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# Antigenic Relationship between Human Coronavirus Strain OC 43 and Hemagglutinating Encephalomyelitis Virus Strain 67N of Swine: Antibody Responses in Human and Animal Sera

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Hemagglutinating encephalomyelitis virus of swine (HEV) was adapted to growth in suckling mouse brain. Electron micrographs of HEV-infected suckling mouse brain, prepared by negative staining and thin-section techniques, exhibited typical morphological characteristics shared with other members of the Coronaviridae. The adaptation of HEV to suckling mouse brain facilitated serologic testing by the use of common host reagents and compatible animal systems. With hemagglutination inhibition, complement-fixation, and neutralization tests, an antigenic relationship was demonstrated between human coronavirus OC 43 and HEV in specific immune and hyperimmune animal sera. Children and adults with seroconversion to OC 43 antigen had diagnostic rises in titer of antibody to HEV antigens. Individuals with seroconversion to human coronaviruses 229E and B814 demonstrated antibody to HEV but not diagnostic rises in titer. Swine with titers of antibody to HEV had lower or no detectable titers of antibody to coronavirus OC 43. Although the prevalence and geometric mean titer of antibody to OC 43 were higher than the titer of antibody to HEV in every group of normal humans tested, significant differences in antibody response to coronavirus OC 43 and HEV were seen between populations that did or did not have possible contact with swine. The evidence suggested that antibody to HEV in humans probably represented a heterologous response to infection with coronavirus OC 43. However, a heterotypic response to unknown or uncharacterized strains of coronavirus cannot be excluded.

In 1967, McIntosh et al. [1] reported the isolation of six strains of virus, similar to infectious bronchitis virus, in organ culture (OC) from adults with upper respiratory illness. Two strains of this virus designated OC 38 and OC 43 were subsequently adapted to growth in the suckling mouse brain (SMB). Reagents prepared from these strains were serologically identical by CF and neutralization tests [2]. These strains were classified as coronaviruses on the basis of their distinctive morphology and according to other fundamental characteristics that they shared with avian infectious bronchitis virus, mouse hepatitis virus (MHV), and human coronavirus strains B814, 229E, and others [3]. Subsequent studies demonstrated the ability of strain OC 38/43 (hereafter OC 43) to agglutinate certain erythrocytes and confirmed the identity of the strains by HAI tests [4]. Several studies with CF, neutralization, and HAI tests have demonstrated the epidemiologic and clinical features of respiratory illness caused by strain OC 43 infection in adults and children [5–8].

In 1963, Greig and Girard reported an encephalomyelitis of swine in Canada caused by a hemagglutinating virus [9]. In 1968, a previously unrecognized disease in swine, characterized by frequent vomiting, decreased appetite, wasting, and a high mortality rate, was observed in European countries [10, 11]. The virus isolated was serologically identical to the strain previously isolated in Canada. In 1971, Phillip et al. [12] described the morphology of the hemagglutinating virus by electron microscopy and reported that it resembled the coronavirus group.

Previously, another disease of swine, characterized by vomiting and wasting and caused by

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a nonhemagglutinating virus called transmissible gastroenteritis virus, had also been proposed as a candidate for the Coronaviridae [13]. However, until recently no outbreaks of encephalomyelitis in swine have been recognized in the United States. In 1972, Mengeling et al. [14] reported the characteristics of a coronavirus isolated in embryonic pig kidney cell culture (EPK) from the nasal cavity of a healthy adult pig. This isolate, designated hemagglutinating encephalomyelitis virus (HEV) strain 67N, was found to be serologically identical to previous isolates. Pathogenicity, characterized by anorexia, listlessness, vomiting, and some respiratory distress, was demonstrated by experimental administration of HEV strain 67N to newborn pigs [15].

Antigenic analyses of certain coronaviruses have been hampered by a lack of suitable host systems and comparable serologic tests. Therefore, the purpose of this study was twofold. We have (*1*) demonstrated the adaptation of HEV to growth in SMB, a finding which facilitated serologic tests with strain OC 43 by the use of common host reagents and (*2*) investigated the possible relationship between HEV and coronavirus strain OC 43 with use of animal and human sera.

## Materials and Methods

*Viruses.* The coronavirus strain OC 43, which had been isolated in human embryonic tracheal organ culture and subsequently adapted to SMB, was used for production of reagent [1]. The 26th passage of HEV strain 67N in EPK was received from Dr. W. L. Mengeling, U. S. Department of Agriculture (USDA), Ames, Iowa. This strain was subsequently passaged in our laboratory by the intracerebral (ic) route in three-day-old SMB derived from pregnant Swiss white (ICR) mice free of MHV. (All colonies bred at the Center for Disease Control [CDC], Atlanta, Ga. [Lawrenceville facility] are monitored monthly by serologic testing for MHV and other endemic viruses of mice.) After three passages of the virus, injected mice developed encephalitis and died 48–72 hr after inoculation. Similar symptoms were not observed in control mice inoculated ic with normal EPK. Suspensions (10%) of HEV-infected SMB in phosphate-buffered saline (PBS), pH 7.2, contained $10^{5.5}$–$10^{6.5}$ $LD_{50}$/0.03 ml. Subsequently, the HEV-infected SMB was successfully repassaged in porcine kidney cell cultures.

*Electron microscopy.* Specimens were prepared by the pseudoreplica technique [16], negatively stained with 2.0% potassium phosphate, pH 7.0, and examined with a Philips EM-200 electron microscope (Philips Electronic Instruments, Mount Vernon, N.Y.). Infected SMB tissues were also cut into 1-mm blocks and fixed at 4 C for 2 hr in 2.5% buffered glutaraldehyde. These blocks were then fixed in 1% $OsO_4$, dehydrated in an ethanol series, and embedded in an Araldite-Epon mixture [17]. Sections were stained with uranyl acetate and lead citrate and examined in a Philips EM-200 or EM-300 electron microscope operating at 60 kV.

*Production of antigen.* Antigens for coronavirus strain OC 43 were prepared as previously described [4]. Antigens for HEV were prepared with a 10% suspension of infected SMB in PBS (pH 7.2) for HAI tests and in Veronal buffered diluent (pH 7.3) for CF tests. Control antigens were prepared from normal SMB in a similar manner.

*Production of antisera.* Immune sera were prepared in weanling mice as described previously [4]. Hyperimmune sera were prepared in six-week-old female mice by four, weekly, 0.5-ml, ip inoculations of antigen plus Freund's complete adjuvant.

*Sera.* Acute- and convalescent-phase sera from children with upper respiratory tract illness and seroconversion to coronavirus strains OC 43 and 229E were collected during a longitudinal survey of respiratory illness conducted from 1960 to 1968 by the Respiratory Virology Branch, Virology Division, CDC, Atlanta, Ga. [6, 18]. Paired sera from adults with respiratory illness possibly due to coronavirus strains OC 43, 229E, and B814 were acquired from Drs. A. Z. Kapikian, National Institutes of Health, Bethesda, Md. and Sylvia Reed, Common Cold Unit, Salisbury, England. Sera from pigs infected with HEV were obtained from Dr. W. L. Mengeling, USDA, Ames, Iowa.

Normal sera, from humans and animals without respiratory illness, were obtained from several sources. Control sera were collected from

children who participated in studies of respiratory illness [6, 18]. Sera were acquired before vaccination from college students and retirees involved in influenza vaccine studies conducted by the CDC. Single samples of sera from employees of three different meat-packing houses (A, B, and C) were donated by Dr. Marshall Fox of the CDC. Single samples of sera from abbatoir employees, veterinary students, and swine producers and from 80 swine were acquired from Dr. George T. Wood, University of Illinois, Urbana, Ill.

*Serologic tests.* HAI tests were performed by the microtiter technique with PBS diluent and 0.5% adult chicken erythrocytes [19]. The Laboratory Branch complement fixation test was also performed by a microtiter technique [20]. All neutralization tests in our laboratory were performed by the constant virus-varying serum method in three-day-old suckling mice via the ic route. Doses of ~100 $LD_{50}$ of virus (ic) were used. All sera were inactivated at 56 C for 30 min. Five mice were used for each dilution of serum and for back-titrations of virus. Tests were concluded after 14 days. Serum titers of virus were calculated by the Karber method [21].

## Results

*Electron microscopy.* The similarity in structure and morphology of HEV to other members of the coronavirus group is shown in figure 1. HEV is ~100 nm in size and possesses widely spaced, club-shaped projections that are ~20 nm in length. The shape and spacing of the projections are the distinguishing features of Coronaviridae that set them apart from negatively staining members of the Orthomyxoviridae and Paramyxoviridae.

In thin sections of SMB, early stages of viral replication are indicated by the accumulation of electron-dense material adjacent to the intracytoplasmic membranes of the Golgi apparatus and endoplasmic reticulum. Bud formation takes place at such sites, and some of the budding particles can be seen in figure 2 (single arrowheads). These typical morphological characteristics are shared by other members of the coronavirus group [22].

*Specificity.* In table 1, the relationship between HEV and MHV is demonstrated by CF tests of specific hyperimmune mouse sera. Reciprocal CF tests showed only a one-way cross-reaction between HEV and MHV. The existence of the cross-reaction could not be confirmed by HAI because of the absence of a MHV hemagglutinin.

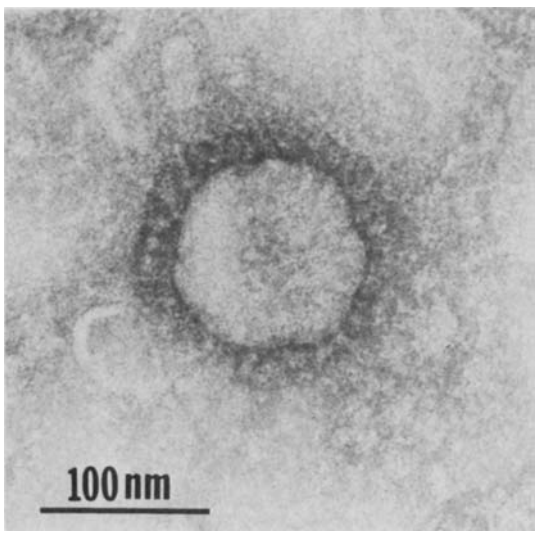


**Figure 1.** Morphologically typical coronavirus particle ~100 nm in size (bar = 100 nm).

*Antigenic relationship.* In table 2, the relationship between coronavirus strain OC 43 and HEV is demonstrated by HAI, CF, and neutralization tests of specific immune and hyperimmune mouse sera. The results reveal a two-way cross-reaction between strain OC 43 and HEV in both types of sera tested by all serologic methods used. However, titers of antibody to OC 43 antigen in HEV-immune serum were at least twofold to fourfold higher than were titers of antibody to HEV antigen in strain OC 43-immune serum.

*Antibody responses.* The HAI antibody response to HEV antigen in the sera of 97 children who showed seroconversion to coronavirus antigen of either strain OC 43 or strain 229E is noted in table 3. Chidren with OC 43 seroconversions demonstrated a higher prevalence of antibody to HEV (24%) in convalescent-phase sera than did children with seroconversion to coronavirus strain 229E (15%). In addition, three children who showed seroconversion to strain OC 43 also had seroconversion to HEV antigens. However,

no child with seroconversion to strain 229E had seroconversion to HEV antigens.

In table 4, antibody responses to OC 43 and HEV antigens by HAI, CF, and neutralization tests are shown for human adults and swine with known infections due to coronavirus. Patients infected with strain OC 43 had antibody and seroconversion to HEV antigens, but titers of HEV antibody were always lower than homologous titers. Patients naturally or experimentally infected with coronavirus strains 229E or B814 did not convert serologically to either OC 43 or HEV. However, patients with B814 infection who also had OC 43 antibody had low titers of antibody to HEV. One of the two pigs infected with HEV converted serologically to strain OC 43, but the titers of antibody to OC 43 were substantially lower than titers of antibody to HEV.

Antibody responses to OC 43 and HEV antigens by HAI tests in normal human populations and swine are demonstrated in table 5. The prevalence of antibody to coronavirus strain OC 43 in every human population studied ranged from 75% in retirees to 99% in abattoir employees. Prevalence of antibody to HEV in humans was

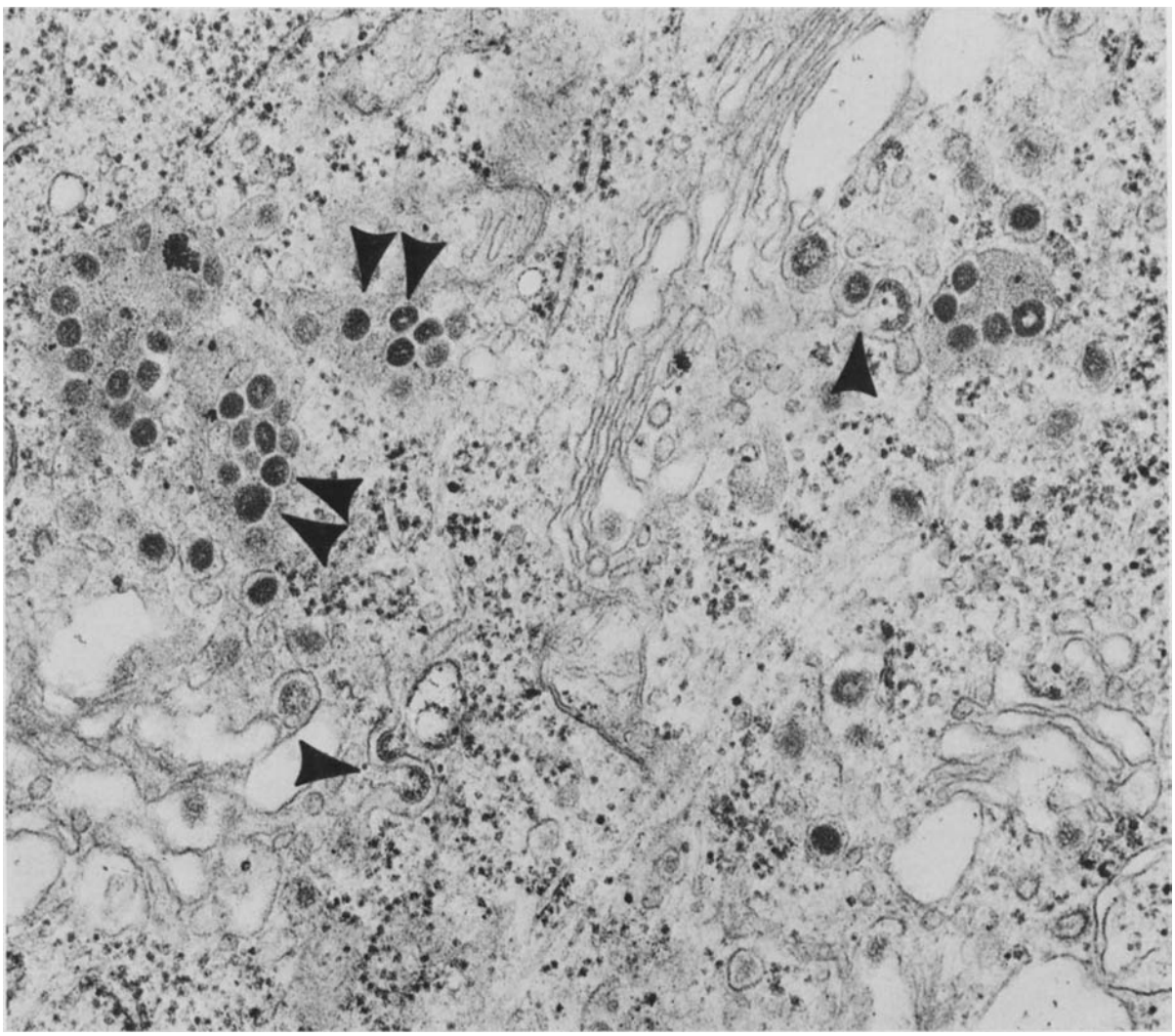

**Figure 2.** Coronavirus particles in suckling mouse brain: portion of a neuron showing virus particles budding into cisternae of the Golgi apparatus (single arrowheads) and completed particles lying within the cisternae (double arrowheads) (×59,700).

Table 1. Antigenic relationship between hemagglutinating encephalomyelitis virus (HEV) and murine hepatitis virus (MHV) as demonstrated by CF tests of specific hyperimmune mouse sera.

| | Antigens | |
|---|---|---|
| Antiserum | HEV | MHV* |
| HEV | 512† | <8 |
| MHV (polyvalent)‡ | 32 | 128† |
| Before inoculation | <8 | <8 |

NOTE. Data are given as reciprocal titers.

*MHV (polyvalent) antigen no. 3-6572 (Microbiological Associates, Bethesda, Md.).

†Titers of homologous antibody.

‡MHV (polyvalent) antisera no. 3-6636 (Microbiological Associates).

lower than the prevalence of OC 43 antibody in every population studied, ranging from 31% in college students to 78% in employees of meat-packing house B. However, significant differences in prevalence of HEV antibody were seen among populations who may or may not have had contact with swine. The observed prevalence of antibody to HEV was significantly higher ($P < 0.05$) in veterinary students (61%), swine producers (71%), abattoir employees (72%), and employees of meat-packing houses A, B, and C (60%–78%) than in college students, children, and retirees (31%–43%).

The reciprocal geometric mean titers (GMT) for OC 43 antibody ranged from 17 to 48, whereas titers of antibody to HEV ranged from 12 to 16 in all human populations tested. However, 22 individuals had HEV antibody titers that were greater than titers of OC 43 antibody by twofold or more. Individuals with antibody only to strain OC 43 were found in all human populations studied. However, significantly lower ($P < 0.05$) prevalences were found among meat-packing house employees, swine producers, abattoir employees, and veterinary students (18%–32%) than among retirees, children, and college students (32%–67%). In addition, there were 15 individuals who had only HEV antibody responses. No titers of antibody to OC 43 were detectable in the normal swine sera tested. However, 38% of the swine revealed HEV antibody with a reciprocal GMT of 60.

## Discussion

Although infections with coronavirus strain OC 43 in humans are fairly well defined, infections caused by other OC isolates are less well characterized [1, 24]. Also, human coronaviruses may be responsible for exacerbations of symptoms in children with asthma and in adults with chronic pulmonary disease [25, 26]. However, the significance of coronavirus-like particles observed by electron microscopy in association with other human diseases remains uncertain [27–31]. Coronaviruses have also been shown to be the etiologic agents in or candidates for a wide variety of diseases in different animal species [32]. Previous studies have demonstrated the presence in humans of antibody to other animal coronaviruses [33–35]. Serologic cross-reactions have also been established among and between certain human and animal strains [2, 4, 12, 23, 35–37]. Specific immune or hyperimmune sera can be used to determine the extent and direction of antigenic

Table 2. Antigenic relationships between coronavirus strain OC 43 and hemagglutinating encephalomyelitis virus (HEV) by HAI, CF, and neutralization (NT) tests of specific immune and hyperimmune mouse sera.

| | Antigen* | | | | | |
|---|---|---|---|---|---|---|
| | OC 43 | | | HEV | | |
| Sera | HAI | CF | NT | HAI | CF | NT |
| Immune | | | | | | |
| OC 43 | (640) | (64) | (1,280) | 10 | 8 | 15 |
| HEV | 40 | 16 | 66 | (160) | (64) | (224) |
| Hyperimmune | | | | | | |
| OC 43 | (20,480) | (2,048) | (26,624) | 80 | 32 | 36 |
| HEV | 320 | 128 | 192 | (20,480) | (1,024) | (16,624) |

NOTE. Data are given as reciprocal titers. Numbers in parentheses are titers of homologous antibody. Sera obtained before inoculation and included in these tests had no detectable antibody to coronavirus strain OC 43 or to HEV antigens.

*Control CF antigens did not fix complement with these sera at a dilution of 1:8.

Table 3. Occurrence of HAI antibody to hemagglutinating encephalomyelitis virus (HEV) antigen among 97 children who showed seroconversion to coronarvirus strains OC 43 or 229E, as demonstrated by serologic tests.

| Coronavirus strain | No. of subjects with seroconversions | No. of subjects (%) with HEV antibody* | No. of subjects (%) with Seroconversion to HEV antigen† |
|---|---|---|---|
| OC 43 | 37 | 9(24) | 3(8) |
| 229E | 60 | 9(15) | 0 |

*Titers of at least 1:10 in the convalescent-phase serum.
†Fourfold or greater rises in titer of antibody.

relationships. However, the interpretation of antibody responses to coronavirus infections in humans is complicated by the knowledge that the response might not be primary; thus, heterotypic antibody may or may not be expected [23, 38–40].

In our study, the adaptation of HEV to growth in SMB was demonstrated by electron microscopy. Specificity was also confirmed by the lack of reciprocal CF antibody response between HEV and MHV and the ability of HEV-infected SMB to agglutinate certain erythrocytes. The adaptation of HEV to SMB facilitated serologic testing with coronavirus strain OC 43 by the use of common host reagents and compatible animal systems.

Serologic testing revealed a two-way cross-reaction between OC 43 and HEV in specific immune animal sera; the cross-reaction became more apparent when the animals were hyperimmunized. However, the titers of heterologous antibody were always at least fourfold lower than the titers of homologous antibody in all serologic tests. Prevalence of antibody to HEV was higher in children infected with strain OC 43 than in children infected with strain 229E. In addition, antibody responses in adults with known infection, who demonstrated conversion to strain OC 43, have also shown seroconversion to HEV. Conversely, sera from adults known to be infected with coronavirus strains other than OC 43 demonstrated no serologic conversion to HEV antigens. However, in all cases, titers of antibody to HEV were lower than titers of antibody to OC 43.

Antibodies to both strain OC 43 and HEV were found in normal human sera collected from the various groups studied; the GMT of OC 43 antibody was higher than the GMT of HEV antibody in every group tested. Furthermore, there were considerably more individuals with only OC 43 antibody in their sera than with only HEV antibody. Sera from normal herds of swine with

Table 4. Antibody responses to antigens of coronavirus strain OC 43 and hemagglutinating encephalomyelitis virus (HEV) by HAI, CF, and neutralization (NT) tests in humans and swine with known coronavirus infection (strain OC 43, 229E, or B814).

| Subject, infecting virus* | Reciprocal antibody titers | | | | | |
|---|---|---|---|---|---|---|
| | HAI | | CF | | NT | |
| | OC 43 | HEV | OC 43 | HEV | OC 43 | HEV |
| 689, OC 43 | <10/160† | <10/20 | <8/32 | <8/8 | 14/320 | <8/100 |
| 712, OC 43 | 20/160 | 10/40 | 8/64 | <8/16 | 56/376 | 13/167 |
| 840, 229E‡ | 20/10 | <10/<10 | <8/<8 | <8/<8 | 32/56 | 14/<8 |
| 844, 229E | <10/<10 | <10/<10 | <8/<8 | <8/<8 | 8/24 | <8/<8 |
| 862, 229E | 20/10 | <10/<10 | <8/<8 | <8/<8 | 74/56 | <8/<8 |
| 865, 229E | 10/10 | <10/<10 | <8/<8 | <8/<8 | 18/11 | <8/<8 |
| Manfield, B814 | 10/10 | <10/<10 | <8/<8 | <8/<8 | 16/36 | <8/15 |
| Burke, B814 | 160/80 | 10/10 | <8/<8 | <8/<8 | 256/160 | 32/32 |
| Pig 17027, HEV | <10/<10 | <10/80 | <8/<8 | <8/<8 | <8/<8 | <8/160 |
| Pig 17039, HEV | <10/20 | <10/640 | <8/<8 | <8/8 | <8/36 | <8/1,432 |

*Sera were supplied by Drs. A. Z. Kapikian, National Institutes of Health, Bethesda, Md.; Sylvia Reed, Common Cold Unit, Salisbury, United Kingdom; and W. L. Mengeling, U.S. Department of Agriculture, Ames, Iowa. Patients were naturally or experimentally infected with virus and/or had fourfold rises in titer of antibody to the infecting virus.
†Titer in acute-phase serum/titer in convalecent-phase serum.
‡Results for 229E/OC 43 NT tests are from [23].

Table 5. Antibody responses to antigens of coronavirus strain OC 43 and hemagglutinating encephalomyelitis virus (HEV) as demonstrated by HAI tests in normal human populations and swine.

| Study population (no. of serum samples tested) | Total no. positive (%) for OC 43 and/or HEV | No. positive (%) for | | Reciprocal GMT | | No. positive (%) only for | |
|---|---|---|---|---|---|---|---|
| | | OC 43 | HEV | OC 43 | HEV | OC 43 | HEV |
| Children (71) | 65(92) | 65(92) | 24(34) | 32 | 13(0)* | 41(58) | 0 |
| College students (328) | 320(98) | 320(98) | 99(31) | 48 | 13(1) | 221(67) | 0 |
| Retirees (93) | 71(76) | 70(75) | 40(43) | 17 | 12(2) | 30(32) | 1(1) |
| Veterinary students (44) | 42(95) | 41(93) | 27(61) | 20 | 13(1) | 14(32) | 1(2) |
| Swine producers (52) | 50(96) | 48(92) | 37(71) | 20 | 16(3) | 11(21) | 2(4) |
| Abattoir employees (50) | 49(99) | 49(99) | 36(72) | 28 | 14(4) | 13(27) | 0 |
| MPH† (A) employees (226) | 220(97) | 219(97) | 170(76) | 30 | 14(4) | 49(21) | 1(0.04) |
| MPH (B) employees (444) | 436(98) | 428(96) | 346(78) | 32 | 13(6) | 82(18) | 8(2) |
| MPH (C) employees (108) | 99(92) | 97(90) | 65(60) | 27 | 13(1) | 32(30) | 2(2) |
| Swine (10 herds) (80) | 30(38) | 0 | 30(38) | | 60(30) | 0 | 30(38) |

NOTE. None of the humans tested had respiratory disease. GMT = geometric mean titer of HAI antibody; titers <1:10 were assigned values of 5 for calculation of GMT. A positive antibody response was determined on the basis of a titer of ≥1:10.

*Numbers in parentheses represent individuals or swine with titers of antibody to HEV at least twofold greater than titers of antibody to coronavirus strain OC 43.

†MPH = meat-packing house.

antibody to HEV revealed no antibody to OC 43 antigens. In almost every instance, the GMT of antibody to HEV in swine was at least fourfold higher than the GMT of HEV antibody in humans. Sera from pigs infected with HEV demonstrated OC 43 antibody and seroconversion, but the titers of this antibody were considerably lower than the homologous titers of antibody to HEV.

The serologic evidence in this study suggests that antibody to HEV in humans probably represents a heterologous response to infection with coronavirus strain OC 43. However, several contradictory points must be considered: (*1*) HEV antibody was found more often in individuals who might have had contact with swine; (*2*) conversely, antibody to OC 43 alone was found more often in individuals who had less possibility of contact with swine; (*3*) 22 individuals had titers of antibody to HEV at least twofold higher than titers of antibody to OC 43; and (*4*) sera from 15 individuals had only antibody to HEV. Of these subjects, 14 had possible contact with swine. However, there is no direct evidence (isolation of virus) at this time that humans in contact with swine acquire any respiratory or nonrespiratory disease caused by HEV. Nevertheless, in view of the serologic relationships between human and animal coronaviruses and the clinical nature of the coronavirus-linked diseases involved, the possibility of human infection with HEV cannot be excluded [41].

In conclusion, the evidence of antibody to HEV in human sera represents (*1*) a heterologous antibody response to infection with coronavirus strain OC 43; (*2*) a heterotypic response to as yet unknown or uncharacterized strains possibly related to OC 43; or (*3*) a subclinical or unrecognized infection with HEV. Therefore, further studies are needed to clarify the serologic relationships among and between human and animal coronaviruses now available, to isolate and characterize "new" coronavirus strains, and to survey properly controlled human populations that have contact with swine for evidence of unrecognized disease associated with coronavirus.

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