Acta virol. 34 : 346—352 1990

ANTIBODIES TO HUMAN CORONAVIRUSES 229E AND OC43 IN THE POPULATION OF Č.R.

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Received January 27, 1988

Summary. — Of 1200 human sera tested by enzyme-linked immunosorbent assay (ELISA), 53% contained antibodies to human coronavirus (HCV) 229E and 88% to HCV OC43. The sera were from persons aged 13 months to 80 years, both males and females, and were collected in four different regions in 1986. The percentage of positives increased with increasing age and tended to vary according to the geographic area. Additional paired human sera from 218 patients with acute respiratory diseases (ARD) were collected between October 1986 and June 1987 in Prague. Significant antibody rises to HCV strain 229E were detected in 7 (3.2%) patients 9 months to 17 years old, to HCV strain OC43 in 4 (1.8%) patients under 2 years of age.

Key words: *coronaviruses*; *ELISA*; *antibodies*

Introduction

To date, no data have been published on coronavirus circulation among the population of Č.R., the presented results being the first contribution in this field.

Materials and Methods

Sera. A set of 1200 sera from healthy persons of both sexes (13 months to 80 years old) originated from Prague (355 sera), Hradec Králové (345 sera), Ostrava (280 sera), and from rural areas of Brno (220 sera). The donors were divided into the following age groups: 2—3 years, 4—5 years, 6—7 years, 8—9 years, 10—14 years, 15—19 years, 20—29 years, 30—39 years, 40—49 years, 50—59 years, 60—80 years. Additional paired sera were coming from 218 patients in Prague, aged 2 days to 66 years, who had been treated as in-patients or out-patients for acute respiratory infections (ARD) in the period between October 1986 and June 1987. Most of them (192 patients) were not older than 30 years, including 88 children under 3 years of age. First serum samples were collected in the acute stage of the illness, second samples were obtained 3 weeks later.

Human coronavirus (HCV) strains 229E and OC43, representatives of two antigenic groups of the human coronavirus, were obtained from K. McIntosh, National Institutes of Health, Bethesda, U.S.A. in 1969. HCV 229E was passaged on human diploid cell cultures LEP 19 (Institute for Sera and Vaccines, Prague), HCV OC43 on 5-day-old suckling mice intracerebrally.

Table 1. Antibodes to HCV 229E and OC43 in the sera of persons from four regions of Č.R.

Region	Percentage of persons with antibodies to HCV	
	229E	OC43
Praha	44	90
Hradec Králové	61	93
Ostrava	39	90
Brno (rural area)	72	76
Total	53	88

Preparation of antigens. Both virus strains were subjected to 30—60 min centrifugation at 3000—5000×g, followed by 2 hr centrifugation of the supernatant fluid at 130 000×g. The sediment was dissolved (or sonicated) in PBS to obtain a 100-fold concentration of the original volume of medium (or 10% brain suspension). The antigen OC43 was additionally centrifuged for 5 min at 3000×g and supernatant was used as antigen. The protein concentration of antigens ranged in the working dilution from 4.5—5.5 μg per 1 ml.

ELISA. Polystyrene microtitration plates type P (KOH-I-NOOR) were used. All reagents were applied in amounts of 100 μl per well; the antigens were diluted with bicarbonate buffer pH 9.6. Antigen binding proceeded overnight at 4°C under humid conditions. For washings 0.85% solution of NaCl with Tween-20 (1 : 2000) was used. Sera and conjugates were diluted with PBS pH 7.4, containing Tween-20 (1 : 2000) and 1% bovine albumin. The sera diluted (from 1 : 100 up) directly in the wells were left overnight at 4°C. After washing, swine anti-human peroxidase-

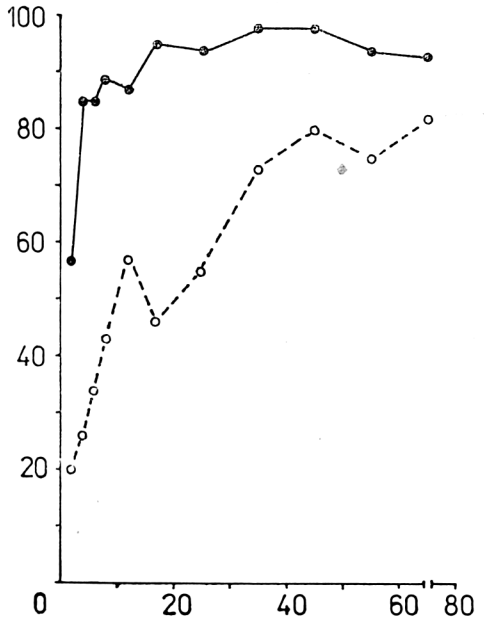

Fig. 1
Antibodies against HCV 229E and OC43 in humans from 4 areas of Č.R.
○— — —○ 229E
●——— ● OC43
Abscissa: percentage of positive persons: ordinate: age in years

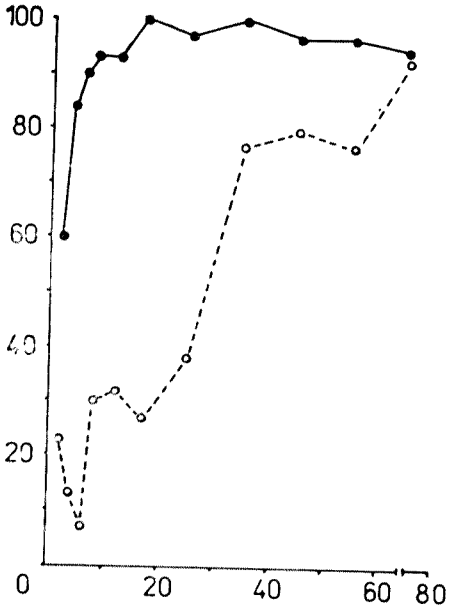

Fig. 2
Antibodies against HCV 229E and OC43 in humans from Prague
For legend see Fig. 1

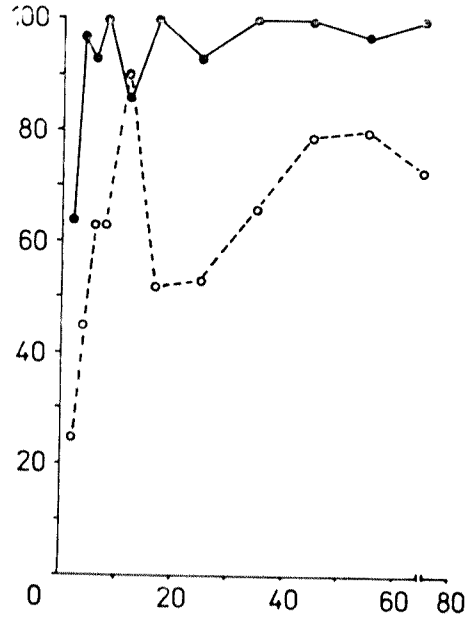

Fig. 3
Antibodies against HCV 229E and OC43 in humans from Hradec Králové
For legend see Fig. 1

Table 2. Elevations in IgG antibody titres to HCV 229E and HCV OC43 occurring in patients with acute respiratory diseases

Patient	Age	Month of illness	Antibody titre to HCV 229E	Antibody titre to HCV OC43	Diagnosis, major symptoms
1	2m.	XII	100*/ < 100	100/400	bronchitis acuta tussis
2	1y. 3m.	II	<100/ < 100	100/400	rhinopharyngitis febris, rhinitis tussis
3	1y. 4m.	IV	400/400	100/400	laryngotracheobronchitis febris, rhinitis, tussis
4	2y.	XII	<100/ < 100	100/400	bronchopneumonia febris, rhinitis, tussis
5	9m.	III	200/800	<100/ < 100	bronchitis obstipans rhinitis, tussis, conjunctivitis
6	11m.	IV	400/3200	1600/3200	bronchopneumonia febris, rhinitis, tussis, tonsilitis
7	10y.	III	100/800	1600/800	others febris, cephalalgia, lymphadenitis, emesis
8	15y.	II	200/800	200/200	others febris, cephalalgia, conjunctivitis, emesis, diarrhoea
9	15y.	XII	200/800	1600/1600	rhinolaryngitis febris, rhinitis, tussis, myalgia, cephalalgia, conjunctivitis
10	15y.	II	100/400	1600/1600	bronchopneumonia febris, tussis, cephalalgia
11	17y.	XII	100/800	400/400	rhinolaryngitis febris, rhinitis, tussis, conjunctivitis, lymphadenitis myalgia

* Antibody titre in acute/convalescent serum

Notes: In some patients simultaneous infection with other respiratory viruses was demonstrated:
patient 2 adenovirus type 2
patient 4 adenovirus type 2
patient 5 adenovirus and parainfluenza virus type 3
patient 6 — influenza virus A (H3N2)
patient 10 — parainfluenza virus type 3

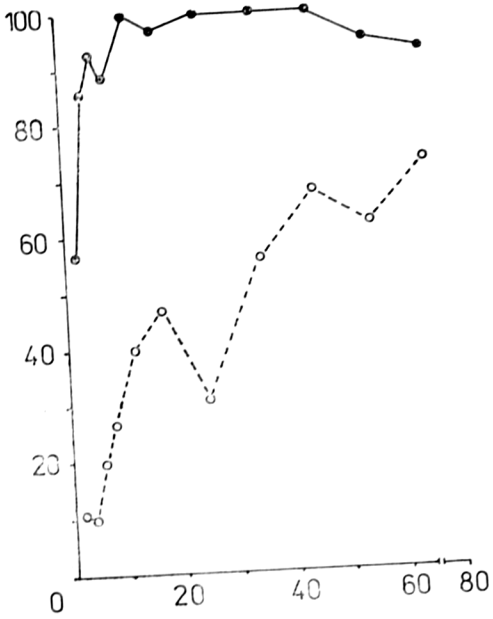

Fig. 4
Antibodies against HCV 229E and OC43 in humans from Ostrava
For legend see Fig. 1

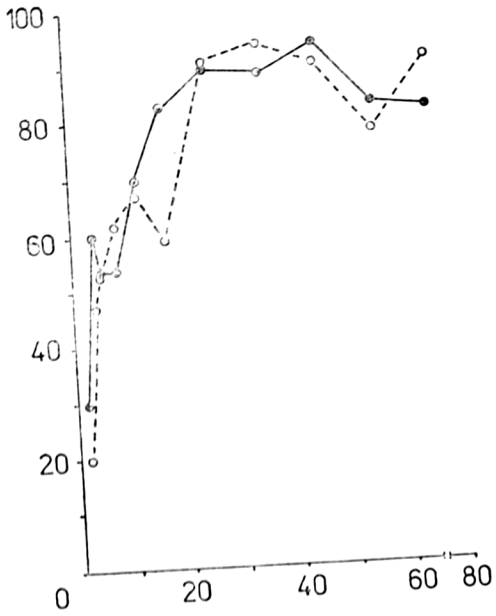

Fig. 5
Antibodies against HCV 229E and OC in humans from Brno (rural area)
For legend see Fig. 1

labelled IgG (SEVAC, Prague) was added and incubated for 2 hr at 37°C under humid conditions. This was followed by 5 washings, addition of substrate (0.1% aqueous solution of 5-amino salicylic ac d with pH adjusted to 6.0 by adding 1N NaOH; 20 μl of 30 % H_2O_2 per 100 ml was added immediately prior to use) and 1 hr incubation at room temperature. The results were read at 450 nm using a MicroELISA Reader. The ELISA antibody titres were expressed as the reciprocal of the highest serum dilution yielding optical density of 0.25.

Results

The frequencies of antibodies to HCV 229E and OC43 in healthy individuals from four different geographic areas of Č.R. are presented in Table 1. The positive examinees irrespective of the geographic area are summarized in Fig. 1. It shows that in the youngest age category antibodies to strain 229E were present in 20% and to strain OC43 in 57% of tested sera. This positive rate was age-dependent tending to rise with increasing age. As evident from Figs. 2 through 5, the age-specific positive rates in different geographic areas varied, especially in the Brno rural area (Fig. 5) where no difference in the number of persons with antibody to HCV 229E and with HCV OC43 antibody existed. Generally, HCV OC43 antibodies were far more frequent than antibodies to HCV 229E.

The behaviour of HCV 229E and OC43 antibody titres in the paired sera of patients with acute respiratory infections is characterized in Table 2. In a group of 218 patients examined between October 1986 and June 1987, a significant rise in IgG antibody titres to HCV 229E occurred in 7 (3.2%) patients, to HCV OC43 in 4 (1.8%) patients. The antibody titre elevations were recorded from December to April; they occurred as concerns strain OC43 in children aged 3 months to 2 years, as concerns strain 229E in the age of 9 months to 17 years.

Discussion

Frequent detections of HCV antibodies in a set of human sera are suggestive of a relatively extensive circulation of the virus in the Č.R. population. The percentage of positivities increased with increasing age, with very fast positive rises of antibodies to HCV OC43 (57% of positives in the age group of 2—3 years versus 85% in children 4—5 years of age). In general, antibodies to HCV OC43 were detected significantly more often (88% of examinees) than antibodies to HCV 229E (53% of examinees). However, this difference in positive antibody rates favoured the antibody to HCV OC43 as result of cross reactivity with enteric CV because of assumed antigenic relatedness of this virus with the whole-virion OC43 antigen used in this study (Gerna *et al.*, 1984).

The positive rates of antibodies to HCV OC43 and 229E antigens in sera from Brno area were almost identical (76% vs 72% of all examinees), breaking thus the pattern common in the remaining areas. This can be explained by the fact that Brno area was the only one with the predominance of rural population where the process of virus circulation might have a quite different course. We are well aware of the fact that our survey is clearly suggestive

of the substantial CV circulation in the population but can hardly serve as an indicator of the degree of population resistence to CV infection and disease. In this study we examined antibody levels in the serum but not secretory antibodies which are known to play an important role in virus induced ARD. According to literature data, the identical CV is capable of including a new illness in man as early as few months after preceding CV infection (Macnaughton, 1982).

Examinations of ARD patients from Prague revealed significant antibody rises to HCV 229E up to 17 years of age, to HCV OC43 in children under 2 years only. These findings, at least to a certain extent, improve our survey data on positive rates in Prague (Fig. 2) which show that antibodies to HCV 229E are relatively infrequent in persons up to the age of 25 years. However, if there were a higher proportion of older persons among our ARD patients, coronaviruses are likely to occur in higher age groups, too. The examined patients were mostly young: 192 examinees out of 218 were not over 30, including 88 children under 3 years of age. However, the positive rate was likely to show year-to-year variations, depending on previous epidemic outbreaks of infection.

References

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