Figure 1. EM appearance of human enteric coronavirus (strain HECV-35). (left), Single virus particle in a fecal specimen; (right), immune aggregate by homologous antiserum.

the antisera to the two HECV strains and, to a lesser extent, antiserum to HCV OC43, reacted with the coronavirus-like particles in stools from all the positive patients by conventional IEM and also by solid-phase IEM [3].

In summary: (1) In infants and young children with acute

gastroenteritis, fecal excretion of HECVs is more frequently detected than in age-matched controls; (2) HECV strains are antigenically related to HCV OC43, and their detection in feces is consistently associated with antibody response to HECV as well as to HCV OC43; (3) our results support the existence of HECV strains [4] and suggest that they could play an enteropathogenic role in infants and young children with acute gastroenteritis.

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References

1. Gerna G, Cattaneo E, Cereda PM, Revelo MG, Achilli G. Human coronavirus OC43 serum inhibitor and neutralizing antibody by a new plaque-reduction assay. *Proc Soc Exp Biol Med* 1980;163:360-6.
2. Kapikian AZ, Dienstag JL, Purcell RH. Immune electron microscopy as a method for the detection, identification, and characterization of agents non cultivable in an in vitro system. In: Rose NR, Friedman H. (ed.). *Manual of clinical immunology*. 2nd edition. Washington: American Society for Microbiology, 1980; 70-83.
3. Shukla DD, Gough KH. The use of protein A, from *Staphylococcus aureus*, in immune electron microscopy for detecting plant virus particles. *J Gen Virol* 1979;45:533-6.
4. Caul EO, Clarke SKR. Coronavirus propagated from patient with non-bacterial gastroenteritis. *Lancet* 1975;2:953-4.

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Pneumocystis carinii in Germ-Free Rats

TO THE EDITOR—I read with interest the letters of W. Beautyman and W. T. Hughes concerning *Pneumocystis carinii* [1, 2] and would like to add a cautionary note from our own experience with "germ-free" Sprague-Dawley rats.

In May 1981, we purchased 70 germ-free, Cesarean-delivered, barrier-sustained Sprague-Dawley rats from the Charles River Company (Wilmington, Mass). These rats were shipped in sterile containers. Five rats that were randomly selected and killed immediately upon arrival in our laboratory were subsequently found to have *Pneumocystis carinii* organisms in their lung tissue. An order placed to the Harlan Sprague-Dawley Company (Indianapolis, Ind) for gnotobiotic rats yielded the same result—all six of the animals shipped were infected with *P. carinii*. The methods and the results of this serendipitous investigation have been published in detail and should serve as a warning to those who employ "germ-free" research animals [3]. The Charles River Company refunded us for their "germ-free" rats after I visited their facility and demonstrated appropriate histological techniques for screening for *P. carinii*. Since then, Dr. Hughes has ascertained that the animals purchased by him for use in his transmission studies [4, 5] were from a different isola-

tor maintained by the Charles River Company (personal communication) than the ones in which our rats were born.

We still do not know how gnotobiotic rats from two highly reputable breeders became infected with *P. carinii*, especially since oral swabs and fecal cultures from the same animals were negative for growth. Possible routes include (1) undetected lapses in technique employed by the companies, (2) unusual resistance of *P. carinii* to physical and/or chemical agents, or (3) in utero transmission. Whatever the route, this experience underscores the need for great caution when attempting to reach conclusions based upon experiments conducted in gnotobiotic animals, especially when the companies breeding the animals do not screen for the infective organisms in question.

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References

1. Beautyman W. The origin of *Pneumocystis carinii* (correspondence). *J Infect Dis* 1984;149:287-8.
2. Hughes WT. Reply (correspondence). *J Infect Dis* 1984;149:288-9.
3. Pifer LL, Lattuada CP, Edwards CC, Woods DR, Owens DR. *Pneumocystis carinii* infection in germ-free rats: implications for human patients. *Diagnostic Microbiology and Infectious Disease* 1984;2:23-36.
4. Hughes WT. Natural mode of acquisition for de novo infection with *Pneumocystis carinii*. *J Infect Dis* 1982;145:842-8.
5. Hughes WT, Bartley DL, Smith BM. A natural source of infection due to *Pneumocystis carinii*. *J Infect Dis* 1983;147:595.

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