Coronavirus-Like Particles in Laboratory Rabbits with Different Syndromes in The Netherlands\textsuperscript{1,2}

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Summary | Virus-like particles were identified from the plasma of rabbits which developed pleural effusion disease after inoculation with different strains of \textit{Treponema pallidum}. These particles were considered coronavirus-like on the basis of their size, morphology, and buoyant density. Clinical and pathological manifestations of pleural effusion disease, which is probably the same disease entity as rabbit cardiomyopathy, resembled those of feline infectious peritonitis which is caused by another probable member of the Coronavirusidae family. Coronavirus-like particles also were demonstrated in the feces of rabbits which had been inoculated with a 450-nm fecal filtrate of rabbits which died from infectious intestinal disease.

Key Words | Coronaviruses — Diarrhea — \textit{Oryctolagus}

Although no members of the Coronavirusidae family have officially been recognized in rabbits so far, coronavirus-like particles have been demonstrated in laboratory rabbits from Scandinavia with a syndrome called rabbit cardiomyopathy and in laboratory rabbits in Canada with contagious diarrheal disease (1,2). In both cases, an antigenic relationship with human coronaviruses was suggested. In the present paper, we describe the presence of coronavirus-like particles in the plasma of laboratory rabbits with pleural effusion disease and in the feces of laboratory rabbits with contagious diarrheal disease in The Netherlands.

Pleural effusion disease has been reported by several authors from different countries, including The Netherlands, as a cause of considerable intercurrent mortality during serial intratesticular passages of certain strains of \textit{Treponema pallidum} in rabbits (3). A probable viral etiology has been suggested by most authors. The disease is clinically characterized by fever, anorexia, lymphocytopenia, leukocytosis, anemia, hypergammaglobulinemia, iridocyclitis, and death. The most conspicuous postmortem finding is the presence of variable amounts of aropy yellowish fluid in the pleural cavity, hence the name pleural effusion disease (4–8). In fatal cases occurring after the first week of infection, peritoneal effusion often is present (5). Focal areas of necrosis and inflammation are found scattered throughout the parenchyma of various organs, particularly liver, kidneys, and lungs. In some cases, the mesenteric lymphnodes are enlarged and may show similar lesions. However, clinical signs and pathological findings of pleural effusion disease vary considerably between different laboratories involved. With the agent from our institute, for example, an iridocyclitis was rarely found, and the amounts of fluid in the pleural cavity was moderate if present at all, whereas alteration in the lungs were pronounced (7). Yet, in an \textit{in vivo} neutralization test in rabbits, it was shown that the Danish pleural effusion disease agent and the Dutch pleural effusion disease agent, associated with different \textit{Treponema pallidum} strains, are identical or serologically closely related (7). Moreover, the Danish pleural effusion disease agent was associated with the same \textit{Treponema pallidum} strain as the agent which causes rabbit cardiomyopathy (2). Therefore, we assume that both conditions, although showing a considerable clinical and pathological variation, are expressions of the same disease entity caused by the same agent.

Another condition in rabbits, which we have also associated with the presence of coronavirus-like particles, is contagious diarrheal disease. As has been the case in many other institutes, it has been a major cause of intercurrent mortality of laboratory rabbits kept under conventional conditions in our Institute. No specific bacterial cause could be found in most cases.

Materials and Methods

\textit{Passage histories:} All rabbits used for passaging of the agents described in the present paper were 8 to 12-week-old female animals, bred at the authors’ institution in a colony which is free of pathogens and kept in pressurized glove boxes during the experiments. Plasma was taken from a rabbit during the acute phase of pleural effusion disease, 4 days after the intramuscular inoculation of a testicular suspension filtered through a 220-nm pore membrane. This material, which contained the Dutch pleural effusion disease agent, was used for the intramuscular inoculation of two rabbits from which a
pool of about 60 ml of plasma was collected 3 or 4 days post-inoculation, during the acute phase of the disease. In the same way, a pool of about 100 ml of plasma was collected from four rabbits which had been inoculated with plasma material containing the Danish pleural effusion disease agent. A 20% fecal suspension, pooled from four rabbits which had died from acute intestinal disease after being taken from the colony into a conventional environment, was filtered through a 450-nm pore membrane after low speed centrifugation. This suspension was inoculated orally into two rabbits.

**Virus purification:** After clarification by low speed centrifugation, the plasma pools were pelleted for 6 hours at 25,000 rpm, the pellets were resuspended overnight in a Tris-EDTA buffer, pH 7.4, and layered on top of a 5-ml 10 to 50% (w/w) linear sucrose gradient. A 15% (w/w) sucrose cushion containing 0.1% formaldehyde was placed in the gradient used for the purification of the Danish agent. The gradients were centrifuged for 3 hours at 35,000 rpm, and the light scattering bands found at a density of about 1.17 g/ml in both gradients were collected and pelleted onto 50% (w/w) sucrose cushions for 3 hours at 35,000 rpm. The interphases were collected and used for negative contrast electron microscopic examination.

**Electron microscopy:** Interphases and 1:10 diluted fecal suspensions were placed on Formvar carbon-coated copper grids. After 5 minutes of adsorption, the grids were negatively stained for 10 seconds using 2% solution of potassium phosphotungstate, adjusted to pH 5.2 using KOH, and examined in the electron microscope.

**Results**

**Pleural effusion disease agent:** In the interphases resulting from the purification procedures carried out on the plasma pools from the rabbits inoculated with the Dutch or the Danish pleural effusion disease agent, high concentrations of pleomorphic virus-like particles were demonstrated (Figures 1 and 2). The particles measured about 70 nm in diameter (range 51 to 98 nm, n = 40) including the projections, which measured about 10 nm (range 7.5 to 13 nm). On the basis of their size, morphology, and buoyant density, they were considered coronavirus-like. In 120 ml of plasma from four control rabbits from the same source, which was treated in the same way, no such particles were observed.

**Contagious diarrheal disease agent:** The two rabbits which had been orally inoculated with the filtered fecal suspension, showed mild symptoms of intestinal disease which disappeared within 24 hours. In the feces of both animals, which were of soft consistency during this period, virus-like particles similar to those described above were demonstrated (Figure 3). The particles measured about 70 nm (range 60 to 90 nm, n = 20) including the projections of about 10 nm. On the basis of their size and morphology, they also were considered coronavirus-like.

**Discussion**

The demonstration of coronavirus-like particles in rabbits with pleural effusion disease or cardiomyopathy and the presence of similar particles in rabbits with acute diarrheal disease indicate that coronaviruses may play a role as pathogens in rabbits. It is possible that the pleural effusion disease agent was originally a virus of humans, which has been transmitted to rabbits with *Treponema*
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infection experiments and serological data will be needed to determine whether pleural effusion disease, rabbit cardiomyopathy, and the enteric disease are caused by the same or different viruses. It is interesting that probable members of the Coronaviridae family were found in association with enteric disease in many animal species including the cat (10).

References