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Prevalence of arbovirus antibodies in sera of animals in Sri Lanka

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The sera of cattle, goats, dogs and crows from the Colombo area were tested for antibodies against seven arboviruses of the families Togaviridae and Bunyaviridae by a plaque-reduction neutralization microtest, using Vero cells and a stable line of pig kidney (PS) cells. The overall percentages of positive sera among the mammals were: Bhanja, 92.5%; Calovo (Batai), 30.6%; Sindbis, 13.8%; Langat, 4.8%; Tahyna, 3.9%; West Nile, 1.6%. Among the birds, 23.8% had antibodies to Bhanja virus and 9.5% to Sindbis. No antibodies against tick-borne encephalitis virus were found. The results show that at least two members of the Bunyaviridae family (Bhanja and Calovo) are highly endemic in Colombo.

In many countries of the tropical and subtropical climatic zone, arbovirus infections appear frequently and, under certain circumstances, can cause a serious public health problem. Information about the occurrence of arboviruses in a particular country may be obtained quickly and conveniently by serological assays of human and/or animal blood samples.

In this study, we examined sera from animals in Sri Lanka for the occurrence of neutralizing antibodies against some arboviruses. Previous research on arbovirus infections in Sri Lanka has been limited mostly to dengue and Chikungunya epidemics (*1-3*).

MATERIALS AND METHODS

Serum samples were collected from 49 cattle and 57 goats in the abattoir of Colombo. These animals came from Colombo and the surrounding area. In addition, serum samples were taken from 29 dogs from the area, and 21 crows (*Corvus* spp.) captured with the use of chloralose. The sera were inactivated at 56 °C for 30 minutes, diluted, and examined for the presence of antibodies against seven arboviruses.

Viruses

The virus strains used in the tests were:

1. Sindbis virus, strain Eg Ar 339, passaged 15 times in suckling mice;[a]
2. West Nile virus, strain Eg 110, passaged 13 times in suckling mice;[a]
3. Tick-borne encephalitis virus, strain Hypr (*4*), passaged 55 times in HeLa cells and 11 times in juvenile mice;
4. Langat virus, strain TP 21 (*5*);
5. Tahyna virus, strain P6b, passaged 7 times in suckling mice (*6*);
6. Calovo virus, strain 184, passaged 7 times in suckling mice (*7*);
7. Bhanja virus, strain 326, passaged 5 times in suckling mice (*8*).

Cell lines

Vero cells were used for the assay with all viruses except Langat, for which PS (stable pig kidney) cells were used. Both cell lines were grown in Eagle's minimum essential medium supplemented with lactalbumin hydrolysate (1 g/litre), sodium bicarbonate (1.1 g/litre), calf serum (100 ml/litre for Vero cells, 50 ml/litre for PS cells), and antibiotics.

Plaque-reduction neutralization test (PRNT)

The procedure for the PRNT was basically that of de Madrid & Porterfield (*9*) modified for microplates (*10*). However, the unit volume of virus and diluted serum was 25 µl instead of 40 µl, and the virus test doses were adjusted so that they caused almost confluent plaques (90-95% cytolysis). The serum samples were incubated with the virus test doses on microplates at 4 °C for 18 hours; the cells were then added and the plates incubated at 36 °C for 4 hours before the addition of carboxymethylcellulose overlay. The incubation medium was Leibovitz L15 with 3% fetal calf serum. The final incubation period was 3 days for Tahyna, 4 days for Calovo, 5 days for Sindbis, West Nile and Bhanja, and 6 days for tick-

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borne encephalitis and Langat viruses. Control immune sera against Sindbis, West Nile, tick-borne encephalitis, Tahyna, and Calovo viruses were prepared at the Institute of Sera and Vaccines (IMUNA, 08222 Šarišské Michalany, Czechoslovakia); the Bhanja antiserum was prepared as described previously (*8*). The dilution of serum that caused an 80–100% reduction in cytolysis of the virus test dose was regarded as the serum titre.

RESULTS

The proportions of sera giving a positive reaction against the various arboviruses are shown in Table 1. In domestic animals, the frequency of antibodies was highest against Bhanja virus (97.9% of cattle, and 91.2% of goats) and Calovo virus (77.1% of cattle and 1.7% of goats); there was a low prevalence of antibodies against Tahyna, West Nile, and Langat viruses, while antibodies against tick-borne encephalitis were not detected.

In dogs, the highest frequency of antibodies was found against Bhanja (86.2%); no antibodies were detected against West Nile, tick-borne encephalitis, Langat and Tahyna viruses.

Crows revealed antibodies against Bhanja and Sindbis viruses (23.8% and 9.5%, respectively) but not against the other viruses tested.

Table 2 shows titres of randomly selected positive sera against Calovo and Bhanja viruses. The distribution of the antibody titres indicates the recent circulation of these viruses in Sri Lanka.

DISCUSSION

The serological assays on free-grazing animals yielded information about the incidence of various arboviruses in the examined areas. Because neutralizing antibodies persist in the infected animals longer than haemagglutination-inhibiting or complement-

Table 1. Frequency of neutralizing antibodies against arboviruses in sera from animals in Sri Lanka

Sera	Prevalence of antibodies[a]						
	Sindbis	West Nile	Tick-borne encephalitis	Langat	Calovo	Tahyna	Bhanja
Cattle	6/47	1/46	0/47	0/15	37/48	3/49	47/48
	(12.8)	(2.2)	(0.0)	(0.0)	(77.1)	(6.1)	(97.9)
Goat	4/55	1/52	0/57	3/31	1/57	2/57	52/57
	(7.3)	(1.9)	(0.0)	(9.7)	(1.7)	(3.5)	(91.2)
Dog	8/28	0/28	0/28	0/16	3/29	0/21	25/29
	(28.6)	(0.0)	(0.0)	(0.0)	(10.3)	(0.0)	(86.2)
Crow	2/21	0/18	0/21	0/6	0/19	0/21	5/21
	(9.5)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(23.8)

[a] No. of positive sera/no. of sera examined. Figures in parentheses give percentage positive. Titres ⩾ 10 were taken as positive.

Table 2. Distribution of neutralizing antibody titres against Calovo (Batai) and Bhanja viruses in randomly selected positive sera

Sera	Calovo (Batai)					Bhanja				
	40	80	160	320	⩾640	40	80	160	320	⩾640
Cattle		3	5	10	1	3	2	8	9	4
Goat			1			1	2	1	2	5
Dog			2					5	2	
Crow							1	1	2	

fixing antibodies, the neutralization test is the most useful assay. The PRNT is an especially appealing technique since it is not only very sensitive, but also markedly specific. In the PRNT, Vero cells were used for most of the viruses tested, because these cells are easy to maintain even under the carboxymethylcellulose overlay. A disadvantage of Vero cells is that flaviviruses of the tick-borne encephalitis subgroup, unadjusted to cell cultures, either do not form plaques, or do so only after 9–15 days (*11*). In these cases, the use of PS cells (which are, however, less easy to maintain) is the method of choice. The micromethod of PRNT, used in this survey, is a little less sensitive than the macromethod (*9*), but is more economical in that only one quarter the amount of each reagent is needed.

We did not detect antibodies against tick-borne encephalitis virus, but sera of three goats contained antibodies against Langat, a closely related virus which was isolated originally from *Ixodes granulatus* ticks in Malaysia (*12*) and which obviously occurs also in Sri Lanka (assuming that the goats were not imported).

The discovery of antibodies against Calovo virus is not surprising because this virus is considered to be a strain of Batai virus (*13*), which was isolated in Malaysia. Calovo virus has been found in other Asian countries, including India (*14*) where the most frequent vector of the virus is *Anopheles stephensi* (*15*). Calovo and Batai viruses have not yet been found to cause symptomatic infections in animals or man, and specific antibodies in man have been detected only sporadically.

The circulation of Tahyna virus in Sri Lanka remains questionable. The reduction of plaques in the five positive animal sera was incomplete, and several plaques always persisted, although the titres were 1:40 to 1:640. It is therefore likely that another virus of the California serogroup, with antigenic composition similar to that of Tahyna virus, might occur in Sri Lanka.

Bhanja virus antibodies were detected in Sri Lanka for the first time, and in a remarkably high proportion, especially among domestic animals. The virus was originally isolated from *Haemaphysalis intermedia* ticks in India (*16*), where a high prevalence of antibodies among goats was also found. The occurrence of antibodies against Bhanja virus in dogs and crows has not previously been reported. Bhanja virus may cause laboratory and natural infections in man (*17, 18*) and the antibodies have been found in the human population of various countries of Africa, Asia, and Europe (*16, 19–21*). It has been found that owners of goats and sheep have a higher frequency of antibodies than other inhabitants of the same area (*22*). The postulated association of Bhanja virus with a paralytic disease in sheep and goats remains unconfirmed, because experimental infections have not demonstrated any significant susceptibility of these animals to the virus (*23–25*).

In a previous study (*3*), antibodies were detected against Chikungunya, dengue, Japanese encephalitis, and Tahyna viruses among animals and man in Sri Lanka. The present study has not covered all arboviruses in Sri Lanka but it has shown that natural foci of at least two bunyavirus (Bhanja and Calovo/Batai) infections exist in the country. Moreover, the circulation of Sindbis and Langat togaviruses and of a possible Tahyna-like bunyavirus has also been demonstrated.

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RÉSUMÉ

FRÉQUENCE DES ANTICORPS ANTI-ARBOVIRUS DANS LE SÉRUM DE CERTAINS ANIMAUX À SRI LANKA

On a recherché la présence d'anticorps dirigés contre 7 arbovirus appartenant aux familles des Togaviridés et Bunyaviridés dans le sérum de 49 bovins, 57 chèvres, 29 chiens et 21 corbeaux des environs de Colombo, à Sri Lanka. On a réalisé une épreuve de neutralisation objectivée par réduction des plages en cellules Vero ou en cellules rénales de porc (PS).

En moyenne, la fréquence des anticorps anti-arbovirus chez les mammifères était la suivante: Bhanja: 92,5%, Calovo (Batai): 30,6%, Sindbis: 13,8%, Langat: 4,8%, Tahyna: 3,9%, West Nile: 1,6%. Chez des oiseaux, 23,8% étaient porteurs d'anticorps dirigés contre le virus Bhanja et 9,5% contre le virus Sindbis. Aucun anticorps dirigé contre le virus de l'encéphalite à tiques n'a été décelé.

L'étude n'a pas couvert tous les arbovirus présents à Sri Lanka, mais elle a montré qu'il existe dans ce pays des foyers naturels d'au moins deux infections à bunyavirus (Bhanja et Calovo). La transmission des virus Sindbis et Langat a aussi été mise en évidence.

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