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Immunological Surveys of Arbovirus Infections in South-East Asia, with Special Reference to Dengue, Chikungunya, and Kyasanur Forest Disease*

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Serological surveys have been widely used in South-East Asia to determine the presence and activity of arboviruses. The haemagglutination-inhibition test has been most frequently employed but complement-fixation and neutralization tests have also been used in some investigations.

Although virus isolations provide the most conclusive evidence, they can be carried out in a few specialized centres only, and serological surveys are very important for studying the distribution of arboviruses.

The surveys have shown that group B arboviruses (principally all four types of dengue, Japanese encephalitis, and West Nile) are widely prevalent. Dengue and Japanese encephalitis viruses are more widespread than West Nile virus, which was not known previously to extend east of India although recent survyes have shown that its range extends to Burma. Japanese encephalitis is frequent in most of South-East Asia but in India is found mainly in eastern and south-eastern parts of the country. Kyasanur Forest disease (KFD) and Langat viruses are the only tick-borne group B arboviruses definitely known to occur in the region, the former in India, the latter in Malaysia. KFD virus has been isolated only from a small focus in Mysore, although human and animal sera containing neutralizing antibodies to this virus have been found sporadically in widely scattered areas. Among the group A arboviruses, chikungunya and Sindbis have been detected in serological surveys, but the former has not yet been found in Malaysia.

Serological surveys to determine the prevalence of arboviruses in South-East Asia are of comparatively recent origin. Some of the earlier surveys in this region were reported by Paterson et al. (1952), Smithburn (1954) for West Malaysia and Indonesia (Borneo), and Smithburn et al. (1954) for India. Sabin et al. (1947) and Pond et al. (1954) studied the distribution of neutralizing antibodies, with particular reference to Japanese encephalitis, in Japan and West Malaysia, respectively. Subsequently, surveys have been made with improved techniques in practically all countries of South-East Asia.

Serum samples have been tested mostly against viruses known, or expected, to occur in the different areas. Haemagglutination-inhibition (HI), complement fixation, and neutralization tests have been used in the surveys but the HI test has been the test most extensively employed.

Although the isolation of a virus is the most conclusive evidence for its occurrence in any area, the facilities are available only in a few specialized centres. Thus, for a long time to come, information on the occurrence and distribution of arboviruses in most parts of South-East Asia will depend, as it does at present, on serological surveys. This review of the serological surveys carried out in

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South-East Asia is based mainly on published reports but the results of some surveys have not yet been published, and other surveys are reported only in official documents.

The arboviruses considered here are those causing dengue, chikungunya, and Kyasanur Forest disease (KFD), but it is necessary also to take into account the distribution of antibodies to closely related viruses.

Dengue is widespread in the whole of South-East Asia in areas where *Aedes aegypti* is present; surveys have indicated that probably no country in the region is altogether free from the disease. Recent studies of the occurrence of jungle dengue in man (aboriginal populations) and monkeys in some areas where *Ae. aegypti* is not prevalent have added a new concept to the epidemiology of this disease.

Chikungunya, which has been recognized as a separate disease only within the last 10 years, is also widespread in South-East Asia but the distribution of the virus differs from that of dengue. It is known to occur in most of the countries in the region except Malaysia; however, its presence is unpredictable. There are very large areas of western and north-western India where the virus has not yet been detected although dengue is known to be present. Unlike dengue, chikungunya does not seem to have established an endemic status in India but it is known to persist year after year in Thailand. While chikungunya seems to have made a fresh entry into South-East Asia during the last decade, there is some serological evidence for the view that the virus had been previously active in this area.

Kyasanur Forest disease, which affects man and monkeys, has so far been found only in a small part of the town of Sagar in Shimoga District, Mysore, India, but antibodies to the virus have been found in a few widely scattered localities. There is no evidence that the infection is present in other countries. A related virus, Langat (TP 21), has been isolated, and antibodies to it have been found in Malaysia, but no evidence has yet been obtained that it occurs in India.

Some of the more important serological surveys carried out in the various countries of South-East Asia are now reviewed.

INDIA

The first extensive serological survey to obtain preliminary information on the prevalence of arboviruses in India was made by Smithburn et al. (1954). Altogether, 588 sera were collected from the indigenous populations of 38 localities in 6 states, and tested for neutralizing antibodies to a number of arboviruses. In many localities, the most frequent antibodies were those against dengue 1 and dengue 2, the highest rates of immunity being found in Broach district (Gujarat) and Nagpur district (Maharashtra). Antibodies to West Nile virus were next in order of frequency, while antibodies to Japanese encephalitis virus were found mainly in sera from some areas of Tamil Nadu (formerly Madras). A few sera from Kutiyana, Saurashtra, were found to neutralize Russian spring–summer encephalitis (RSSE) virus. Banker (1952) made a brief survey in the city of Bombay and obtained serological evidence of West Nile virus infections in man.

Surveys of Tamil Nadu, Kerala and the South Kanara district of Mysore were made in 1955 and 1956 (Banerjee & Desai, personal communication). Antibodies to group B arboviruses were found in sera from different parts of Tamil Nadu, being present in nearly 35% of the children under 10 years of age and in 50% of the total population. Antibodies neutralizing dengue viruses were most frequent and there was a low, but significant immunity to Japanese encephalitis. However, antibodies to group B arboviruses, including dengue, were less frequent in the population of Kerala. In South Kanara district, antibodies to group B arboviruses were also less frequent but those to dengue viruses predominated. None of the sera from children under 10 years of age in those districts contained HI antibodies to this group of viruses. Carey & Myers (1968) showed that antibodies to Japanese encephalitis and West Nile viruses were present in rural populations in Vellore (Tamil Nadu).

A serological survey in rural areas of Kolaba district (Maharashtra) in 1955 indicated that antibodies to dengue viruses were either absent or very infrequent in the 5–50 years age group. Antibodies to West Nile virus were found mostly in the older age groups (Virus Research Centre, unpublished data). It is interesting to note that *Ae. aegypti* does not occur in this area.

A high rate of immunity to dengue 1 virus was found in 1955 in Calcutta, Diamond Harbour (West Bengal), and Jamshedpur (Bihar) (Virus Research Centre, unpublished data). Immunity to dengue virus was found also in the city of Indore and the surrounding areas of Madhya Pradesh in 1958 (Virus Research Centre, unpublished data).

A serological survey in 1958 in Ramtek, Nagpur district (Maharashtra), showed that dengue virus was highly endemic, practically all persons becoming infected before the age of 6 years (Virus Research Centre, unpublished data). Sarkar & Chatterjee (1962) reported that 81.1% of the population in the city of Calcutta had antibodies to group B viruses but in Parbatpur (a rice-growing area about 40 miles (64 km) west of Calcutta), Belpukur (a seaside village 50 miles (80 km) south of Calcutta), and Darjeeling (a town at an altitude of 7000 feet (2000 m) the corresponding rates ranged from 6.2% to 12.2%.

In 1959, HI tests on nearly 700 sera collected at Anantnag, Baramulla, Bandipur, Srinagar, and Jammu districts (Jammu and Kashmir) showed that arbovirus antibodies were practically absent (Virus Research Centre, unpublished data; Rodrigues, personal communication).

An examination in 1963 of 377 sera from North Lakhimpur and Darrang districts, situated north of the Brahmaputra River (Assam), showed that 22% of the population had antibodies to Japanese encephalitis. Antibodies to dengue viruses were much less frequent and were detected only in sera from the Darrang district. At the same time, tests were made on 859 sera collected in high-altitude areas of Kameng and Subansiri Frontier Divisions of the North-East Frontier Agency, and in low-altitude areas of Siang and Luhit Frontier Divisions. In sera from the higher altitudes the prevalence of antibodies was found to be low but group B arbovirus antibodies were demonstrated in 18% of the sera from low-altitude areas, antibodies to Japanese encephalitis being most frequent (12%). The prevalence of dengue 2 neutralizing antibodies was low except in sera from the Luhit Division (Virus Research Centre, unpublished data; Rodrigues, personal communication).

Serological surveys for neutralizing antibodies to KFD virus in different areas in Mysore, outside the known KFD-infected zone of Sagar and Sorab in Shimoga district, showed that only 2 sera out of 287 were positive (Upadhyaya & Murthy, 1967). Antibodies to KFD virus, or a related virus, were reported in 1960 and 1965 in man and animals from the semi-arid areas of Saurashtra and Kutch, which are over 700 miles (1200 km) from the KFD-infected Sagar area (Virus Research Centre, unpublished data).

The antibodies to RSSE (now tick-borne encephalitis—TBE) virus found by Smithburn in the first survey in Saurashtra (Madras) and Ramtek (near Nagpur) may have been related to KFD infections since TBE virus has not so far been detected in India. Sarkar & Chatterjee (1962) also found neutralizing antibodies to KFD virus in two human sera from Parbatpur (Bengal).

HI tests were made on 880 sera from hill areas in the states of Uttar Pradesh and Himachal Pradesh in 1968. The prevalence of arboviruses was very low; only 36 sera were found to be positive for group B antibodies, 6 sera were positive for antibodies to chikungunya virus, and only 1 serum was positive for antibodies to Sindbis virus (Virus Research Centre, unpublished data; Rodriguez, personal communication).

In 1969, serum samples were collected in different localities of Poona district, all age groups being represented. Altogether, 202 sera out of a total of 673 contained HI antibodies to one or more group B arboviruses. Antibodies to West Nile virus were most frequent, followed by antibodies to dengue and Japanese encephalitis viruses. These results were confirmed by neutralization tests (Virus Research Centre, unpublished data; Marlihalli et al., personal communication). None of the sera contained KFD neutralizing antibodies. Deodhare (1969) examined 119 sera collected from the city of Poona and the surrounding area and found antibodies to one or more group B arboviruses.

The occurrence of chikungunya virus antibodies has been reported (Chatterjee et al., 1967; Pavri, 1964) in human sera in Jamshedpur (Bihar) and Calcutta (West Bengali), collected several years prior to the epidemics of 1963. Antibodies to chikungunya virus were also detected in sera collected in 1956 in several parts of Madras state (Banerjee, 1965). These studies showed that a few persons above the age of 30 or 40 years had antibodies to chikungunya virus, indicating that the virus had been present in India at some time in the past but had been inactive for many years until it reappeared in 1963. In a post-epidemic survey carried out in 1964–65 in the city of Madras, the frequency of chikungunya antibodies was found to be 38.4% (Dandawate et al., 1965). Some recent chikungunya epidemics in India have been reviewed by Ramachandra Rao (1966).

The presence of HI antibodies to chikungunya virus has been demonstrated in a few sera from Hoshiarpur and Kapurthala districts (Punjab), indicating that chikungunya virus or a related group A arbovirus occurs in those areas. The results also

appear to show that more than one virus group B (West Nile and dengue viruses) was prevalent in the Punjab (Saha & Thomas, 1967).

MALAYSIA AND SINGAPORE

Surveys of arboviruses have been carried out in Malaysia over a much longer period than in any other country of South-East Asia. Paterson et al. (1952), Pond et al. (1954), and Smithburn (1954) were among the first to make such surveys in West Malaysia, particularly in and around Kuala Lumpur, and surveys were also made in Singapore. Since then, studies made by Hale & Lee (1955) Hale et al. (1956), Gordon Smith (1958), Pond (1963), and several other workers, have established that Japanese encephalitis and dengue viruses are the most important mosquito-borne arboviruses affecting man in Malaysia. Although several epidemics of dengue, including haemorrhagic dengue, have been studied in Malaysia, chikungunya virus has not so far been detected there (Chan et al. 1967; Rudnick, 1966).

Pond (1963) reported that most of the serum samples from Malaysian adults contained antibodies to Japanese encephalitis, Ntaya, Zika, and Murray Valley encephalitis viruses and stated: "Many sera also contained antibodies against West Nile, Ilheus, Semliki Forest and RSSE viruses". Although antibodies to Japanese encephalitis and Ntaya viruses were present in nearly 90% of sera from Malaysians of all age groups, neutralizing antibodies to Zika, Ilheus, and Semliki Forest viruses reached their highest levels only in adults over the age of 30 years. Antibodies to both Murray Valley encephalitis and West Nile viruses were frequently observed, but the author doubted whether these viruses were present in Malaysia and suggested that the antibodies might have resulted from cross-reactions with Japanese encephalitis virus. Surprisingly, no reference is made to dengue. It is interesting to note that Zika virus has now been isolated in Malaysia (Marchette et al., 1969).

The work of Gordon Smith (1956) and Rudnick et al. (1967) in Malaysia is of outstanding interest and provides firm evidence for the existence of jungle dengue. Serological evidence shows that dengue occurs in monkeys and that in 1965 and 1966 dengue 3 was the most frequent dengue virus in forest-dwelling Malaysian aborigines (Rudnick et al., 1967).

The epidemics of dengue haemorrhagic fever in Singapore (Chan et al., 1967; Lim et al., 1964) Penang (Lucas, 1967; Rudnick, 1966), etc., have extended our knowledge about the distribution and prevalence of the arboviruses in this part of South-East Asia.

THAILAND

Although no extensive serological surveys have been made in Thailand, all four known types of dengue virus, and possibly even six types, have been isolated (Hamilton et al., 1961a). Antibodies to the Bunyamwera group of viruses and to Sindbis virus have been found in children in Bangkok (Yamarat & Udomsakdi, 1961). Serological studies of apparently normal children in Bangkok during the epidemics in 1958 and 1960 showed that the infection rate with chikungunya virus exceeded 50% (Hamon et al., 1961b).

Mild or asymptomatic infection seems to have occurred predominantly after the third year of life. Neutralizing antibodies to chikungunya virus have been found in human sera collected in all parts of Thailand (Halstead & Udamsakdi, 1966).

Work on dengue haemorrhagic fever in Thailand has produced much valuable information on all aspects, including the immunology, of dengue and chikungunya infections (Halstead, 1966; Halstead et al., 1967; Jatanasen, 1966). Sekeyova & Gresikova (1969) have reported a recent serological survey in Thailand. Unfortunately, only HI tests were used in the study. Thus the conclusions on dengue, Japanese encephalitis and chikungunya generally confirm previous findings, but the presence of antibodies to eastern equine encephalitis, western equine encephalitis, tick-borne encephalitis, West-Nile and yellow fever viruses needs to be confirmed by neutralization tests.

CEYLON

Although previous, unpublished, serological studies had indicated a high frequency of group B arboviruses in Ceylon, the occurrence of group A arbovirus infections was not seen until February 1965, when there was an epidemic of a dengue-like illness in Colombo. A survey made before the epidemic showed that 8% of persons aged 11–40 years and 45% of those over 40 years of age had antibodies to chikungunya (Hermon, 1967). This suggests that chikungunya, or a closely related virus, had previously been active in Ceylon for many years. Among 363 paired and single sera that were tested, antibodies to chikungunya virus

were found in 32%; 16% of the sera were positive for group B viruses alone, and 12% were positive for both chikungunya and group B viruses. Presumably, the group B antibodies were all dengue-neutralizing, but it is only recently that a dengue virus (type 2) has been isolated from a human case in Ceylon (Hermon et al., 1970). Since conditions in Ceylon are very similar to those in Tamil Nadu, India, further serological studies in Ceylon may show results similar to those obtained in South India. Japanese encephalitis virus has also been isolated recently from a human case in Ceylon (Hermon, unpublished data).

VIET-NAM

One of the earliest serological surveys in Viet-Nam (Halstead et al., 1965) revealed the existence of antibodies to dengue and chikungunya viruses in the residents of Saigon. It is evident that chikungunya was at that time endemic in urban and rural Viet-Nam. A study by Mirovsky et al. (1965), who used serology mainly for diagnostic purposes, indicated that an epidemic in parts of Viet-Nam was due to a dengue virus, although it is possible that some cases may have been due to chikungunya virus. Vu-qui-Dai & Kim-Thoa (1965) and Vu-qui-Dai et al. (1967) made regular serological surveys in children. They found that 82% of children in the 10–15 years age group had HI antibody titres for dengue 1 virus of 1: 40 or greater and that a similar proportion had HI antibodies to chikungunya virus.

INDONESIA

A survey made by Hotta et al. (1967) showed that antibodies to group B arboviruses, particularly Japanese encephalitis, dengue 1 and dengue 2, are frequent in Indonesians. Japanese encephalitis antibodies were more frequent in Lonbuck Island than elsewhere, perhaps on account of environmental conditions. Since HI tests were generally used, a few sera were also positive for yellow fever, possibly as a result of immunological overlapping between group B viruses. The occurrence of antibodies to dengue viruses had been earlier reported in the country.

BURMA

There are no records of serological surveys in Burma except for a casual observation by Halstead & Udomsakdi (1966) of neutralizing antibodies in human sera from Rangoon. A recent study of 238 sera collected in Rangoon produced evidence for the activity of chikungunya, all four types of dengue, Japanese encephalitis and West Nile viruses (Ghosh et al., unpublished data). The detection of antibodies to West Nile virus in sera from Rangoon is of considerable significance since it extends the known eastern range of this virus from India to Burma.

CAMBODIA

No records are available of extensive human serological surveys in Cambodia but antibodies in reptiles were studied by Chastel (1966); antibodies to chikungunya, dengue and Japanese encephalitis viruses were found in snakes and lizards. Chastel (1963) had previously reported the isolation of chikungunya virus in Phnom Penh. Halstead et al. (1967) also refer to the isolation of dengue 1 and 4 viruses in Cambodia.

CONCLUSIONS

This review shows that serological surveys have been extremely useful in mapping the distribution of chikungunya dengue. Japanese encephalitis and West Nile viruses in South-East Asia. The serological findings generally have been consistent with reports of virus isolations, although there have been fewer isolations.

In serological surveys, the possibilities of cross-reactions to closely related antigens cannot be completely eliminated. Fortunately, however, chikungunya and dengue viruses can usually be distinguished by serological techniques from closely related viruses. The only virus of group A other than chikungunya that is prevalent in South-East Asia is Sindbis, which can readily be distinguished in serological tests. Getah virus, which has also been reported from Malaysia, and which is perhaps present in some parts of India, is also easily distinguishable from chikungunya and dengue viruses.

The four types of dengue, Japanese encephalitis and West Nile viruses are the principal group B, mosquito-borne viruses in South-East Asia. It is usually difficult to distinguish between them in HI tests, but by means of complement-fixation and neutralization tests, dengue antibodies can easily be distinguished from antibodies to Japanese encephalitis and West Nile viruses. Difficulties of identification, however, arise with Japanese encepha-

litis and West Nile viruses. Although these two viruses have been unmistakably identified in many surveys, their close similarity is a source of confusion

Kyasanur Forest disease presents a slightly different problem. The virus has been isolated only in Mysore, but antibodies have occasionally been demonstrated in human or animal sera from other, scattered parts of India. In such areas, the specificity of antibodies to KFD merits further investigation and efforts should be made to isolate the virus. Langat virus that is known to occur in South-East Asia, can easily be distinguished serologically from KFD virus, and there is little possibility of the two viruses being confused.

Although much information is available on the distribution of arboviruses in South-East Asia, there are still vast areas of this region that have not yet been studied virologically and several areas in which more adequate studies are required. Serological surveys are the most useful and convenient means for carrying out these investigations.

RÉSUMÉ

ENQUÊTES IMMUNOLOGIQUES SUR LES INFECTIONS À ARBOVIRUS (NOTAMMENT LA DENGUE, LE CHIKUNGUNYA ET LA MALADIE DE LA FORÊT DE KYASANUR) EN ASIE DU SUD-EST

On a eu amplement recours aux enquêtes sérologiques pour déceler la présence et la circulation des arbovirus en Asie du Sud-Est. L'épreuve la plus couramment utilisée a été la réaction d'inhibition de l'hémagglutination, mais les épreuves de fixation du complément et de neutralisation ont été également mises à profit dans certaines investigations.

Ces enquêtes ont fait ressortir une forte prévalence des arbovirus du groupe B, représentés surtout par les virus de la dengue (4 types), de l'encéphalite japonaise et West Nile. Les deux premiers de ces virus sont plus fréquemment rencontrés que le virus West Nile, considéré autrefois comme ne circulant pas à l'est de l'Inde mais qui a été récemment décelé en Birmanie. Le virus de l'encéphalite japonaise est très répandu dans la majeure partie de l'Asie du Sud-Est, mais en Inde, on le trouve principalement dans l'est et le sud-est du pays. Parmi les arbovirus du groupe B transmis par des tiques, seuls le virus de la maladie de la forêt de Kyasanur (virus KFD) et le virus Langat ont été identifiés avec certitude dans la région, le premier en Inde, le second en Malaisie. Le virus KFD n'a été isolé que dans un foyer limité dans l'Etat de Mysore, mais la présence d'anticorps neutralisant ce virus a été démontrée sporadiquement dans des sérums humains et animaux prélevés à des endroits très éloignés de ce foyer.

Les virus chikungunya et Sindbis, du groupe A, ont été identifiés lors d'enquêtes sérologiques, mais le premier n'a pas encore été décelé en Malaisie.

L'auteur met l'accent sur l'importance des enquêtes sérologiques pour l'étude de la répartition des arbovirus.

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