

Coronaviridae: Second Report¹

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Further experience has confirmed our earlier recommendation that coronaviruses appear to form a quite closely related group. Firm evidence on the nucleic acids of several indicates that they have a single, large molecular weight, single-stranded RNA genome which carries a covalently attached poly(A) sequence at the 3'-terminus. Consistent with this result, the early reports of the presence of a viral particle polymerase have not been confirmed. The viruses are presumably positively stranded; the genomic RNA has been shown to be infectious, although it has not as yet been shown to code for viral polypeptides in a cell-free system. The position with the viral polypeptides is confusing; different numbers and molecular weights are found when the same viruses are studied in different laboratories and when different viruses are studied in the same laboratories. High and low molecular weight polypeptides can apparently be produced during extraction and separation. However, the internal polypeptide associated with the RNA seems to be of molecular weight about 50,000 in all cases which have been adequately studied. The lipids of only one virus have been examined. More work is needed on the polypeptides and their antigenic and biochemical characteristics. New viruses, which seem to have typical coronavirus morphology, have been recognized and tentatively added to the group, but further information is needed about their structure. There is still not enough evidence to justify subdividing the group.

References included in the review by MCINTOSH [1] and in the report by TYRRELL *et al.* [2] are not repeated in this report.

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- 1 Taxonomy
 - 1.1 Family: Coronaviridae
 - 1.3 Taxonomic status: Family with one genus *Coronavirus*.
- 2 The virion
 - 2.1 Chemical composition
 - 2.1.1 Nucleic acid
 - 2.1.1.1 RNA
 - 2.1.1.2 Single-stranded: HCV² (229E) [3]; HCV (OC43) [4]; HEV [5]; IBV [6–9]; TGEV [5].
 - 2.1.1.4 Number of pieces: One or several [3–9].
 - 2.1.1.5 Sedimentation coefficients: HCV (229E), 52S [3]; HCV (OC43), 70S [4]; HEV, 60–70S [5]; IBV, 48–64S [6,8,9]; TGEV, 60–70S [5].
 - 2.1.1.6 Molecular weight: HCV (229E), 5.8×10^6 [3]; HCV (OC43), 6.1×10^6 [4]; IBV, $5.6\text{--}9.0 \times 10^6$ [6–9].
 - 2.1.1.11 Infectivity: IBV RNA is infectious [6,8].
 - 2.1.1.12 Other features: HCV (229E) contains a covalently attached poly(A) tract of about 70 nucleotides at or near the 3'-terminus [3]. HCV (OC43) contains a covalently attached poly(A) tract of about 19 nucleotides [10]. IBV [6–8] and TGEV [11] also contain covalently attached poly(A) tracts.
 - 2.1.2 Proteins
 - 2.1.2.2 Number of polypeptides: HCV, 6 or 7 polypeptides [12,13]; HEV, 5 polypeptides [14]; IBV, 7 polypeptides [15]; MHV, 4–6 polypeptides [16]; TGEV, 4–6 polypeptides [17].
 - 2.1.2.3 Molecular weights of polypeptides: HCV, 15,000–196,000 [12,13]; HEV, 26,500–180,000 [14]; IBV, 33,000–130,000 [15]; MHV, 23,000–180,000 [16]; TGEV, 28,000–200,000 [17].
 - 2.1.2.5 Enzymes: RNA-dependent RNA polymerase not found in HCV (OC43) [10] or IBV [8]. There are unpublished reports that RNA- and DNA-dependent RNA polymerases are not found in HCV or IBV.
 - 2.1.2.6 Other functional proteins: Hemagglutinin in HCV, IBV [18,19], HEV and NCDCV [20]. Hemagglutinin inhibited by specific antisera.
 - 2.1.3 Lipids: TGEV contains phospholipid and glycolipid resembling those of host cell [21].

² See 10.3 for abbreviations of species.

- 2.1.4 Carbohydrates: Several peptides are glycosylated, probably the surface peptides: HCV [12, 13]; HEV [14]; IBV [15]; MHV [22]; TGEV [17].
- 2.2 Physicochemical properties
 - 2.2.1 Density: 1.16–1.23 g/cm³ in sucrose; 1.23–1.24 g/cm³ in CsCl.
 - 2.2.2 Sedimentation coefficient: HCV (229E), 378–400S; HCV (OC43), 374–416S; IBV, 330S; TGEV, 495S [23].
 - 2.2.4 Stability of infectivity
 - 2.2.4.1 pH: IBV: optimum stability between pH 6.0 and 6.5. TGEV: optimum stability at pH 6.5. Conflicting or no evidence for other viruses.
 - 2.2.4.2 Heat: Rapidly inactivated at 56°; slow inactivation at 37°; moderately stable at 4°, assuming optimal suspending medium.
 - 2.2.4.5 Other agents: Unstable with common disinfectants and detergents.
- 2.3 Structure
 - 2.3.1 Nucleocapsid: RNA associated with peptide of molecular weight 50,000: IBV [24]; MHV [22]; TGEV [23]. Helical RNP by negative staining [25, 25a].
 - 2.3.2 Envelope: Lipid-containing envelope present, including glycopeptides. See 2.1.4.
 - 2.3.3 Cores: Electron-dense inner shell visible in thin section. HCV: ribonucleoprotein core, density 1.31 g/cm³ (CsCl), sedimentation coefficient 180S; linear appearance by negative staining. TGEV: ribonucleoprotein core, density 1.295 g/cm³ (CsSO₄), sedimentation coefficient 650S [23].
 - 2.3.3.1 Dimensions: 50–70 nm in diameter in thin section.
- 2.4 Morphology
 - 2.4.1 Overall shape: Flattened, dough-nut shape with some elongated forms. Spherical when freeze-dried.
 - 2.4.2 Dimensions: 60–220 nm.
 - 2.4.3 Surface projections: Characteristic bulbous projections, 12–24 nm long, widely spaced.
 - 2.4.4 Special features in thin sections: Inner and outer shells, sometimes separated by electron-lucent space.
 - 2.4.5 Other features: Fragile attachment of projections to surface of virion. Inner tongue-shaped membrane in IBV visible by negative staining [26].
- 3 Replication
 - 3.1 Site of accumulation of viral proteins: Cytoplasm.

- 3.3 Mode of nucleic acid replication
- 3.3.2 Effect of inhibitors: Sensitive to 6'-azauracil and virazole. Insensitive to 5'-iododeoxyuridine, 5'-bromodeoxyuridine, 5'-fluorodeoxyuridine, cytosine arabinoside, aminopterin and actinomycin D.
- 3.4 Site and mechanism of maturation: Matures in cytoplasm by budding through endoplasmic reticulum.
- 3.5 Other features: No budding at plasma membrane.
- 4 Cooperative interactions: No information available.
- 5 Host range
- 5.1 Natural: Generally restricted to normal host species.
- 5.2 Experimental
- 5.2.1 *In vivo*: Generally specific for species of origin.
 HCV (some strains): suckling hamsters, suckling mice.
 IBV (some strains): suckling hamsters, suckling white rats, newborn rabbits.
 MHV: hamsters.
 TGEV: cats, dogs, humans, muskrats, opossums, skunks, starlings.
- 5.2.2 *In vitro*:
 CCV: 1^o dog kidney cells.
 FIPV: None known.
 HCV (some strains; cf. serotype 229E): 1^o and 2^o human embryonic cells. Others: human embryo trachea organ cultures subsequently adapted to suckling mice and monkey kidney cells.
 HECV: organ culture of human intestine.
 HEV: 1^o porcine cells.
 IBV: 1^o chicken and chicken embryonic cells, chicken tracheal organ cultures, 1^o monkey kidney cells, VERO cells.
 MHV: 1^o mouse and mouse embryonic cells, mouse macrophages, L cells, WI-38 cells, NCTC-1496 cells.
 RCV and SDAV: 1^o rat kidney cells.
 TGEV: 1^o porcine cells (especially thyroid), 1^o canine kidney cells, organ culture.
- 6 Pathogenicity
- 6.1 Association with diseases:
 CCV: Diarrhea in dogs [27].
 CET: Diarrhea in turkeys.

³ 1^o = First passage.

FIPV: Infectious peritonitis in cats [28].

HCV: Common colds in humans.

HECV: Possibly diarrhea in humans [29].

HEV: Encephalitis in pigs.

IBV: Acute respiratory disease, nephritis and gonadal damage in chickens.

MHV: Acute hepatitis and/or encephalitis in mice, also chronic immunologically mediated diseases [30]. Also causative agent of LIVIM (lethal intestinal virus of infant mice) disease [31].

NCDCV: Gastroenteritis of calves.

RCV: Pulmonary infections in rats.

SDAV: Sialodacryoadenitis in rats.

TGEV: Gastroenteritis in pigs.

6.2 Tissue tropisms:

HCV: Upper respiratory tract, occasionally lower.

HECV: Alimentary tract.

HEV: Intestine, brain.

IBV: Respiratory and reproductive tract.

MHV: Brain, liver, spleen, macrophages.

NCDCV: Small and large intestine.

RCV: Lung.

SDAV: Salivary glands.

TGEV: Small intestine, lung.

6.3 Cytopathology: Cellular vacuolation leading to cell disintegration, sometimes syncytium formation.

7 Geographic distribution: Information limited and patchy. HCV, HECV, IBV, MHV and TGEV are certainly present in several continents and probably worldwide.

8 Transmission

8.1 Vertical: IBV, yes. No data available for other strains.

8.2 Horizontal: Yes.

8.3 Vectors

8.3.1 Biological: None recognized.

8.3.2 Mechanical: HCV, airborne; IBV, contaminated equipment, personnel, airborne, etc.; TGEV, fecal-oral route. No data for other strains.

9 Antigenic properties

9.1 Number of distinct antigenic molecules in virion: HCV, 3; HEV, 3; IBV, 3; MHV, 2; TGEV, 3.

- 9.2 Antigenes involved in virus neutralization: (i) Viral surface glycopolypeptides. (ii) Anti-host antibody + complement.
- 9.3 Number of distinct nonstructural antigens: No adequate information.
- 9.4 Specificity of different antigens: No information.
- 10 Classification
- 10.1 Definition of family Coronaviridae: Pleomorphic enveloped particles, averaging 100 nm diameter, containing RNA and essential lipid. Bear unique definitive projections. Multiply in cytoplasm, mature by budding through endoplasmic reticulum. No defined subgroups, but a tentative grouping may be made on basis of serology though data are incomplete and somewhat contradictory. IBV has many recognized serotypes; however, all seem to be inter-related, possibly by a common antigen. No clear interrelationship demonstrated with any of the other coronaviruses, although may be related to TGEV. HCV, several serotypes, two main groups – those isolated in tissue culture and those isolated in organ culture. Serologically related to HEV, MHV and NCDCV. The three rodent coronaviruses – MHV, RCV and SDAV – are interrelated serologically and also related to HCV. No adequate information on relationship or diversity between individual strains of MHV. TGEV, no antigenic diversity between strains, possible relationship to FIPV and HEV. HEV, no antigenic diversity between strains, possible relationship to HCV, NCDCV and TGEV. CET, only one report available, no relationship shown to other coronaviruses.
- 10.2 Only one genus, *Coronavirus*. Type species: IBV.
- 10.3 Species: Avian infectious bronchitis virus (IBV)
 Canine coronavirus (CCV)
 Coronavirus enteritis of turkeys (CET) (bluecomb disease)
 Feline infectious peritonitis virus (FIPV) (feline coronavirus)
 Human coronavirus (HCV)
 Human enteric coronavirus (HECV)
 Murine hepatitis virus (MHV)
 Neonatal calf diarrhea coronavirus (NCDCV)
 Porcine transmissible gastroenteritis virus (TGEV)
 Porcine hemagglutinating encephalitis virus (HEV)
 Rat coronavirus (RCV)
 Sialodacryoadenitis virus of rats (SDAV)

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