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PREVALENCE OF ANTIBODY TO HUMAN CORONAVIRUSES 229E, OC43 AND NEONATAL CALF DIARRHEA CORONAVIRUS (NCDCV) IN PATIENTS OF NORTHERN ITALY

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A seroepidemiological study for detection of antibody to human coronaviruses OC43, 229E, and neonatal calf diarrhea coronavirus (NCDCV), has been carried out using sera collected from hospitalized patients or healthy persons through routine laboratory tests in Northern Italy. Patients tested were children and adults with different pathological diseases. Antibody detection was performed by using an indirect immunoperoxidase staining technique (for all viruses) and, in the case of OC43 and NCDCV, antibody detection was obtained even with a hemagglutination inhibition test and a plaque reduction neutralization assay. Results obtained show a significant difference in the prevalence of antibody to 229E between children and adult group. Furthermore, a different titer was observed, within the two groups, between patients affected by hematological diseases (leukemia) and patients with other diseases. Finally, our data seem to confirm previous studies reporting a very high prevalence of antibody to coronavirus OC43 but a less detectable seropositivity to coronavirus 229E.

INTRODUCTION

Human coronaviruses OC43 and 229E are well known as etiological agents of respiratory infections generally affecting the upper respiratory tract and responsible for common colds during winter-spring seasons Isolation of these viruses is particularly difficult and, consequently, all epidemiological and reliable data about their diffusion are based upon serological studies performed using reference strains of cell culture adapted coronaviruses. To date, viruses are included in the coronavirus group for some properties (14):

i) the presence of one molecule of infectious ssRNA of MW ranging from 5.5×10^6 and 6.1×10^6 daltons with polyadenylated 3' terminus;

ii) morphology showing pleomorphic enveloped particles with diameter ranging from 80 to 160 nm and surface projections (spikes) protruding 12-24 nm from envelope;

iii) common physicochemical properties such as banding at 1.16.-1.23 g/cm^3 in sucrose gradients, disruption by ether, chloroform, and detergents, removal of surface projections by bromelain and trypsin.

Consequently, viruses could be included in the coronavirus group independently of their antigenic cross-reaction but, particularly, for their characteristic morphology (spikes), as revealed by electron microscopy.

Human coronavirus OC43 and 229E are the prototypes of all coronaviruses affecting humans. No particular relatedness has been, to date, re-

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ported between these two viruses; furthermore, in vitro, 229E shows an easy growth in human lung fibroblast cell cultures with evident cytopathogenic effect; on the other hand, OC43 can grow in cell cultures (VERO cells or human fibroblast cell cultures) only after adaptation of virus strains cultured in suckling mouse brain. Despite their morphological and antigenic differences, these viruses are responsible, in humans, for common colds not easily distinguishable by symptoms or seasonality of appearence.

Previous reports showed a high prevalence of antibody to human OC43 and 229E coronavirus in sera collected from patients of northern Italy (1, 2). Results were obtained using very complex tests such as Immune-Adherence Hemagglutination (IAHA), in the case of 229E specific antibody detection, or not highly sensitive and reliable tests, as complement-fixation and hemagglutination-inhibition, for OC43 antibody detection (1, 2).

Another interesting test was utilized for serodiagnosis of OC43 infection, based upon a microneutralization method, but, despite its high degree of specificity and sensitivity, its performance was economically very expensive (3). Furthermore, the presence of a non specific inhibitor for OC43 and NCDCV coronavirus present in mammalian sera and in fetal calf serum (FCS) that can be removed by phospholipase C (PLC) treatment, prompted us to develop a new sensitive and specific hemagglutination inhibition test and a new plaque reduction assay (4). By the use of these new tests for OC43 and by an indirect immunoperoxidase staining technique (IIP), for 229E, we carried out a seroepidemiologic study in order to verify previously reported data (2, 3).

In addition, the strict antigenic relatedness between OC43 and NCDCV (5) suggested the investigation of the prevalence of a human immune response against this bovine virus.

MATERIALS AND METHODS

Viruses

Coronavirus 229E was obtained from American Type Culture Collection (ATCC - Rockville, Md., USA) and adapted to grow in human embryo lung fibroblast cells (HELF).

OC43 and NCDCV coronaviruses were cell culture adapted strains originally obtained from suckling mouse brain adapted virus supplied by Dr. H.S. Kaye (C.D.C., Respiratory Virology Unit, Atlanta, Ga., USA) and Dr. C.A. Mebus, (Dept. of Veterinary Science, University of Nebraska, Lincoln, Neb., 68503). Adaptation to growth in HELF has been previously reported (5).

Sera

Three hundred and twenty nine sera were collected from hospitalized patients and healthy persons having routine laboratory tests in the University Hospital of Pavia, Italy.

Sera from six age groups were studied for specific antibody to 229E, OC43 and NCDCV coronaviruses. A number of sera were also collected from leukemic infants and children.

Indirect immunoperoxidase staining technique (IIP)

HELF cells were cultured in sterile microplates for tissue culture; when the monolayer was confluent, all cell wells were infected with 0.05 ml of a suspension of 229E containing 100 infectious virus particles, incubated at 33°C for 60 min. To each well was then added 0.1 ml of 199 medium containing 2% fetal calf serum. Infected cultures were incubated at 33°C for 36 hours, washed 3 times with PBS Dulbecco B and then fixed with absolute ethanol. Fixed preparations were immediately used for IIP or stored at —70°C until use.

IIP for specific human antibody detection was performed as previously described for other viruses (5-8), except that the 3-amino -9-ethyl-carbazole-H_2O_2 colour developing system was used.

Hemagglutination-inhibition test (HI)

Antibody titer to OC43 and NCDCV was detected by HI following a reported procedure (10) and a suggested treatment of sera in order to avoid non-specific inhibitors of OC43 and NCDCV hemagglutination (9), except that the inactivation of phospholipase C (PLC) with heat treatment (56°C for 60 min) was used in substitution of phenanthroline treatment.

Plaque reduction assay (PRA).

PRA for OC43 and NCDCV antibody detection was performed as previously described (4). Briefly, a titered virus suspension, known to contain 40-50 infectious units of virus, was mixed with an equal volume of serial twofold dilution of PLC - treated sera diluted in serum - free 199 medium with addition of glutamine, penicillin, streptomycin and gentamycin.

Mixtures were incubated at 33°C for 60 min and then inoculated in wells of microplate cell culture of HELF (0.05 ml/well). After adsorption at 33°C for 60 min, plaquing medium (medium 199 with fetal calf serum at the final concentration of 10% and 5% for OC43 and NCDCV, respectively), was added for 24 h and then cells were fixed with absolute ethanol and stained by the indirect immunoperoxidase staining technique described using mouse hyperimmune sera.

RESULTS

As reported in Figure 1, antibody to 229E coronavirus was present in a high percentage of sera studied; its prevalence and the specific antibody titer increased with increasing age. Antibody titers in leukemic patients seemed to be consistently lower than in other patients (Figure 1). The results obtained were not particularly different from those observed in previous reports (2, 11, 12), except for the fact that, because of the greater sensitivity of IIP technique used, antibody titers were greater. Furthermore, the IIP test was very easy to read, as shown in Figure 2, very simple to perform and can avoid some problems (i.e. false results and/or difficulties in interpreting data) frequently occurring using other conventional and tedious methods like complement fixation and immune adherence hemagglutination.

Finally, the IIP technique for 229E antibody detection had other advantages: it allowed

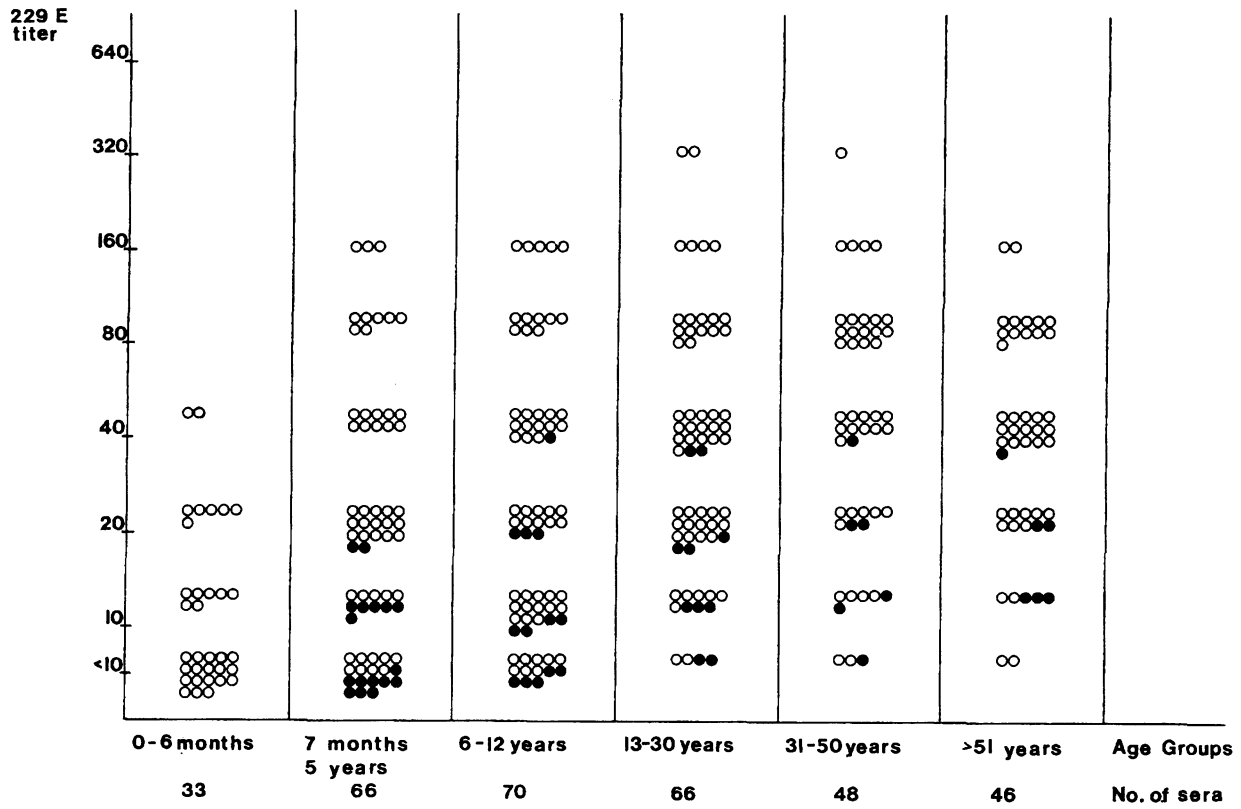

Figure 1. - Reciprocal of antibody titer to 229E coronavirus as detected in different age groups by IIP.
● Antibody to 229E in sera from leukemic patients.
○ Antibody to 229E in non leukemic sera.

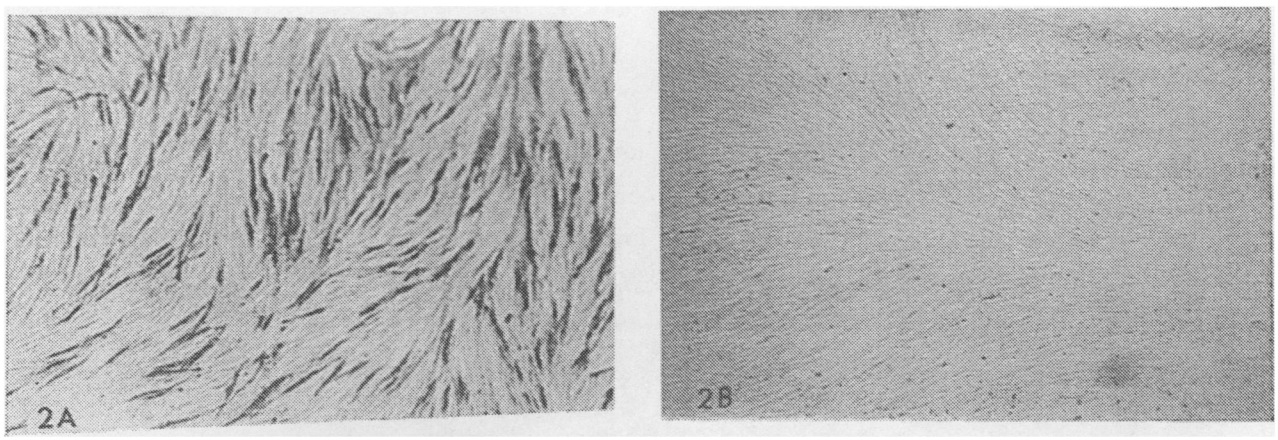

Figure 2. - IIP for 229E antibody detection on infected human embryonic fibroblasts. 2A: positive human serum; 2B: negative serum.

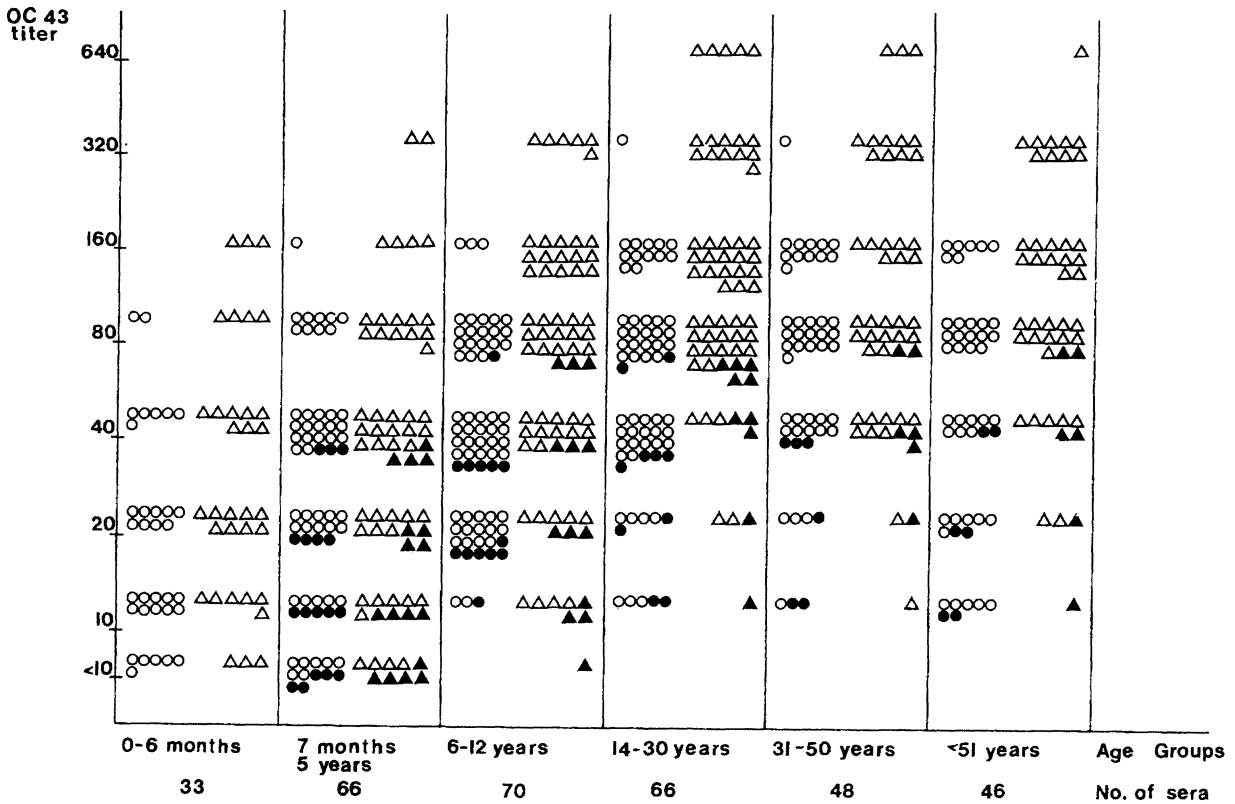

Figure 3. - Reciprocal of antibody titer to OC43 as detected in different age groups by HI and PRA tests. Antibody to OC43 in leukemic patients as detected by HI ● and PRA ▲. Antibody to OC43 in non leukemic patients as detected by HI ○ and PRA △.

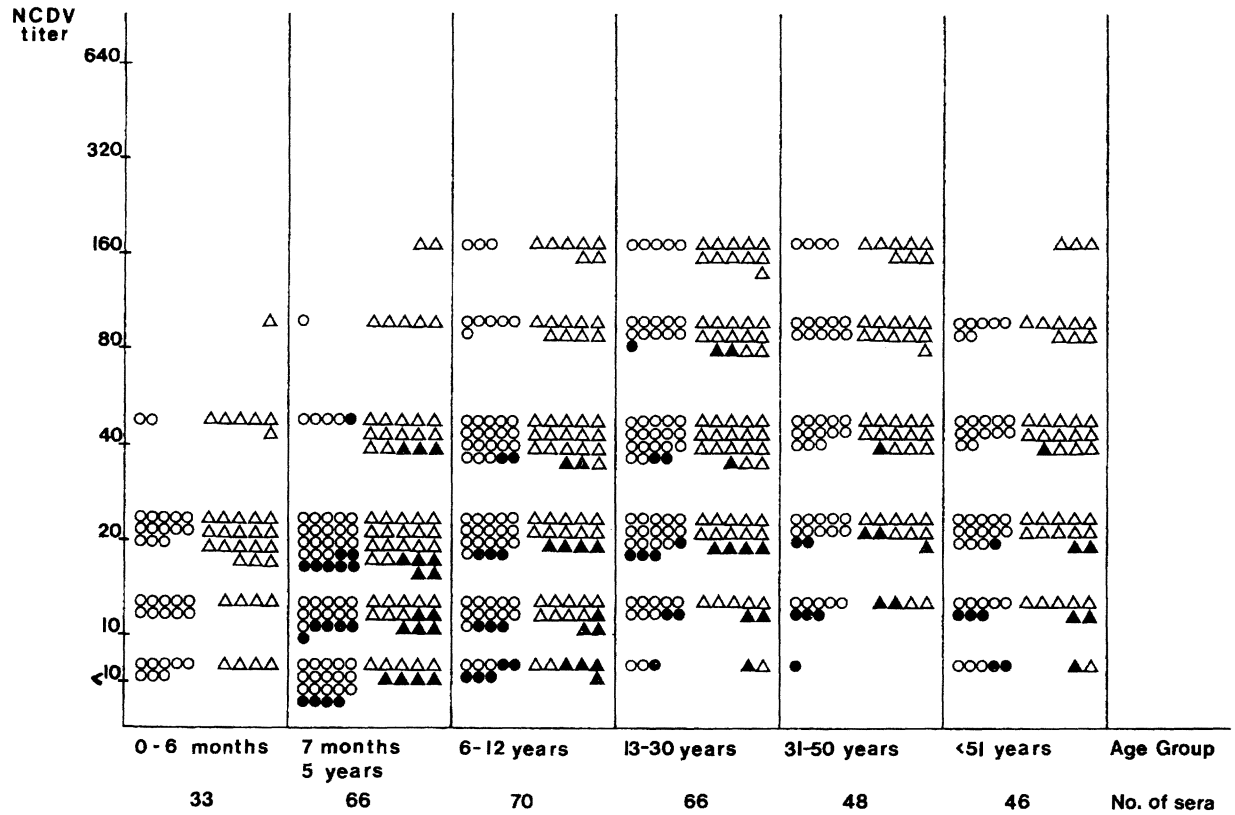

Figure 4. - Reciprocal of antibody titer to NCDCV as detected in different age groups by HI and PRA tests. Antibody to NCDCV in leukemic patients as detected by HI ● and PRA ▲. Antibody to NCDCV in non leukemic patients as detected by HI ○ and PRA △.

prolonged storage of infected and fixed substrates, and was inexpensive.

Antibody to coronavirus OC43 was very frequent in the studied population. Absence of specific immune response was an exceptional finding in the course of our survey.

Detection of specific antibody by using hemaglutination inhibition test (HI) gave results not particularly different from those previously reported for sera collected from patients of the same geographical area (1) thus confirming a very high prevalence of antibody response to this coronavirus.

In parallel to OC43 antibody detection, we investigated the immune response to NCDCV, a bovine coronavirus antigenically strictly related to OC43. Antibody titer to OC43 was generally two-fourfold dilutions higher than the titer to NCDCV. We never observed sera positive for OC43 and negative for NCDCV and viceversa.

The PRA test showed a greater sensitivity than HI (titer obtained were generally four-fourfold dilutions higher) but the immune response to homologous (OC43) and heterologous (NCDCV) virus were always in the same proportions as observed by using the HI test (Figures 3 and 4).

Even in the case of OC43 and NCDCV coronaviruses, sera from leukemic patients showed specific antibody titers lower than titers observed in non leukemic patients in the same group of age.

As in the case of IIP for 229E, results obtained by PRA for OC43 and NCDCV were easily readable and interpretable as shown by Figure 5.

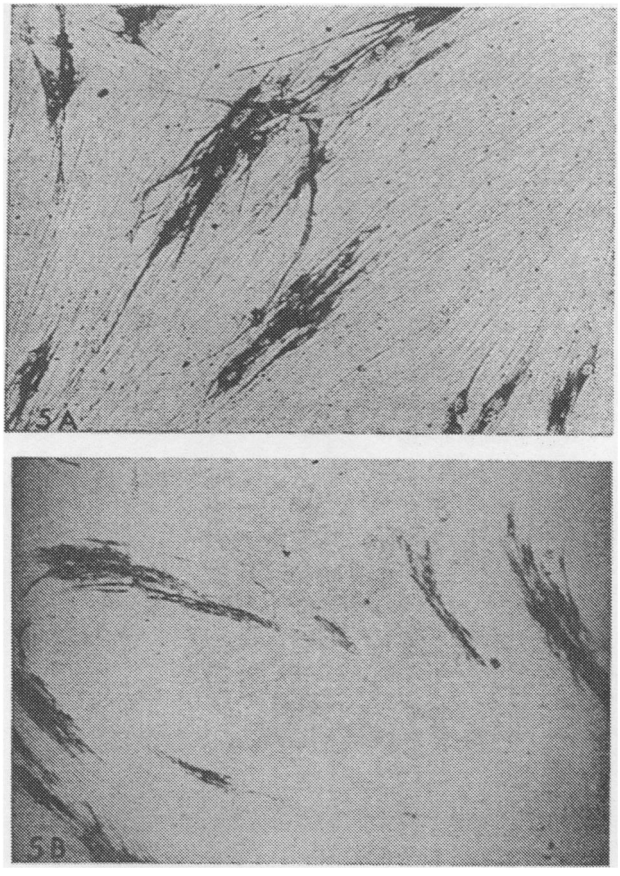

Figure 5. - PRA for OC43 and NCDCV positive sera identification. 5A: OC43 positive serum; 5B: NCDCV positive serum.

DISCUSSION

Coronaviruses are members of a group of virus characterized by a high incidence in many mammalian species, including man.

Human coronaviruses are generally involved in the common colds and in upper respiratory tract disease: OC43 and 229E are the most important and studied human coronaviruses.

In the present work we searched for antibody titer to 229E and OC43 coronavirus using very sensitive tests. Furthermore, we assayed sera in order to detect antibody response to NCDCV, a coronavirus known to cross-react strongly with human OC43. Our data, obtained by more sensitive and reliable methods, appear to consistently confirm previous reports showing a high prevalence of OC43 and 229E coronavirus in northern Italy and are in agreement with results obtained in other countries (1, 10-12).

As reported above, we used tests which are sensitive and not particularly tedious and expensive. Hemagglutination inhibition of OC43 and NCDCV performed after destroying non specific inhibitors is a very simple and reliable test. Sensitivity and specificity of IIP (for 229E) and PRA (for OC43 and NCDCV) were described and are not here discussed.

Finally, the detection of immune response to NCDCV (the bovine coronavirus antigenically related and cross-reacting with OC43), was due to our interest in confirming these antigenic relationships and excluding that human sera positive for OC43 were not reacting for NCDCV. These latter data are in agreement with previously reported results (5) and render questionable other reports referring to the presence of specific human antibody to NCDCV (13). In fact, the detection of immune reactivity to human sera to NCDCV (which however must always be performed after destroying specific serum inhibitors), is definitely confirmed to be dependant on the strict antigenic relatedness between OC43 and NCDCV and not consequent to human immunization by NCDCV.

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