

## The Study of Coronavirus OC43 Infection

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**OBJECTIVE:** To determine the past incidence of coronavirus infections in people living in the tropical environment of Thailand.

**BACKGROUND:** Coronaviruses are a newly described group of RNA viruses isolated from patients with upper respiratory tract illness. At present, at least 3 distinct strains of coronaviruses have been isolated—B814, 229E, and NIH O.C. (organ culture) strains. Strain B814 was isolated and has only been grown in human embryonic tracheal organ cultures. Strain 229E was isolated in human cell cultures and can be grown in various human derived cell lines. The NIH O.C. viruses (OC38 & OC43), originally isolated in human organ culture, have been successfully adapted to grow in suckling mouse brain. Although the antigenic characteristics of these 3 prototype viruses are not completely described, strains 229E and OC43 appear to be antigenically distinct.

Coronaviruses produce upper respiratory disease as shown by studies in England and the United States. Infections tend to occur in autumn and winter months in temperate climates and in some years about 20% of upper respiratory diseases are associated with coronavirus infections. Although their detailed epidemiology is not yet clear, the URI's produced by this virus have a tendency to appear as epidemic outbreaks in addition to more sporadic illness. Serological surveys of patients with lower respiratory disease in temperate and tropical environments have been negative for coronavirus infections. The incidence of coronavirus URI in the tropics is still unknown.

**MATERIALS AND METHODS:** Virus Seed and HA Antigen Preparations: Strain NIH OC43 was obtained from Dr. D.J. Tyrrell in the lyophilized form of a 10% suspension of infected mouse brain containing 5% calcium gluconate lactobionate. After reconstitution in 1 ml of phosphate buffered saline, pH 7.2, the suspension contained approximately  $10^{4.5}$  suckling mouse lethal dose<sub>50</sub> (SMLD<sub>50</sub>). A dose of 0.02 ml containing  $10^3$  SMLD<sub>50</sub> was inoculated intracerebrally into 3–5 day old suckling mice. Infected mice showing typical encephalitic symptoms, usually within 48–60 hours after inoculation, were sacrificed and their brains pooled and made up into the following antigen preparations.

a. Virus seed. A 10% suspension of brain was made in tryptose phosphate broth containing 0.5% gelatin. Brain suspensions were distributed in 2 ml aliquots into ampules, lyophilized and then kept at  $-20^{\circ}\text{C}$ . The infectivity titer of the suckling mouse brain seed prepared was  $10^{4.6}$  SMLD<sub>50</sub>.

b. Hemagglutinating antigen: Antigen was prepared by making 10% suspensions of infected brain in phosphate buffered saline pH 7.2, which were clarified by refrigerated centrifugation at 600g for 20 minutes, kept at  $-70^{\circ}\text{C}$ , and used as HA antigen. As an antigen control, normal mouse brains were pooled and made up to 10% suspension in veronal buffered diluent and clarified in the same manner as the antigen. The hemagglutinating antigen prepared agglutinated chicken red blood cells at a 1:640 to 1:1280 dilution.

Hemagglutination inhibition test. The reaction was carried out by microtiter technique at room temperature, using adult chicken erythrocytes at 0.5% concentration with PBS pH 7.2 as a diluent and 4 units of HA antigen. Sera were heated at  $56^{\circ}\text{C}$  for 30 minutes prior to dilution for the test.

Neutralization test. Neutralizing antibody was measured by adding serial virus dilutions with 1:2 dilution of serum tested. The virus-serum mixture was incubated for 1 hour in room temperature before inoculation into suckling mice. A neutralization index (NI) of  $1.7 \log_{10}$  or greater was considered to reflect neutralizing antibody.

Sucrose density gradient centrifugation. A 0.25 ml volume of human serum was layered on a preformed 10–40% sucrose density gradient and centrifuged at 35,000 RPM for 18 hours in an SW-39 rotor. Twelve fractions were collected, and each fraction tested for OC43 HI antibody and IgG and IgM concentrations.

DEAE–Sephadex extraction. Selected sera were extracted by the DEAE–Sephadex method described by Altemeier, et al (Applied Microbiology 19:785, 1970) which removes all serum proteins except IgG. Purity of the extracts obtained was tested by immunoelectrophoresis of whole and treated serum using goat anti-whole human serum.

RESULTS: Prevalence of Coronavirus OC43 HI antibody. Two populations were studied; 832 adult male blood donors in Bangkok and 476 residents of the Chiangmai Valley. This latter group involved residents of village Maerim (88), Sanpatong (86), Sankampang (81), Saraphi (104) and school children in Chiangmai City (117). As shown in Table 1, only about 7% of Bangkok adults and 13% of Chiangmai residents lacked OC43 HI antibody. The majority of both populations had antibody at a titer between 1:10 and 1:40. The age-prevalence of OC43 antibody is shown in Table 2. Although the majority of children less than 2 years lacked antibody, 65% of the 3–4 year olds tested, 85% of the 5–9 year olds tested, and 95% of the population 10 years or older had OC43 HI antibody.

Persistence and acquisition of OC43 HI antibody. Sera from Chiangmai villagers were collected in November 1969, March 1970, July 1970, and November 1970. Sequential sera over a 9 to 12 month period from 278 villagers were tested for antibody persistence. As shown in Table 3, 248 villagers had HI antibody in November 1969, and 90% had no significant change in antibody titer; 21(9%) of these had  $\geq 4$  fold antibody rises. Of the 30 villagers lacking HI antibody to OC43, 13(43%) had significant rises in antibody suggesting infection with this virus or an antigenically related virus during the year.

Identification of HI activity in human sera as antibody. Since sera from most people sampled had HI activity, it was important to determine whether this activity could be related to antibody. Results of fractionating 4 human sera by sucrose density gradient centrifugation are shown in Table 4. HI antibody was found only in fractions containing IgG.

In addition, 7 sera with HI titers  $\geq 1:40$  were extracted with DEAE–Sephadex. Serum electrophoresis of 2 extracted sera revealed 2 lines—a strong IgG line and a faintly visible line toward the anode. All 7 sera tested had HI activity after DEAE–Sephadex extraction, suggesting that serum HI activity was associated with IgG.

Neutralization tests were performed on sera from 16 individuals. Sera of the 9 persons tested who lacked HI antibody lacked neutralizing activity, while sera of 6 of the 7 persons who had HI activity had neutralizing activity (Table 5).

SUMMARY: The association of HI activity with the IgG fraction of serum and the correlation between serum HI activity and serum neutralizing activity suggests that the assay used is detecting HI antibody rather than non-specific inhibitors. The antibody data is consistent with the possibility that viruses identical or serologically related to OC43 commonly cause infections in Thais and indeed infect most persons by 3–4 years of age.

Table 1.  
Distribution of Coronavirus OC43 Antibodies in 2 Thai Populations

HI antibody titer	Adults (Bangkok)		All ages (Chiangmai)	
	No. with HI titer	(%)	No. with HI titer	(%)
<10	61	7.2	63	13.4
10	126	15.5	93	20.1
20	299	35.9	181	38.5
40	254	30.5	102	21.3
80	180	9.6	29	5.8
160	12	1.4	3	0.6
Total	832		476	

Table 2.  
Prevalence of Coronavirus OC43 HI Antibody by Age, Chiangmai Population.

Age	No. studied	No. with antibody	% with antibody
0-2	15	2	12.5
3-4	32	20	64.5
5-9	183	155	84.7
10-14	84	80	95.2
15-19	37	35	94.6
20-29	32	30	93.7
30-39	75	74	98.6
40	18	18	100.0
Total	476	409	86.8

Table 3.  
Persistence of Coronavirus OC43 HI Antibody, Chiangmai Villages

Initial Antibody status	No.	No. without rise or fall	No. with $\geq 4$ -fold rise	No. with $\geq 4$ -fold fall
$\geq 10$	248	221	21	6
<10	30	17	13	-

Table 4.  
 Immunoglobulin Concentration and HI Antibody Activity of Fractions  
 from Sucrose Gradient Centrifugation

Serum No.	HI Whole Serum	Fraction No.	Immunoglobulin IgG mg%	concentration IgM mg%	HI titre		
1849	<10	3	<10	23	<4		
		4	<10	24.5	<4		
		5	<10	30.5	<4		
		6	—	—	<4		
		7	<10	0	<4		
		8	—	—	<4		
		9	94	0	<4		
		10	20	0	<4		
		2418	<10	3	<10	0	<4
				4	<10	16	<4
5	<10			13.8	<4		
6	—			—	<4		
7	<10			0	<4		
8	138			0	<4		
9	—			—	<4		
10	<10			0	<4		
1856	40			3	<10	0	<4
				4	<10	16	<4
		5	—	—	<4		
		6	10	0	<4		
		7	10	0	4		
		8	74	0	16		
		9	82	0	32		
		10	—	—	8		
		2410	40	3	<10	20	<4
				4	<10	24.5	<4
5	—			—	<4		
6	<10			0	<4		
7	<10			0	4		
8	58			0	16		
9	74			0	32		
10	—			—	4		

Table 5.  
 Correlation between OC43 HI and Neutralizing Antibody

HI Antibody	Log Neutralization Index	
	<1.7 (No.)	≥ 1.7 (No.)
<10	9	0
≥ 10	1	6