalgia, anorexia, and low back pain; less commonly, retro-orbital pain, photophobia, chills, nausea and vomiting were recorded. A marked leucopenia with persistent neutropenia continuing beyond the period of clinical illness was characteristic (Bartelloni and Tesh, 1976). In endemic areas, most clinical infection probably occurs in the younger age groups and does not present a serious public health problem except under circumstances involving the movement of a large non-immune population into such an area.

Clinically, sandfly fever has been recognized in the Peshawar-Khyber pass area of Pakistan from many years (Young et al., 1926; Anderson, 1941; however, the first, definitive isolation of sandfly fever viruses were made from the sera of febrile patients and from sandflies collected during a 1959 survey (PMRC Ann. Rep., 1963-69; Barnett and Suyemoto, 1961). The severity and frequency of symptoms recorded in Pakistan appear to be similar to those reported in other studies (Bartelloni and Tesh, 1976; Anderson, 1941).

Dengue viruses: Dengue (DEN) like WN is a mosquito-transmitted flavivirus. Currently, 4 types of DEN viruses are recognized (types 1, 2, 3) and all of which occur in the tropical areas of Asia and the Western Pacific. Dengue types 1, 2 and 3 also have reported from the New World; however, distribution of these viruses in Africa remains poorly defined (Theiler and Downs, 1973). The main vector of the DEN viruses throughout their range is *Aedes aegypti*. *Ae. aegypti* is a semi-domestic mosquito that breeds extensively in artificial containers—such as discarded cans, jars and old automobile tires, but it also can be found breeding in natural sites such as tree holes (Gould et al., 1968). *Ae. aegypti* is anthropophilic, and most DEN virus transmission occurs in an urban man-mosquito-man cycle. Other mosquito species such as *Ae. albopictus* and members of the *Ae. Scutellaris* complex also have been implicated as vectors of DEN (Jumali et al., 1979; U.S. Public Health Service, 1977).

Clinically, 2 forms of DEN illness are recognized. The classical or normal DEN usually produces an acute febrile disease with symptoms such as headache, myalgia, malaise, anorexia and fatigue. A maculopapular rash is often present 3-4 days after onset of fever, appearing initially on the trunk and spreading to the face and limbs. Leucopenia and lymphadenopathy also are common signs. The expression of this disease syndrome is age dependent, however, with infants and young children usually experiencing a milder febrile response or subclinical infection. Classical DEN fever usually is a self-limiting illness with a low case-fatality rate; although convalescence may be prolonged over several weeks (WHO Tech. Adv. Comm., 1975).

A more serious form of disease associated with DEN virus infections, dengue hemorrhagic fever (DHF), frequently has been recorded during epidemics in Southeast Asia and the Western Pacific areas over the past 2 decades. DHF is an acute febrile disease characterized by hemorrhagic manifestations such as positive tourniquet test, petechiae, epistaxis and hema-temesis (Eram et al., 1979). Unlike the classical dengue fever, this illness is seen most frequently in children. Patients with DHF have a tend-

ency to develop hypovolemic shock termed dengue shock syndrome (DSS) which may be fatal. The occurrence of thrombocytopenia ($100,000/\text{mm}^3$) and haemoconcentration (20%-increase in haematocrit) are useful laboratory markers for the differential diagnosis of DHF/ DSS from other infections (WHO Tech. Adv. Comm., 1975).

The status of DEN in Pakistan is not well defined. The potential for the disease to exist endemically or to spread epidemically in the country is probably limited geographically by the distribution of *Ae. aegypti*. According to Aslam-khan (personal communication), *Ae. aegypti* has been collected in Sind Province from Karachi and Larkana, in the North West Frontier Province from Peshawar and Dera Ismail Khan, and in Punjab from Attock. This species also had been reported from Lahore in the past, but has not been collected in the city since 1950.

A virus which appears to be a member of the DEN complex (probably DEN-3) was isolated from a sick child during an epidemic of encephalitis which occurred in Lahore during the summer of 1968 (Table I). During this outbreak, a total of 14 patients aged 1-10 years, were examined at the Sir Ganga Ram Hospital. The clinical syndromes consisted of sudden onset with high fever followed by convulsions, vomiting and unconsciousness. Most of the children gave a history of a "measles-type" illness 2-3 weeks prior to admission. With the exception of the abnormal neurological findings, the physical examination was normal, and the white blood cell count also was normal. The cerebrospinal fluid had elevated levels of glucose and a low protein concentration except in one patient in which the picture was reversed. Pleocytosis also was common. Four of the 14 patients died within 48 hours of hospitalization (PMRC Ann. Rep., 1963-69; Wisseman, personal communication). The vectors involved in this epidemic were not identified. The Lahore epidemic was unusual in that dengue infections normally are not associated with encephalitis; however, several cases of encephalitis in children infected with either DEN-2 or DEN-3 virus recently were reported from Indonesia (Sumarmo et al., 1978).

Sero-surveys have revealed a low-percentage of sample positive for antibodies to dengue-type viruses in human population groups around Rawalpindi and Lahore; however, many of these positives probably represent cross-reacting antibody from previous infection with the related flavivirus, WN (PMRC Ann. Rep. 1963-69; Burney, 1966). Similar may be the case with Jap B viruses against which low titre antibodies were described in human population in Rawalpindi (Burney, unpub. data).

Other Arboviruses from Pakistan:

Bakau virus originally was recovered from mosquitoes collected in Malaysia. Although serosurveys in man show an immunity rate of about 20% in Malaysia, infection with this virus has not yet been associated with human disease (Int. Cat. of Arboviruses, 1975). A single isolated of Bakau has been recovered in Pakistan (Table I), but sero-surveys to determine the extent of human infection in Pakistan have not been conducted (PMRC Ann. Rep., 1963-69).

Hazara virus, isolated in the Kaghan Valley from ticks collected off a vole (Table I),

Table: History of Arboviruses Isolated in Pakistan

<i>Virus</i>	<i>Group</i>	<i>Original isolations</i>	<i>Source of original isolations</i>
Bakau	Bakau	Oct. 1964 at Mahmoodbooti, 6 miles N.E. of Lahore.	Pool of adult ticks (<i>Argas abdu-salami</i>) collected from Gyps Bengalensis roost in Dalbergia tree.
Congo-Crimean Hemorrhagic Fever	Congo	Nov. 1965 at Changa Manga	1) Pool of ticks (<i>Hyalomma a. anatolicum</i>) collected from a cow. 2) Pool of ticks (<i>Boophilus microplus</i> and <i>H.a. anatolicum</i>) collected from a cow. 6 isolates from human blood. 4 isolates from human blood. 1 isolate from human blood.
Dera Ghazi Khan	Dera Ghazi Khan	Jan-Feb. 1976, Rawalpindi. May 1976, Quetta, Baluchistan. July 1978, Rawalpindi. April 1966, Sakhi Sarwar, Dera Ghazi Khan, District.	Pool of tick larvae (<i>Hyalomma redikorzevi</i>) collected from a camel.
Hazara	Congo	July 1964, at Gitidas Kaghan Valley, Hazara District.	Pool of adult ticks (<i>Ixodes redikorzevi</i>) collected from the vole, <i>Aticola Roylei</i> .
Manawa	Uukuniemi	August 1964 at Manawa 13 miles east of Lahore. 1964-66, Changa Manga Forest, Lahore district and Baltit, Hunza, Gilgit Agency.	1) Pool of ticks (<i>Argas abdu-salami</i>) collected from Gyps Bengalensis roost in Dalbergia tree. 2) Pool of tick larvae (<i>Rhipicephalus ramachandrai</i>) collected from a gerbil (<i>Tatera indica</i>). 3) Pool of ticks, mixed stages (<i>R. sanguineus turanicus</i>) collected from a goat.
Wad Medani	Kemerovo	1965 at Changa Manga Forest, Lahore district.	Pool of adult ticks (<i>H.a. anatolicum</i> and <i>Boophilus microplus</i>) collected from a cow.
Karimabad	Phlebotomus Fever	1959 at Said Pur, Rawalpindi, district.	Pool of sandfly females (<i>Sergentomyia</i> spp)
Sandfly Fever (Napels)	Phlebotomus Fever	1959 at Rawalpindi.	1) Human plasma (Male 22 years of age). 2) Human plasma (Male 45 years of age).
Sandfly Fever (Sicilian)	Phlebotomus Fever	1959 Rawalpindi and Bazid Khel.	1) Human plasma (Male 22 years of age). 2) Pool of sandfly females (<i>Phlebotomus</i> spp).
West Nile	B	1962-65, Lahore district. 1963-65, Rawalpindi Area.	6 isolates from pools of mosquitoes (<i>Culex tritaeniorhynchus</i>) 5 isolates from human plasma from febrile patients. 2 isolates from human blood from febrile patients.
Dengue	B	Summer of 1968 in Lahore.	Cerebrospinal fluid from a child.

is anti-genically related to C-CHF virus but has not been associated with disease even though sero-surveys indicate that human infections occur in Pakistan (PMRC Ann. Rep. 1963-69). To date, this virus has not been reported to occur in any other country. An agent apparently related to these viruses was isolated in 1976 from ticks-parasitising goats in Chitral but full characterization has not been done (Burney).

Wad Medani virus has been reported from Egypt, Sudan, India, and Jamaica as well as Pakistan. All of the isolates have come from ticks (Int. Cat. of Arboviruses, 1975). The single isolate of this virus in Pakistan was from the Changa Manga National Forest (PMRC Ann. Rep. 1963-69) (Table I). This virus also has not been associated with human disease.

The original isolation of 2 other arboviruses, Manawa and Dera Ghazi Khan, were made in Pakistan (Table I). Both viruses were recovered from ticks. Limited sero-surveys have not detected antibodies to

these viruses in man (Begum et al., 1970a; Begum et al., 1970)

Conclusion

In addition the above mentioned arboviruses known to occur and cause human infection in Pakistan, several other important human pathogenic arboviruses such as Japanese encephalitis, members of the group B tick-borne encephalitis-complex such as Russian spring-summer encephalitis, Omsk hemorrhagic fever, and Kayasanur Forest disease are prevalent in surrounding countries and if introduced into Pakistan could cause severe epidemics. Japanese encephalitis (JE) should be of particular concern because *Cx. tritaeniorhynchus*, the main vector of JE virus throughout most of its geographic range, is a very common mosquito in many areas of Pakistan (Reisen, 1978). Several epidemics of JE have occurred in West Bengal, India, in recent years (Rao, 1975) and in 1978, a large outbreak of encephalitis attributable to this virus was reported as far North as Uttar Pradesh (Morbidity and Mortality Rep., 1978). Also the possibility of epidemics of dengue resulting from the introduction of virus from adjacent areas such as India where it is endemic remains a considerable public health threat. Such an introduction of virus may have precipitated the Lahore epidemic of 1968 since an outbreak of dengue was reported from New Delhi during October-November, 1967 (Virus Res. Centre, Poona, Ann. Rep., 1967).

Physicians should remain alert to the possible arbovirus etiology of acute febrile illness particularly during epidemic conditions and also when associated with hemorrhagic or encephalitis syndromes.

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What is an arbovirus? "Arbovirus," short for arthropod-borne virus, doesn't refer to one particular virus. Rather, it refers to a type of virus transmitted via insects that bite and feed on blood. This includes a large family of viruses. In fact, there are over 600 known arboviruses, and more than 80 of them are known human pathogens. The resulting illnesses can range from mild to severe. On a global scale, dengue virus may be the most challenging, infecting 100 to 390 million people and leading to 12,500 deaths per year. It spreads mostly through mosquitos in underdeveloped, highly populated Health indicators in Pakistan were dismal even before the floods, with maternal mortality at 230 (190-280) per 100 000 live births and under-5 mortality at 89 per 1000 live births.² Most of the flooded populations comprise the lowest socioeconomic quintiles that were already facing neglect. This crisis has worsened their plight. Adequate hygiene and sanitation are of primary importance in diarrhoeal disease prevention. Public-private partnerships define the modern international health and development landscape.⁹ Close alignment of values and interests between public and private public-health entities, as well as the recipient countries, is critical to the initial surge effort as well as for post-crisis efforts.⁹ Hundreds of viruses, designated as arboviruses, are transmitted by arthropod vectors in complex transmission cycles between the virus, vertebrate host, and the vector. The hemorrhagic form of the disease is likely to have the highest public health significance based on the case numbers. This is largely due to the increased disease burden created by DENV, which is the etiological agent for dengue hemorrhagic fever (Gubler, 1998). Because of its importance in limiting virus infections in vertebrate hosts, apoptosis signaling pathways were evaluated in a number of studies; however, the role of apoptosis in arbovirus infection in arthropod vectors remains contradictory (Wang et al., 2008, 2012; Neill et al., 2015; Eng et al., 2016).