

Prevalence of Human Coronavirus Antibody in the Population of Southern Iraq

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Sera from adults in Southern Iraq were collected during winter and screened by an enzyme-linked immunosorbent assay for the presence of antibodies to the two antigenic groups of human coronaviruses, the 229E and the OC43 groups: 91% of the sera had antibodies to at least one of the groups, whereas 4 and 5% of the sera had antibodies to only the 229E or OC43 groups, respectively. There was significant correlation between the levels of antibody to the 229E and OC43 group coronaviruses in these sera.

Key words: HCV antibodies, 229E and OC43 groups, HCV in Iraq, Iraqi serum antibodies

INTRODUCTION

Human coronaviruses (HCVs) are an important cause of common colds in man and up to 25% of colds may be caused by these viruses [Bradburne and Tyrrell, 1971; Larson et al, 1980; McIntosh, 1974; Monto, 1974]. Recent reports have suggested that these viruses may be associated with more severe diseases, and in particular gastroenteritis, but as yet there is no clear evidence to support this [Macnaughton and Davies, 1981]. Several reports have analyzed the extent of infections in various populations in America and Europe by estimates of HCV antibodies in sera from patients and volunteers [Bradburne and Somerset, 1972; Candeias et al, 1970; Cavallaro and Monto, 1970; Hamre and Beem, 1972; Hendley et al, 1972; Hovi et al, 1979; Kaye et al, 1971; McIntosh et al, 1970; Zakstelskaya et al, 1972]. A number of assays have been used, including neutralization, complement fixation, hemagglutination inhibition, and radioimmunoassay. The numbers of subjects with significant HCV antibody in their sera varied considerably depending on the population studied, time of assay, assay system used, and method used to determine significant levels of antibody. In one report most of the adult population was found to have serum antibodies to HCV OC43 as detected

Accepted for publication January 7, 1982.

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by radioimmunoassay [Hovi et al, 1979] indicating that infections with these viruses are very common.

Two serological groups of human coronaviruses, the HCV 229E and the HCV OC43 groups, have been recognized and show no antigenic cross-reaction with each other [McIntosh et al, 1969; McIntosh, 1974; Macnaughton et al, 1981; Pedersen et al, 1978]. All HCVs, so far identified, fall into one or other of these groups, suggesting that only two antigenically distinct HCV antigens should prove adequate in detecting all HCV infections [Macnaughton et al, 1981].

In this study representative viruses from the 229E and OC43 antigenic groups were used in ELISA to measure the antibodies to these viruses in sera collected during the winter from patients and volunteers from the Basrah area of Southern Iraq. The distribution of antibodies to these two HCV groups in the sera of the Iraqi population and the correlation between the amount of antibody to the two HCV groups in individual sera are described. The importance of these results is discussed in the light of previous work.

MATERIALS AND METHODS

Viruses

Two prototype HCV strains, 229E [Hamre and Procknow, 1966] and OC43 [McIntosh et al, 1967b], and seven HCVs, AD, GI, HO, PA, PR, TO, and RO, isolated from the nasal washings of subjects with natural colds [Larson et al, 1980] were used. In addition, a coronavirus of possible human origin, called CV Paris, was also used. This virus was isolated from the feces of a neonate with necrotizing enterocolitis [Sureau et al, 1980], although it shows close morphological and structural similarities to a bovine coronavirus [Macnaughton and Davies, 1981] also studied in that laboratory. The HCV strains, 229E, PR, and TO, were readily adapted to growth in monolayer cultures of MRC continuous cells, which were originally obtained from Dr. A.F. Bradburne. CV Paris readily grew in HRT 18 cells, a cell line derived from a human rectal adenocarcinoma. HCV OC43 grew to only low titers in tissue culture [Macnaughton et al, 1981], although readily in suckling mouse brain [McIntosh et al, 1967a]. The other five HCV isolates, strains AD, GI, HO, PA, and RO, could only be passaged in human fetal tracheal and nasal organ cultures [Larson et al, 1980].

Virus Antigens for ELISA

HCV 229E was grown in MRC continuous cells as described previously [Macnaughton and Madge, 1978]. CV Paris was obtained from Dr. J. Laporte and grown in HRT 18 cells in RPMI-1640 with 10% fetal calf serum containing antibiotics at 37°C and harvested after 30 hr. For both viruses, the cells were subjected to one freeze-thaw cycle, and the resulting suspension was clarified at 2000g for 30 min. Control antigens were prepared from uninfected cells by the same method. HCV OC43, grown in suckling mouse brain, was obtained from Dr. S.E. Reed of the Common Cold Unit, Salisbury and used as a 10% suspension of suckling mouse brain. Uninfected suckling mouse brain was used as a control antigen. Preparations of HCV 229E and CV Paris containing between 10^7 and 10^8 particles/ml and HCV OC43 containing between 10^8 and 10^9 particles/ml, as determined by electron microscopy [Macnaughton et al, 1980], were used.

Serum Samples

Sera were obtained during 1976 from adult volunteers taking part in experiments at the Common Cold Unit, Salisbury. The paired sera used in this study were collected from volunteers who developed colds after an inoculation of HCV or from controls given a saline inoculation. Postinoculation sera were collected about 3 weeks after virus inoculation. The sera were mixed with equal volumes of bovine calf serum and held at 4°C for 16 hr and stored at -20°C. The experiments were approved by the Ethical Committee of Northwick Park Hospital, Harrow.

258 serum samples were collected during February and March, 1981, from students at the Agricultural College, Basrah University, Iraq, or from patients' samples sent to the Central Public Health Laboratory at Basrah, the Al-Jumhury Hospital or to the Basrah Medical College Teaching Hospital, Iraq. Sera were absorbed for 16 hr at 4°C with equal volumes of bovine calf serum or tissue culture fluid from uninfected cell monolayers and stored at -20°C before testing by ELISA.

ELISA Procedure

The ELISA method used for detection of antibodies to HCVs in human sera was based on a previously described method [Kraaijeveld et al, 1980b]. Flat-bottomed polystyrene microtiter plates (Dynatech) were coated with duplicate 0.2-ml amounts of antigen diluted in 0.1 M carbonate-bicarbonate buffer (pH 9.6) and incubated overnight at room temperature. After incubation the plates were washed four times with phosphate-buffered saline containing 0.05% Tween 20 and 0.02% sodium azide (PBST) and shaken dry. Portions of 0.2 ml of sera diluted in PBST were added to the wells and incubated for 4 hr at room temperature. After incubation, the plates were washed four times in PBST and shaken dry. Anti-human immunoglobulin G, directed against heavy and light chains, and labeled with alkaline phosphatase conjugate (Miles Laboratories) at a dilution of 1:800, was added in 0.2-ml quantities and left overnight at room temperature. After four additional washes with PBST, 0.2 ml of phosphatase substrate, consisting of a 0.1% solution of p-nitrophenylphosphate in 10% (wt/vol) diethanolamine buffer (pH 9.8) with 0.02% sodium azide and 0.01% MgCl₂·6H₂O, was added to each well. Absorbance values were read after 30 min at 405 nm in a Flow Titertek Multiscan photometer.

RESULTS

Selection of Viruses for ELISA

HCV strains 229E and OC43 and CV Paris, which were representative members of the two HCV antigenic groups, were used. CV Paris was tested by ELISA against serum pairs from 46 volunteers, given OC43 group virus strains OC38/43, GI, HO, and RO; 229E group virus strains 229E, AD, PA, and TO; or saline (Table I). The highest ratio of postinoculation to preinoculation absorbance values, obtained at serum dilutions of 1:50, 1:100, and 1:200, was called the ELISA ratio, and ratios of 2 or more were considered to represent significant antibody rises [Kraaijeveld et al, 1980b]. Significant ELISA ratios (antibody rises) were detected with CV Paris antigen in the serum pairs from volunteers given OC43 group viruses, but not in those given 229E group viruses or saline. This indicates that CV Paris is antigenically related by ELISA to the OC43 group.

TABLE I. Antibody Rises to CV Paris in Paired Sera of Volunteers Inoculated with HCV Strains or Saline

Volunteer inoculum	Number of serum pairs	Number with positive ELISA ratios ^a	Range of ELISA ratios	Mean ELISA ratios
OC38/43	7	3	1.0-3.1	1.7
GI	2	1	1.3-2.0	1.6
HO	3	3	2.3-3.3	2.7
RO	8	5	1.3-3.1	1.9
229E	3	0	0.8-0.9	0.9
AD	7	0	0.8-1.4	1.0
PA	9	0	0.7-1.3	1.0
TO	4	0	0.6-1.1	0.9
Saline	3	0	1.0-1.1	1.0

^aELISA ratios of 2 or more were considered to be positive.

CV Paris was the only OC43 group virus that grew to high titers in tissue culture: HCV OC43 grew only to low titers in tissue cultures, although to high titers in suckling mouse brain [Macnaughton et al, 1981]. Tissue culture-grown viruses were more suitable for ELISA than suckling mouse brain-grown material, as less nonspecific reactions were observed and more material was available. Thus in this study CV Paris and HCV 229E, respectively, were used in ELISA as representative members of the OC43 and 229E antigenic groups.

Determination of Antibodies to HCVs

The 258 Iraqi serum samples were tested against HCV 229E and CV Paris (Fig. 1). For comparative purposes all sera were tested at the same dilution of 1:50. Controls were absorbance values obtained with PBSA or 1:50 dilutions of tissue culture fluid from uninfected cell monolayers. We considered an individual serum sample to contain significant antibody to HCVs when it had an absorbance value of at least twice the average of the controls, represented by the dotted lines in Figure 1. However, this criterion for deciding that a particular serum sample was positive was very rigorous, and some positive sera may therefore have been missed. There was no marked variation in the number of positive sera or their relative absorbance values on using different serum dilutions or on reading the absorbance values after different times after developing the plates.

These absorbance values were related to ELISA titers defined as the reciprocal of the highest dilution to produce an absorbance value of twice the control absorbance value at the same dilution. Thus, absorbance values in the range of 1.40 to 1.99 corresponded to antibody titers of 2000 to 4000 for both viruses, and lower absorbance values corresponded to proportionately lower antibody titers.

Figure 1 shows that 86 and 87% of the sera contained antibody to HCV 229E and CV Paris, respectively, with a wide range in the amount of antibody to both viruses in positive sera. 50 of the sera, taken at random, were tested in ELISA against the prototype HCV OC43 at the same dilution of 1:50 as for CV Paris, and the same number of sera were positive with each virus.

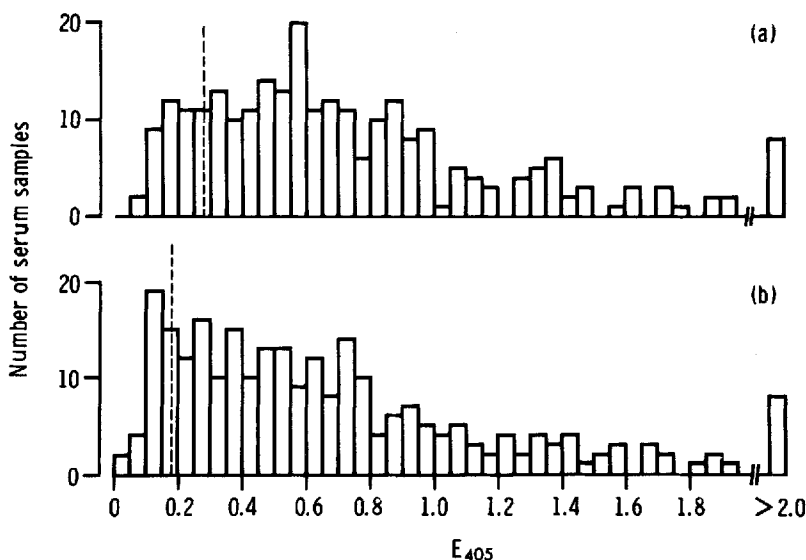


Fig. 1. HCV antibody levels. (a) HCV 229E; (b) CV Paris. Antigen and serum dilutions of 1:50 were used. All readings were taken after 30 min at 405 nm. Serum samples to the right of the dotted line were considered positive for HCV antibodies.

Correlation of Antibodies to HCV 229E and CV Paris

Figure 2 showed a strong correlation between the distribution of antibody to HCV 229E and CV Paris in the sera (Spearman's rank correlation coefficient of 0.73, $P < 0.001$). All points below and to the left of the lines in the figure represent sera with no HCV antibody. The serum samples showing significant antibody levels to either HCV group formed 91% of the total. Some sera in Figure 2 had significant antibody levels to HCV 229E (4%) and none to CV Paris, whereas other samples showing significant antibody levels to CV (Paris 5%) were negative for HCV 229E.

Antibody in Human Sera from England

67 random sera were collected from healthy adult volunteers at the Common Cold Unit, Salisbury, before inoculation with any viruses, and tested by ELISA for antibodies to HCV 229E and CV Paris.

Similar antibody levels as those from the Iraqi sera were observed, and 100 and 94% of these volunteers had serum antibodies to HCV OC43 and 229E group viruses, respectively.

DISCUSSION

This is the first report to describe the prevalence of HCV antibodies in a Middle Eastern population. Our results show that clinical or subclinical HCV infections in an adult population from the Basrah region of Southern Iraq are widespread with a high proportion, 91% of this population, having serum antibodies to HCV 229E and/or

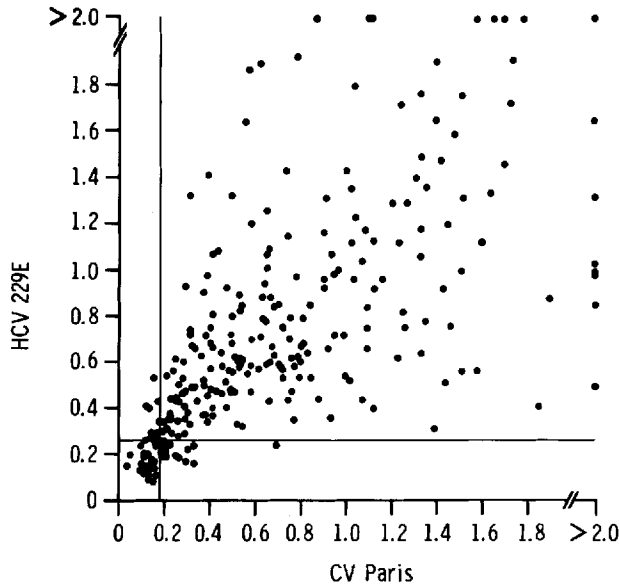


Fig. 2. Correlation between the level of antibodies to HCV 229E and CV Paris (Spearman's rank correlation coefficient of 0.73, $P < 0.001$). Antigen and serum dilutions of 1:50 were used. All readings were taken after 30 min at 405 nm. Serum samples above and to the right of the lines were considered positive for HCV antibodies.

OC43 group viruses. However, it is not clear how frequently individuals are infected with HCVs, as we do not know how long HCV serum antibodies remain after infection and how much antibody is produced by natural HCV infections. Serum samples were taken during the winter when HCV infections have been reported to occur most frequently [McIntosh et al, 1970; Cavallaro and Monto, 1970; McIntosh, 1974; Monto, 1974]. A similar prevalence of HCV antibodies was detected in sera of volunteers at the Common Cold Unit, Salisbury, England. However, in this study a smaller number of serum samples was examined and they were taken randomly throughout the year. These results imply that HCV infections occur frequently in both populations. We cannot say at present that HCV infections are less common in Iraq than in England, as considerable variation has been reported in HCV antibody prevalence in sera depending upon when samples are taken [Bradburne and Tyrrell, 1971; Monto, 1974].

Most previous studies have revealed no antigenic relationship between the HCV 229E and HCV OC43 group viruses [McIntosh et al, 1969; Monto, 1974; Macnaughton et al, 1981], although generally only reactions involving external antigens were examined in these studies. However, one group has described an antigenic relationship between these virus groups [Bradburne and Tyrrell, 1971; Bradburne and Somerset, 1972], which may reflect the type of sera used and the presence of contaminating serum and other components [Kraaijeveld et al, 1980a]. In our study we observed a strong correlation between the amount of antibody to the two HCV groups in individual Iraqi sera. This may be due to the detection of antibody directed against antigenically related internal antigens: such antigens are present in significant amounts in human sera (Macnaughton, unpublished results). However, further studies are required into both the

antigenic relatedness of HCV antigens and into the nature, duration, and role of HCV serum antibodies produced during HCV infection, in order to resolve this problem.

Previous studies have analyzed the prevalence of HCV antibodies in the sera of various populations in North America [Cavallaro and Monto, 1970; Hamre and Beem, 1972; Hendley et al, 1972; Kaye et al, 1971; McIntosh et al, 1970], South America [Candeias et al, 1972], and Europe [Bradburne and Somerset, 1972; Hovi et al, 1979; Zakstelskaya et al, 1972] and shown that HCV antibodies are widespread in these populations. Several of these reports describe variations in the incidence of antibodies to HCVs depending on a number of factors including the population studied and the season the samples were taken.

Most previous studies have reported considerably lower prevalence of HCV antibodies, than our study. This probably does not reflect significant differences in antibody prevalence in these populations, but rather the sensitivity of the assays used; our ELISA is more sensitive for detecting HCV antibody than many other assays [Kraaijeveld et al, 1980b] and as such should prove to be a useful technique for HCV antibody prevalence studies. Future studies are required to determine the variation in the levels of HCV antibodies in sera from Iraq from season to season, to identify epidemics, and to relate the severity of infections with those from other parts of the world.

ACKNOWLEDGMENTS

We thank staff at the Agriculture College, Basrah University, the Central Public Health Laboratory, Basrah, and the Department of Microbiology, Basrah Medical College Teaching Hospital for assistance during this study. In particular we are grateful to Dr. A. Jabbar for help in the collection of samples. We also thank Dr. S.E. Reed for supplying us with sera from adult volunteers taking part in experiments at the Common Cold Unit, Salisbury.

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