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Prevalence of antibody against influenza A viruses in the Kren-Akorore, an Indian tribe of Central Brazil, first contacted in 1973

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SUMMARY

Influenza A antibodies in serum samples obtained in 1980 from two Indian populations in Central Brazil were compared. The Kren-Akorore, who were first contacted in 1973 and two years later transferred to the Xingu Indian Park (PIX), were compared with Indians from other tribes already living in the PIX before 1975. An analysis was made of the prevalence and distribution of antibodies against the influenza A viruses which have circulated in the civilized world since 1918. Antibodies to the early influenza A viruses were absent in both Indian populations, but A/Hong Kong/1/68 (H3N2) virus apparently circulated in the PIX. No antibody to influenza A/Bangkok/1/79 or to A/Brazil/11/78 (H1N1) was found in any of the sera, whereas antibodies to these viruses were commonly found in urban populations in Brazil. The evidence from influenza antibodies agrees with the information that the Kren-Akorore Indians had been living in complete isolation until 1973, when they were first contacted.

INTRODUCTION

The existence of an isolated Amazon Indian tribe named Kren-Akorore in the north of the state of Mato Grosso had been known for many years, but there was no particular reason to contact them. This situation changed when a new road crossing the Kren-Akorore territory was proposed. Contact was inevitable and was made in 1973. Two years later the situation became extremely bad for the Kren-Akorore, and they were transferred to the Xingu Indian Park (PIX), a federal Indian reserve 300 km to the east of the Kren-Akorore territory. In January 1975, 80 Kren-Akorore Indians entered the PIX (Baruzzi *et al.* 1977) and joined other Indian tribes already living there (Baruzzi & Franco, 1981).

The paper describes the prevalence of influenza A antibodies among the Kren-Akorore and other tribes that were living in the PIX before 1975. It was hoped that the influenza A antibody status might demonstrate the degree of

contact which had existed among the Kren-Akorore, the PIX tribes, and the general population in Brazil.

MATERIALS AND METHODS

Sera

Blood samples were collected in 1980 from 40 Kren-Akorore Indians and from 43 Indians belonging to other tribes that live in the PIX. The age distribution was approximately the same in the two groups, the average age of the Kren-Akorore being 28 and that of the other tribes 26. Sera were stored at −20 °C until tested.

Antigens

Prototype strains of the main influenza A subtypes were supplied by the WHO Collaborating Centre for Reference and Research on Influenza, London. These were: A/New Jersey/5/76 (HSw1N1), antigenically close to the swine influenza virus thought to have been responsible for the 1918 influenza pandemic; A/PR/8/34, representing the H1N1 subtypes which circulated in the world between 1934 and 1957; A/Singapore/1/57, representing the H2N2 subtypes which circulated in the world between 1957 and 1968; A/Hong Kong/1/68, representing the H3N2 subtype which has circulated from 1968 up to the present time.

Variants of influenza virus which have appeared since 1972 were obtained from various sources. A/England/42/72 (H3N2), prevalent between 1972 and 1974, was kindly provided by Dr R. D. Machado, Universidade Federal do Rio de Janeiro. A/Victoria/3/75 (H3N2), prevalent between 1975 and 1978, and A/Bangkok/1/79 (H3N2), prevalent between 1979 and 1983, were both provided by the WHO Collaborating Centre for reference and Research on Influenza, Atlanta; this Centre also supplied A/Brazil/11/78, a variant of A/USSR/90/77, an H1N1 subtype which had reappeared in the world after a gap of 20 years.

Serological tests

The sera were tested by haemagglutination-inhibition (HI) after prior treatment with receptor-destroying enzyme (received from CDC Atlanta). When all the sera inhibited haemagglutination by A/Singapore/1/57, a failure of the receptor-destroying enzyme to remove non-specific inhibitors was suspected. Potassium periodate and trypsin were then used to see if this treatment would be more effective than RDE. Besides this, single radial haemolysis was employed to confirm the results. All the serological methods were done as recommended by Kendal, Pereira & Skehel (1982).

RESULTS

The results of HI tests show that none of the sera from either the Kren-Akorore or other Indians in the PIX had antibodies against the early influenza A subtypes, A/New Jersey/1/76 (HSw1N1), A/PR/8/34 (H1N1) or A/Singapore/1/57 (H2N2). As described in the Methods section, in the first instance all the sera reacted with A/Singapore/1/57 (H2N2). As this result needed confirmation, alternative treatment was attempted to see if the apparently positive results were

Table 1. *HI antibody against the main subtypes of influenza A virus in sera from Indians living at the Xingu Indian Park (PIX), Central Brazil, 1980*

Indian population	Results of HI tests with the stated virus							
	A/New Jersey/1/76 (Hsw1Ni)		A/PR/8/34 (H1N1)		A/Singapore/1/57 (H2N2)		A/Hong Kong/1/68 (H3N2)	
	< 10	> 10	< 10	> 10	< 10	> 10	< 10	> 10
Kren-Akorore ($n = 40$)	40	0	40	0	40	0	38	2 (gmt = 1·2)*
Other tribes in PIX ($n = 43$)	43	0	43	0	43	0	19	24 (gmt = 5·9)

* Geometric mean titre.

Table 2. *HI antibodies against H3N2 variants in sera from Indians living at the Xingu Indian Park (PIX), Central Brazil, 1980*

Indian population	HI antibody results against stated variants					
	A/England/42/72		A/Victoria/3/75		A/Bangkok/1/79	
	< 10	> 10	< 10	> 10	< 10	> 10
Kren-Akorore ($n = 40$)	18	22 (gmt = 4·5)	40	0	40	0
Other tribes in PIX ($n = 43$)	17	26 (gmt = 7·2)	35	8 (gmt = 1·7)	43	0

due to a failure to remove non-specific inhibitors. After treatment of sera with potassium periodate all the sera were negative in HI tests, a result which was confirmed by single radial haemolysis when none of the sera gave any zone of haemolysis in plates containing A/Singapore/1/57 (H2N2) virus.

However, as shown in Table 1, antibody to the H3N2 subtype virus, A/Hong Kong/1/68, was demonstrated in over half the Indians who had been living in the PIX for several years. Two of the Kren-Akorore were also found to have antibody to this virus, but at only just detectable levels. It was suspected that this might reflect cross-reactive antibody from possible infection with later variants. The results of tests with later variants are shown in Table 2. With the first of the H3N2 variants, A/England/42/72, over half the sera from both the Kren-Akorore and the other tribes had clear evidence of antibody. The two members of the Kren-Akorore tribe who had the low titres to A/Hong Kong/1/68 (H3N2) were found to have higher titres to A/England/42/72 (H3N2), supporting the idea that the antibody detected against A/Hong Kong/1/68 (H3N2) was cross-reactive. The later variants A/Victoria/1/75 (H3N2) and A/Bangkok/1/79 (H3N2) do not appear to have circulated widely, if at all, in the PIX. The Kren-Akorore were found to have no antibody to either, and the few positive sera from the other tribes against A/Victoria/1/75 could well be cross-reactive due to earlier exposure to A/Hong Kong/1/68 virus rather than actual infections with this later variant. The low geometric mean titres in this group and in the Kren-Akorore with A/Hong Kong/1/68 virus would support this.

DISCUSSION

Influenza A virus has certain properties that made it the infectious agent of choice for a study of the kind described here. One of these properties is that the virus undergoes major antigenic change every so often to produce a new subtype, and lesser changes every two or three years to produce new variants. Another characteristic is its ability to spread. A new subtype may be found all round the world in a matter of a few months from its first appearance; all age groups are vulnerable and infection is accompanied by a specific antibody response. This antibody is long-lasting and acts as an indicator of previous infection, however distant in time.

There are now few, if any, peoples in the world who are completely isolated. Island populations may have infrequent contact with the mainland and their immunity to some infectious agents may be low, but usually they will be found to have some antibodies which indicate that their isolation has not been total (Tyrrell, Peto & King, 1967). In isolated mainland communities where contact with civilization has been thought to be unlikely, this may be shown to be not the case when evidence of infection can be demonstrated by serological means. In a study of a remote tribe of hunter-gatherers living in N. W. Tanzania (Bennett *et al.* 1973) antibody profiles were found to be not unlike those of people living in towns. Further investigation revealed that this tribe had occasional visits from persons with a link to the outside. This appeared to have been sufficient for the introduction of all the common infectious agents.

In the present study the search for antibody to influenza A was intended to indicate whether the Kren-Akorore had really had no contact with civilized man before 1973, and whether their exposure to outside populations after this had resulted in infections with currently circulating influenza viruses.

Only a few of the Indians of the Kren-Akorore tribe would have been alive when the pandemic of 1918 occurred, caused by a virus closely related to swine influenza virus (HSw1N1), and it is not surprising that no antibody to this virus could be demonstrated. However, many of the tribe would have been alive when it was possible for them to have been infected with subsequent influenza subtypes, H1N1, H2N2 and H3N2.

How extensive the spread of the H1N1 and H2N2 viruses was in Brazil up to 1968 is not known precisely, but serum surveys have shown that antibody is common in the age groups likely to have been exposed to these subtypes during their periods of prevalence. Takimoto, Barbosa & Salles-Gomes (1978) showed that antibody to the swine influenza virus (HSw1N1) was present in a considerable proportion of sera from people in São Paulo aged 50 or more. The isolation of influenza A viruses in epidemics in Rio de Janeiro in 1945, 1949 and 1951, during the period of prevalence of the H1N1 subtype, was reported by Lacorte, Monteiro & Loures (1949, 1951, 1957). In 1957 H2N2 viruses were isolated in Rio de Janeiro by Vasconcellos *et al.* (1957) and in several areas of Brazil by Lacorte, Monteiro & Loures (1958).

It is clear from our results that these Indians were not infected by H1N1 viruses when this virus reappeared in 1977, although H1N1 viruses were isolated in Belém (Mello, Freitas & Pinheiro, 1978) and São Paulo (Takimoto *et al.* 1982); and

serological surveys have shown that this virus circulated among young people under 20 years of age in Rio de Janeiro (Chaves, Nascimento & Pereira, 1982).

Information is also available on the spread of the H3N2 subtype, which first appeared in 1968. Candeias & Pereira (1972) described the extensive circulation of the prototype A/Hong Kong/1/68 virus in São Paulo, and a serological study of Indians living in the PIX at that time indicated that a high proportion had been infected (Pereira, Baruzzi & Carvalho, 1971). However, the Kren-Akorore Indians had not yet been contacted and their introduction into the PIX occurred only in 1975.

The H3N2 variant prevalent in 1973–4 was one designated A/England/42/72. It is known that it circulated in Brazil in 1973 in Rio de Janeiro (Machado, 1974) and in São Paulo (Anraku *et al.* 1977). This appears to have been the first influenza virus ever encountered by the Kren-Akorore, and their exposure led to over half of them becoming infected.

The next major variant, A/Victoria/3/75, was also identified in Brazil, in Rio de Janeiro (Marques *et al.* 1978) and in São Paulo (Takimoto *et al.* 1982), but the Kren-Akorore appear to have escaped infection entirely and it is doubtful if this variant ever circulated in the PIX. The antibody demonstrated in the sera of the tribes long established there would seem more likely to reflect their extensive exposure to earlier viruses of the H3N2 subtype.

Within a subtype, such as the H3N2, cross-reacting antibody between variants does not allow a precise identification as to which virus circulated; but had, for example, A/Victoria/3/75 reached the PIX, antibody would surely have been detected in the sera of the Kren-Akorore.

Viruses like the next significant variant, A/Bangkok/1/79, were isolated in Rio de Janeiro, Belém and São Paulo (Kendal, personal communication), and Nascimento *et al.* (1983) showed that antibodies were frequent in all age groups in Rio de Janeiro in 1980. No antibody to this virus was found in the Indian sera. It seems that this variant had not yet spread to the PIX when the blood samples were taken in 1980.

Our results suggest that the Kren-Akorore really were a completely isolated tribe until their first contact with civilization in 1973. Their exposure to influenza seems to have occurred shortly after, causing infection in over half of the tribe with the influenza A variant circulating at that time.

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