Acta virol. **28**: 325–328, 1984

SEROLOGICAL STUDIES ON SANDFLY FEVERS IN THE REPUBLIC OF BANGLADESH

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Received October 11, 1983; revised January 20, 1984

Summary. — Blood samples from 160 inhabitants of the Republic of Bangladesh were studied by haemagglutination inhibition (HI), indirect HI, and radial haemolysis in gel tests. The sera were found to contain antibodies to the arboviruses of Sicilian (6.25%) and Naples (1.25%) sandfly fevers and to the Karimabad virus (11.25%) which are transmitted by *Phlebotomus papatasi*. Antibodies to the Karimabad virus were found among the Bangladesh population for the first time.

Key words: Phlebovirus; sandfly fever; antibodies; indirect haemagglutination test; radial haemolysis test

Introduction

The sandfly fever virus group (*Phlebovirus*, the *Bunyaviridae* family) now includes more than 30 representatives. These viruses have been isolated at different locations throughout the world; 8 of them are endemic in the countries of the Old World, namely in Europe, Asia and Africa. In these continents the habitants of *Phlebotomus papatasi*, the Sicilian and Naples (SFS and SFN) sandfly fevers can be found, while the Karimabad and Selehabad viruses have been isolated only in Asia and Africa. Epidemic outbreaks in Old World countries are associated only with SFS and SFN. The contribution of the other viruses remains unclear, although antibodies in humans have been found to the Karimabad, Arumovot, Gordil and Saint Floris viruses (Tesh *et al.*, 1976).

The incidence of sandfly fever viruses in Bangladesh has not been investigated sufficiently. In a single report Tesh *et al.* (1976) demonstrated the occurence of SFS and SFN virus antibodies in Bangladesh, while antibodies to Karamabad virus remained undetected. In this study we examined sera of Bangladesh residents for the presence of antibodies against the SFS, SFN and Karimabad viruses. The tests employed were HI, indirect HI and radial haemolysis (RH) in gel. The two latter tests were developed at the Laboratory of Biology of Arboviruses, Ivanovsky Institute of Virology of the U.S.S.R. Academy of Medical Sciences, and have been widely used in serological studies on various arboviruses (Gaidamovich *et al.*, 1980*a*).

Materials and Methods

The SFS, SFN and Karimabad viruses were obtained from the virus collection of the Ivanovsky Institute of Virology. The viruses were maintained in the laboratory by means of passaging in suckling mice.

Test antigens were prepared by sucrose-acetone extraction technique (Clark and Casals, 1958). The immunoglobulin-treated red blood cells for indirect HI to the Karimabad, SFN and SFS viruses were prepared as described (Klisenko *et al.*, 1981), by sensitizing formalinized, tannin--treated sheep erythrocytes with γ-globulins isolated from immune ascitic fluids of mice, individually for each virus.

The blood serum was collected from feverish patients at a hospital of Dakka, the Republic of Bangladesh, on filter paper discs (Schleicher-Schuell) calibrated for 0.1 ml of serum. The discs were stored at −20 °C until use. Elution from the discs was carried out for 18 hr at 4 °C with 1 ml of phosphate buffer (pH 7.2), so that 1:10 dilutions were obtained. Before examined, the sera were heated for 20 min at 56 °C to destroy the inhibitors, and the normal red blood cell agglutinins were removed.

The HI and indirect HI tests were run on Takachi type plates using a micromodification of the method. HI was performed as described by Clark and Casals (1958) with goose red blood cells at pH 6.0 and pH 6.2 and with 4 and 8 haemagglutinating antigen units. Indirect HI was carried out as described before (Gaidamovich *et al.*, 1974). The sera were titrated at 2-fold dilutions beginning with 1:10. To 0.025 ml of each serum dilution 0.025 ml of antigen titrated in indirect haemagglutination test was added (2 to 4 units), and after 20 min of incubation, 0.025 ml of 1% suspension of immunoglobulin-treated red blood cells was added. The dilution that inhibited haemagglutination by at least 2+ was taken as the serum titre. RH was run according to Melnikova and Gaidamovich (1980) except for chrome coupling (Goding, 1976) employed in sensitizing sheep red blood cells. To 2.5 ml of agarose gel (A grade agarose, Biochimreactiv, Latvian S.S.R) at 42 °C 0.3 ml of antigen-sensitized sheep red blood cells and 0.1 ml of complement were added. The mixture was stirred rapidly and poured onto a 7 by 2 cm polystyrene plate (Medtekhnika, Moscow). Wells 2 mm in diameter were then cut in the solidified agarose layer, and 10 μl of serum in 1:10 dilution was added into each of them. Sera were regarded positive if they produced at least 4 mm zones of haemolysis.

Results

The total number of patient sera included in the study was 160. The results of indirect HI were as follows: 10 sera were SFS-positive (6.25%), 2 sera SFN-positive (1.25%), and 18 sera Karimabad-positive (11.25%) Noteworthy was the high percentage of Karimabad-positive sera.

The available amount of each serum was not enough to screen them for all 3 viruses in each test. Therefore, since indirect HI test suggested the highest incidence of Karimabad antibodies, we employed HI and RH tests to study this type of antibodies only. According to these tests, 24 were (15%) Karimabad-positive of the total of 160 sera. This suggests that HI and RH tests are also suitable for detecting antibodies to Karimabad virus, although the frequency recorded was somewhat less than in a case of indirect HI test. Thus, the number of Karimabad-positive sera was 18 (11.25%) for indirect HI, 15 (9.37%) for HI and 13 (8.17%) for RH test, respectively. Nine sera (Table 1) were positive in all the tests (No. 89, 93, 96, 101, 131, 140, 148, 152, and 156). Six sera were only indirect HI-positive (No. 132, 133, 142, 143, 147, and 153), all in low titres (1 : 10 or 1 : 20). Of 15 HI-positive sera, 3 reacted in this test only (No. 98, 103 and 115). In RH test, no antibodies were usually detected unless the indirect HI titre was 40 or higher. Two sera (No. 81 and 122), however, were positive in RH and HI tests but negative in indirect

Table 1. Titres of antibody to Karimabad virus in human sera as revealed by indirect HI, HI and RH tests

Serum No.	HI indirect	RH	HI
81	0	7**	20
89	80*	9	40
93	40	11	80
94	40	7	0
98	0	0	20
101	80	7	20
102	20	0	0
103	0	0	10
115	0	0	10
122	0	12	80
131	80	7	20
132	20	0	0
133	10	0	0
138	0	0	40
140	80	7	80
141	40	7	0
143	10	0	0
147	20	0	0
148	80	7	20
152	10	7	20
153	10	0	0
156	80	7	40
96	80	7	20
142	10	0	0

0 = megative result; * Dilution reciprocals; ** Plaque diameter (in mm).

HI test, which is within the technical error of the experiment. As already mentioned, we had only 0.1 ml of each serum at our disposal and, besides, we were not sure that the discs were soaked to saturation. This might be a source of error in preparation of the 1 : 10 dilution of serum in course of elution. Nontheless, the high degree of coincidence in the 3 or 2 tests suggest the reliability of our results.

Discussion

The result of this study is the first demonstration of the contact of Bangladesh population with the phlebovirus Karimabad. It also confirms the previous data on the occurence of antibodies to SFS and SFN viruses (Tesh *et al.*, 1976). The incidence of Karimabad antibodies (11.25%) was higher than that of SFS and SFN, and was close to that found in Egypt, Sudan and Iran (Tesh *et al.*, 1976) or in the Tadjik and Turkmenian Republics of the Soviet Union (Gaidamovich *et al.*, 1978). A high sensitivity of the indirect HI test should also be noted. The HI test is almost as sensitive as HI test, while RH test gives positive results only with the sera that were

positive in HI and indirect HI tests in dilutions of 1 : 20 — 1 : 40 and negative in dilution 1 : 10. This suggests a lower threshold sensitivity of RH test as compared to the other two methods. Inspite of this lower sensitivity, the RH test may be recommended for those cases when a large-scale serological or epidemiological screening is to be performed in a short time and with a small quantity of serum. Our data supplement the information on the arbovirus incidence in Bangladesh proving that besides the dengue and Japanese encephalitis viruses (Gaidamovich *et al.*, 1980*b*) also natural foci of the SFS, SFN and Karimabad phleboviruses exist in this country.

Acknowledgment. The autors wish to express their deep gratitude to Drs Rouf and Altafuddin for their assistance in collecting the sera samples.

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