J. Vet. Med. B **35**, 709–712 (1988)

ISSN 0931–1793

Short Communication

WHO Collaborating Centre for Collection and Evaluation of Data on Comparative Virology, Institute of Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, Ludwig-Maximilians-Universität, Munich, FRG

Enteric Coronaviruses in Primates

W. EICHHORN and C.-P. CZERNY

Address of authors: Institute of Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, Ludwig-Maximilians-Universität, Veterinärstr. 13, 8000 Munich 22, FRG

With two figures

(Received for publication June 22, 1988)

Summary

Coronaviruses antigenetically related to bovine coronaviruses and human coronavirus strain OC43 were detected in fecal samples from two primates (moor macaque and a chimpanzee) by electron-microscopy and ELISA. This ELISA is specific for coronaviruses belonging to the antigenic group 2. Attempts to propagate the primate coronaviruses in cell culture or suckling mouse brain failed.

Key words: Coronavirus, primates, ELISA, antigenic relationship

Coronaviruses have been isolated from a variety of mammalian and avian species. They are known to have pathogenic potential and one of the disease entities caused by these viruses is diarrhea as observed in calves, piglets, dogs, cats, horses, turkeys and other species (WEGE et al., 1982). Mammalian coronaviruses have been divided into at least two antigenic groups (PEDERSEN et al., 1978). Porcine epidemic diarrhea virus (PEDV) is antigenically unrelated to these groups. Additionally, coronavirus-like particles have been demonstrated in diarrheal stool samples from humans (CAUL et al., 1975; BATTAGLIA et al., 1987). There are only a few reports on the propagation of these particles in organ cultures or cell lines (CAUL and EGGLESTONE, 1977; LAPORTE and BOBULESCO, 1981). Polypeptides of particules purified from stools have been shown to be related to polypeptides of the human coronavirus strain OC-43 (BATTAGLIA et al., 1987) indicating that they are members of the family coronaviridae and related to human respiratory coronaviruses. In primate species enteric coronavirus-like particles have been detected by electron microscopy (CAUL and EGGLESTONE, 1979; SMITH et al., 1982), but no attempt has been made to

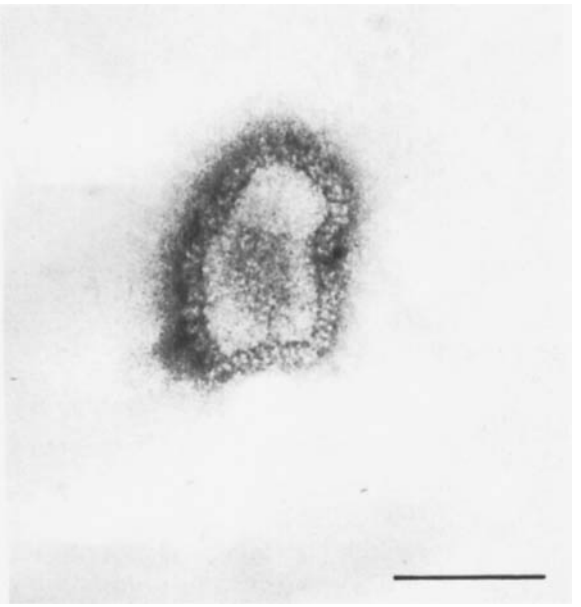

Fig. 1. Negatively stained preparation of a fecal sample from a moor macaque *(Macaca maura)*. The particle is of typical coronaviral morphology, but details of the projections can not be defined. Bar represents 100 nm

demonstrate antigenetic relationships to established coronaviruses. Therefore the nature of these particles remains unclear.

Recently, fecal samples from two primates which sequentially suffered from acute diarrhea and finally died were sent to our laboratory. Both animals had been kept in a zoological garden in South Germany. The first case was a moor macaque *(Macaca maura)* about one and a half years old. The animal had been brought in from another zoo about two weeks before. The second animal was a chimpanzee aged about 37 years. This animal was housed in the neighbourhood of the first and died with diarrhea about two months later.

Fecal samples were routinely processed for virological diagnosis, i. e. diluted about 1 : 5 in PBS, thoroughly suspended in a Labblender (Seward Lab, London), and centrifuged. Supernatants were assayed by ELISA specific for rotavirus (BACHMANN, 1979) or coronaviruses (antigenic group 2; CZERNY and EICHHORN, in preparation) or negatively stained and examined by electron microscopy. By EM, coronavirus-like particles were easily detected. Additionally, particles with typical coronaviral morphology were detected less frequently (Fig. 1). The size range of these particles was about 90—160 nm. Surface projections were clearly visible (about 18—25 nm) but details of their shape could hardly be distinguished. This morphological injury may be due to the time elapsed between the death of the animal and the arrival of the samples in the laboratory ("Waterloo station effect", REYNOLDS et al., 1984). No other viruses were detected by EM.

Both samples scored negative in the polyclonal rotavirus-ELISA. On the other hand, in the coronavirus-ELISA both samples scored clearly positive. This assay is based on a mixture of two monoclonal antibodies generated against the bovine coronavirus strain Munich V 270/84. They recognize different epitopes (determined by competition ELISA) and serve as catching antibodies. Rabbit hyperimmune serum is used as detecting antibody in an indirect assay. The assay proved to be highly specific for bovine coronaviruses (BCV) and human coronavirus strain OC-43. Reaction patterns of these viruses are nearly

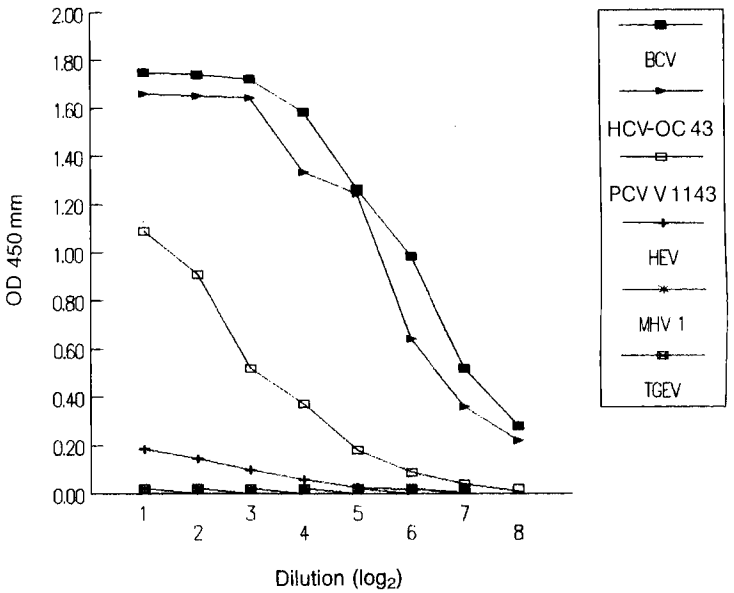

Fig. 2. Optical densities obtained with different coronaviruses assayed in a monoclonal antibody based ELISA. Bovine coronavirus (BCV) and human coronavirus strain OC 43 (HCV-OC 43) react identically. The reaction pattern of the primate coronavirus (PCV V 1143) is similar, although lower extinctions are observed. Reactivity of porcine hemagglutinating encephalomyelitis virus (HEV) is strikingly different. Coronaviruses of the antigenic group 1, like porcine transmissible gastroenteritis (TGEV) or mouse hepatitis viruses (MHV) do not react at all

identical (Fig. 2). Other members of the coronavirus antigenic group 2 react with minor intensity. Members of the other antigenic groups of the family coronaviridae do not react at all. Fecal samples from primates gave similar reaction patterns in this assay (Fig. 2). The patterns were similar to BCV and HCV OC-43 although the extinctions obtained were clearly lower. This may be due to proteolytic damage that occurred before the material could be assayed. On the other hand, this behaviour may also be explained by minor antigenic differences of epitopes recognized by ELISA between primate coronaviruses and BCV or HCV OC-43. Nevertheless, the results clearly indicate that the particles observed by EM are viruses to be classified within the family coronaviridae.

Propagation of the primate coronaviruses was attempted in Vero-cells, human embryonic lung cells and additionally in secondary bovine embryonic lung cells. Samples were inoculated both with or without the addition of trypsin (2.5 µg/ml).

Cultures were observed daily for cpE and passaged after 7 days. In the course of three passages no cpE was evident. Examination of freeze-thawed cell cultures by ELISA and EM proved to be negative during all passages.

Additionally, suckling mice (Balb/c, 2–3 days old) were inoculated i.c. with fecal supernatants and observed daily. No disorders were observed over the next 10 days. Passage in suckling mouse brains was negative as was examination of brain suspensions by ELISA and EM.

The failure to propagate the strains in cell cultures or suckling mouse brain probably results from destruction of infectivity during transport and other manipulations. This is in agreement with the observation of only a small percentage of complete particles with coronaviral morphology in feces.

The pathogenic potential of coronaviruses of primates remains unclear, although both animals had suffered from diarrhea. However, in the first animal a number of bacterial pathogens (salmonella enteritidis, beta-hemolysing streptococci and staphylococci) were

identified. The second animal did not only show diarrhea, but suffered a general breakdown of the organism, probably due to ist age.

The use of the monoclonal antibody-based ELISA which is specific for antigens of BCV, HCV OC-43 and obviously primate coronaviruses, may give more insight into the prevalence, epidemiology, and pathogenicity of these viruses.

Acknowledgements

We thank SHEILA SCOTT for her help in preparing the manuscript and MONIKA HEILMANN for her technical assistance. This work was supported in part by the German Federal Ministry of Youth, Family, Women and Health.

Zusammenfassung

Enterale Coronaviren bei Primaten

In Kotproben von zwei Primaten (Moormakake und Schimpanse) wurden im Elektronenmikroskop sowie im ELISA Coronaviren nachgewiesen, die antigenetisch mit bovinen Coronaviren und dem humanen Coronavirus Stamm OC-43 verwandt sind. Der verwendete ELISA ist spezifisch für Coronaviren der Antigen-Gruppe 2. Versuche, die Primaten-Coronaviren in Zellkulturen oder Babymäusen zu vermehren, schlugen fehl.

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