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# Coronaviruses Associated with Outbreaks of Transmissible Enteritis of Turkeys in Quebec: Hemagglutination Properties and Cell Cultivation

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**SUMMARY.** Coronaviruses were observed by electron microscopy in the intestinal contents of turkeys in Quebec flocks where repeated outbreaks of enteritis occurred. Three isolates could be serially propagated in turkey embryos inoculated by the amniotic route with clarified intestinal contents. Purification and concentration of viral particles contained in intestinal contents of infected embryos were achieved by precipitation with polyethylene glycol and ultracentrifugation on sucrose density gradients. Three particle types were demonstrated: intact virions with a density of 1.18 to 1.20 g/ml and incomplete particles with densities of 1.14 and 1.24 g/ml. Hemagglutination of rabbit and guinea pig erythrocytes was demonstrated with the intact viral particles; the hemagglutinin was not dependent on incubation temperature. All the isolates were antigenically related, as shown by hemagglutination-inhibition. The turkey coronaviruses did not cross-react with antisera against coronaviruses of avian infectious bronchitis, porcine transmissible enteritis, bovine neonatal calf diarrhea, or mouse hepatitis. One of the Quebec isolates was shown to induce syncytia formation on its third passage in primary chicken-embryo kidney cell cultures. Electron-microscopic examination of infected cell-culture fluids revealed characteristic coronavirus particles identical to those found in intestinal contents of infected turkeys.

**RESUMEN.** Coronavirus asociados a brotes de enteritis transmisible en pavos en Quebec (Canadá): propiedades hemaglutinantes y propagación en cultivo celular.

Por medio de microscopía electrónica se observó la presencia de coronavirus en el contenido intestinal de parvadas de pavos en Quebec con historia de brotes repetidos de enteritis. Tres aislamientos obtenidos a partir de contenido intestinal fueron propagados en embriones de pavo inoculados por la vía amniótica. La purificación y concentración de las partículas virales presentes en el contenido intestinal de los embriones infectados fueron llevadas a cabo por medio de precipitación con polietileno glicol y ultracentrifugación en gradientes de densidad con sucrosa. Se demostró la presencia de 3 tipos de partículas: viriones intactos con una densidad de 1.18 a 1.20 g/ml y partículas incompletas con densidades de 1.14 y 1.24 g/ml. Se observó que la aglutinación de eritrocitos de conejo y cobayo ocurrió con viriones intactos y que esta hemaglutinación no dependió de la temperatura de incubación. De acuerdo a los resultados obtenidos en la prueba de inhibición de la hemoaglutinación, todos los aislamientos resultaron estar relacionados antigénicamente. Estos coronavirus de pavo no tuvieron reacciones cruzadas con antiseros contra coronavirus de pollo (virus de la bronquitis infecciosa), de porcino (virus de la gastro enteritis transmisible), de bovino (virus de la diarrea neonatal en terneros) y de roedor (virus de la hepatitis tipo 3). Uno de estos aislamientos indujo la formación de sincitio celular al tercer pasaje en cultivos primarios de células renales de embrión de pollo. En el fluido de los cultivos celulares se demostró la presencia de partículas de coronavirus por medio de la microscopía electrónica. Estas partículas eran idénticas a las encontradas en el contenido intestinal de los pavos infectados.

Transmissible enteritis (bluecomb disease) of turkeys is an acute, highly infectious disease af-

fecting turkeys of all ages, but especially poults. It is characterized by anorexia, watery droppings, marked dehydration, weight loss, and higher than average mortality (18,19). The lesions are limited to the intestinal mucosa and consist of

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marked shortening of villi, loss of microvilli, epithelial desquamation, and hemorrhage in the jejunum, ileum, and cecum (1,19,21). The entity was first observed in 1951 in Washington state (17), and in the early 1960s, it was recognized as the most costly disease of turkeys in Minnesota (6,18). In Canada, the disease has been encountered in Ontario (7) and Quebec for several years, where at times it has become a serious problem.

A coronavirus is now accepted as the cause of transmissible enteritis (8,13,14). No cross-reaction of this virus with other coronaviruses has been found by immunoelectron-microscopy (13), and different isolates of the turkey coronavirus (TCV) were shown to be antigenically identical or closely related (20). Failure to propagate the TCV in conventional cell-culture systems has limited the development of serologic tests; for the same reason, an attenuated virus vaccine is not yet available.

In winter 1984–85, coronaviruses were recovered by electron microscopy (EM) from the intestinal contents of turkeys in Quebec flocks where repeated outbreaks of enteritis occurred. Three isolates could be serially propagated in turkey embryos. This paper describes the purification procedures used to prepare adequate amounts of viral antigen and our observations on hemagglutination and cultivation properties of these isolates and the Minnesota strain.

## MATERIALS AND METHODS

**Source of virus.** Intestinal contents were obtained from diarrheic poults purchased from three different flocks in southern Quebec. The specimens were homogenized in 10 volumes of 0.05 M Tris-HCl buffer (pH 8.0) and clarified by centrifugation at  $5000 \times g$  for 30 min at 4 C. The supernatants were then filtered through Millipore membrane filters 450-nm-pore size and kept at 4 C for less than 1 week.

The Minnesota isolate of turkey coronavirus, which had been serially passaged in embryonated turkey eggs (5), was kindly provided by Dr. B. S. Pomeroy (College of Veterinary Medicine, Univ. of Minnesota, St. Paul, Minn.).

Clarified clinical specimens were inoculated into the amniotic cavity of 22 to 24 one-day-old embryonated turkey eggs obtained from a source known to be free from all of the usual specific pathogens of turkeys. After inoculation, the eggs were incubated at 37 C for 3 to 4 days. Thereafter, embryo intestines were harvested and homogenized in 10 volumes of phosphate-buffered saline (PBS), pH 7.2, using a Waring commercial blender. The homogenates were clarified at  $10,000 \times$

$g$  for 20 min, and supernatants were used for subsequent inoculations and preparation of purified viral antigen.

**Concentration and purification.** Clarified suspensions of intestinal contents (200 ml) were adjusted to contain 8% polyethylene glycol (molecular weight 8,000), stirred 4 hr at 4 C, and centrifuged at  $10,000 \times g$  for 40 min. The pellets containing the viruses were then dispersed in 5 ml of PBS and layered on top of a step gradient consisting of 3 ml each of 30, 40, 50, and 60% (w/v) sucrose solutions. After overnight centrifugation at  $100,000 \times g$  (Beckman L5-65 ultracentrifuge, rotor SW27), opalescent bands were collected from the top of the tubes, dialyzed overnight against PBS, and recentrifuged at  $100,000 \times g$  for 3 hr on a similar sucrose gradient. Fractions were collected from the bottom of the tubes (Hoefer fraction collector, Hoefer Scientific, San Francisco, Calif.), and the absorbance (280 nm) and the sucrose density were determined for each sample. Virus-containing fractions were then dialyzed overnight and stored at  $-70$  C.

Samples of the clarified specimens and fractions of the sucrose gradients were negatively stained with 2% phosphotungstic acid, pH 7.0, and examined by EM, as previously described (3).

**Hemagglutination (HA) and hemagglutination-inhibition (HI).** Serial twofold dilutions of purified viral antigen were prepared in 0.05-ml amounts and mixed with 0.05 ml of 0.5% erythrocyte suspensions. The mixtures were then incubated at 4 C, 22 C, and 37 C for 1 hr, and the tests were read. The HA titer was expressed as the reciprocal of the highest antigen dilution showing complete HA.

For HI tests, sera were first adsorbed with kaolin and then mixed with packed erythrocytes to remove non-specific HA activity. Four units of HA antigen in 0.025 ml and 0.025 ml of serial twofold dilutions of the treated sera were mixed and then incubated at 37 C for 1 hr before adding 0.025 ml of erythrocyte suspension. The microplates were incubated at room temperature for 1 hr, and the tests were read. The HI antibody titer was expressed as the reciprocal of the highest serum dilution showing complete HI. The diluent used throughout was PBS 0.01 M, pH 7.2, containing 0.1% bovine serum albumin.

**Antisera.** Hyperimmune sera were prepared in guinea pigs against the Minnesota and Quebec isolates. On day 1, guinea pigs received a mixture of 0.5 ml purified antigen (250  $\mu g$  of viral proteins) and 0.5 ml complete Freund's adjuvant (Difco, Detroit, Mich.) subcutaneously at multiple sites (0.1 ml per site). On days 14 and 28, they were re injected intraperitoneally with a mixture of 0.5 ml antigen and 0.5 ml incomplete Freund's adjuvant; they were bled on day 40. The antisera were inactivated at 56 C for 30 min.

Convalescent sera were also obtained from turkeys in one flock from which we have received fecal samples. Antisera against bovine diarrhea, avian infectious bronchitis (Beaudette strain IBV), porcine transmis-

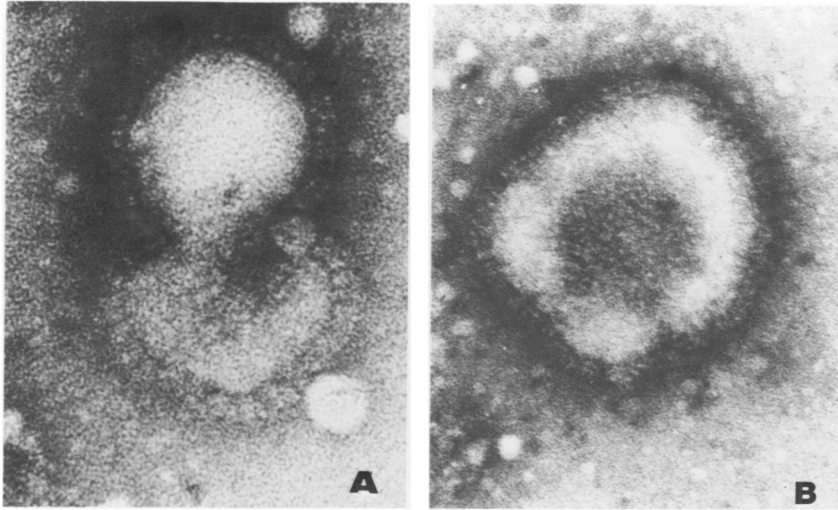


Fig. 1. Electron micrograph of coronavirus particles observed in the intestinal contents of diarrheic turkeys. 260,000 $\times$ .

sible gastroenteritis (TGEV), and mouse hepatitis (MHV-3) coronaviruses produced in our laboratory were used for comparison.

**Cell cultures.** Primary and secondary chicken and turkey embryo skin, kidney, and fibroblast cell cultures were prepared as previously described (4,24). They were grown as monolayers in Eagle's minimum essential medium (MEM) with Earle's salt and glutamine (Flow Labs., Inc., Mississauga, Ontario) supplemented with

10% heat-inactivated bovine fetal serum, 100 U of penicillin/ml, and 100  $\mu$ g of streptomycin/ml. The maintenance medium consisted of MEM without serum. A continuous human rectal tumor (HRT-18) cell line (9), kindly provided by Dr. J. Laporte (Laboratoire de virologie vétérinaire, Thiverval-Grignon, France) was also used.

Confluent cell monolayers in 25-cm<sup>2</sup> tissue-culture flasks were rinsed twice with PBS and inoculated with

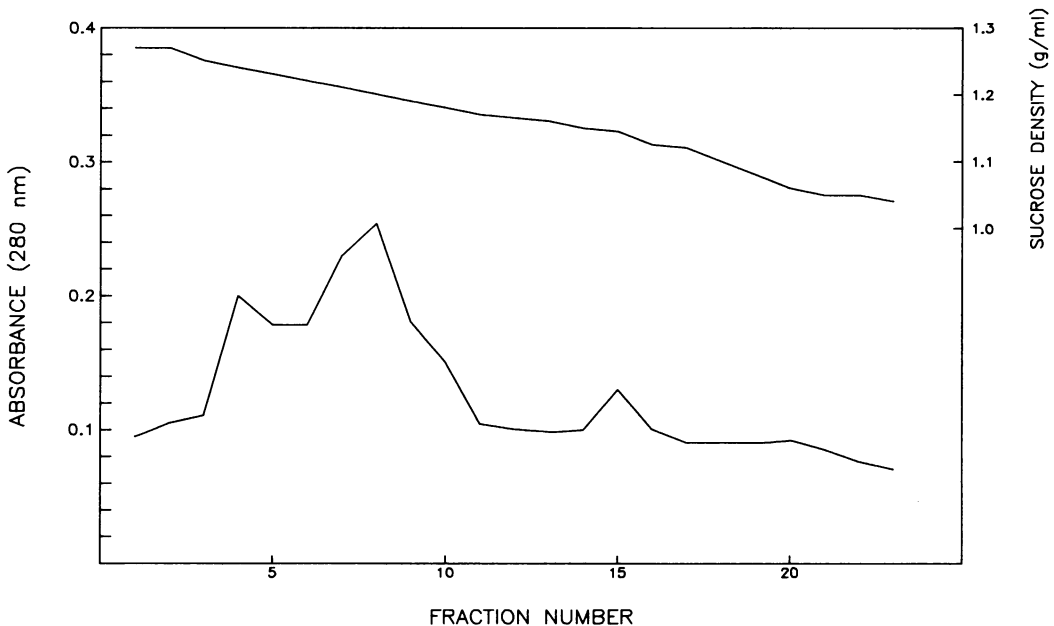


Fig. 2. Isopycnic centrifugation on sucrose gradient of the Minnesota isolate of turkey coronavirus passaged in embryonated turkey eggs.

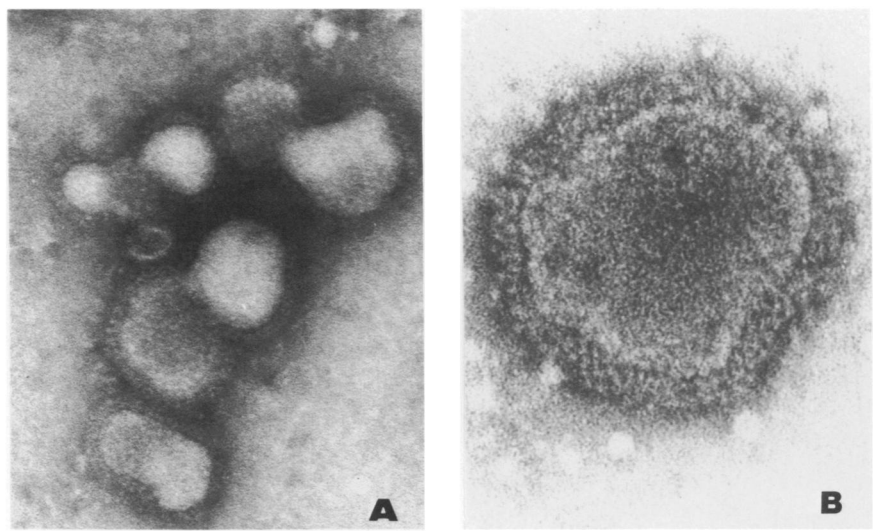


Fig. 3. Electron micrograph of viral particles observed in sucrose gradient fractions of density 1.18 to 1.20 g/ml. A: aggregated coronavirus particles. 125,000 $\times$ . B: an intact coronavirus particle. 260,000 $\times$ .

0.5 ml of clarified viral suspension. After adsorption for 2 hr at 37 C, the cultures received 5 ml of maintenance medium adjusted to pH 8.0 with sodium bicarbonate. They were incubated at 37 C and checked daily for the appearance of any cytopathic effect (CPE). Subpassages were done at 6-to-7-day intervals; the cultures were harvested by two freeze-thaw cycles.

RESULTS

Numerous pleomorphic coronavirus-like particles were recognized by EM of the intestinal

Table 1. Hemagglutinating activity of bluecomb coronavirus isolates propagated in embryonated turkey eggs.

Erythrocyte species	Isolates			
	Minnesota	84-2303	84-2204	85-403
Rabbit	512-1024 <sup>a</sup>	4-8	512-2048	32-64
Bovine	0	0	0	ND <sup>b</sup>
Guinea pig	256	0	256	0
Horse	0	0	0	ND
Sheep	0	0	0	ND
Mouse	8-16	0	0	ND
Goose	0	0	0	ND
Monkey	0	0	0	ND
Rooster	0	0	0	ND
Chicken	0	0	0	ND

<sup>a</sup>Reciprocal of the highest antigen dilution showing complete HA.

<sup>b</sup>Not done.

contents of diarrheic poult s purchased from the Quebec flocks. The particles were generally spherical to oblong in shape, enveloped, and centrally depressed. They were surrounded by a single fringe of regularly spaced petal-shaped projections attached to the particles by a short, thin stalk 7.3 to 17.4 nm in length. The virions ranged in diameter from 48 to 230 nm, with an average of 120 nm (Fig. 1).

Isolates from these flocks and the Minnesota strain were propagated by inoculation of embryonated turkey eggs. Three successive passages increased the viral titers and provided adequate stocks for purification. After 3 days of incubation, few embryos had died and gross lesions were confined to the intestinal tract. The contents of the duodenum, jejunum, and ceca were watery and gaseous. The ceca were markedly distended and filled with watery, greenish contents. The other organs were normal in appearance.

The coronaviruses were more easily recognized following precipitation with polyethylene glycol than following precipitation by ultracentrifugation. The viruses showed less damage, resulting in fewer collapsed particles with more projections on their surfaces. Sucrose-density-gradient centrifugation of the partly purified preparations yielded two opalescent bands and three absorbance peaks of 1.14, 1.18-1.20, and 1.22-1.24 g/ml (Fig. 2). The predominant peak was located between sucrose densities of 1.18



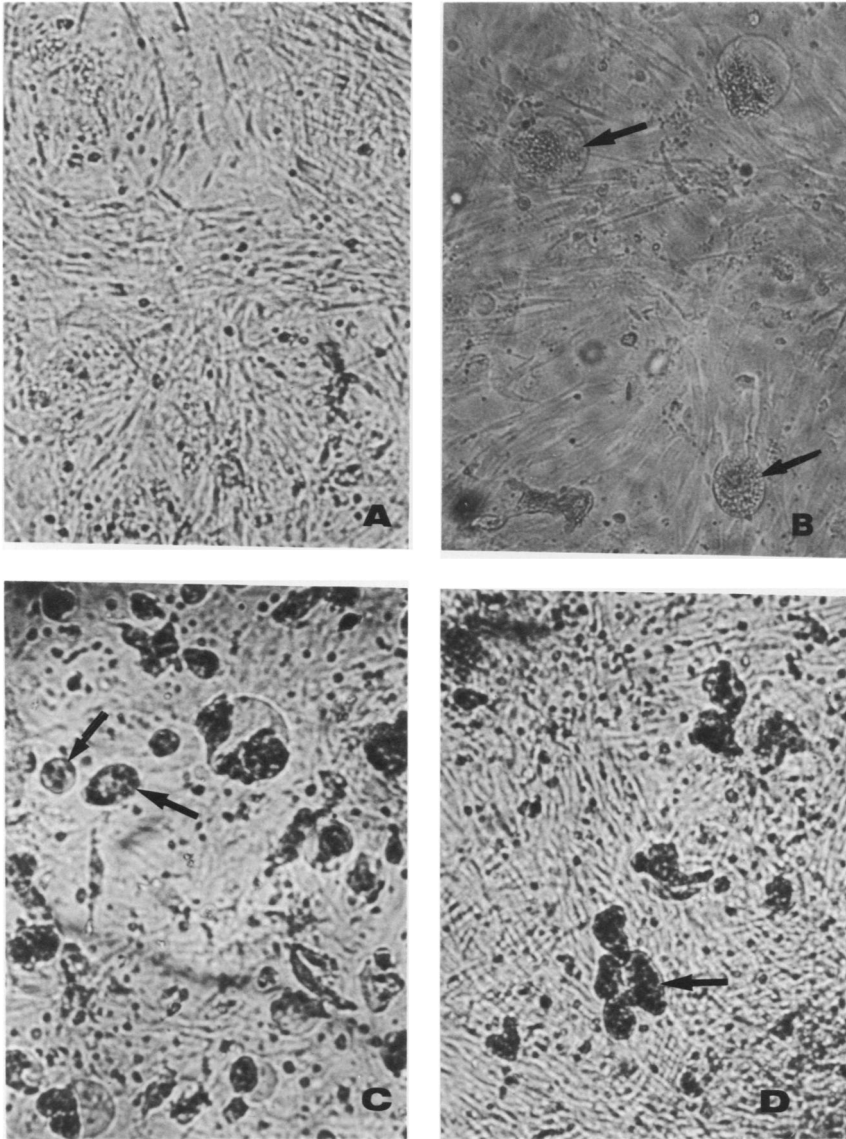


Fig. 4. Cytopathic effect induced by the Quebec isolate 85-403 of turkey coronavirus at its third passage in primary chicken-embryo kidney cells. A: control at 72 hours; B: infected monolayers after 48 hours. The arrows indicate morphologically altered infected cells; C: infected monolayers after 72 hours. The arrows indicate multinucleated giant cells; D: infected monolayers after 96 hours. The arrow indicates detached syncytia floating in the culture fluid. Note the integrity of the monolayer. 100 $\times$ .

and 1.20 g/ml. This peak contained numerous intact virions (Fig. 3), whereas other fractions contained damaged particles only. Particles of 1.24 density were without projections and averaged 80 nm in diameter, whereas those of 1.14 density appeared to possess an envelope but few projections. Numerous cellular debris were ob-

served in this peak. All Quebec isolates showed a similar ultracentrifugation profile.

The American prototype and the Quebec isolates agglutinated rabbit and guinea pig erythrocytes spontaneously (Table 1). No hemagglutination was noted with bovine, horse, sheep, mouse, goose, monkey, rooster, or chicken cells.

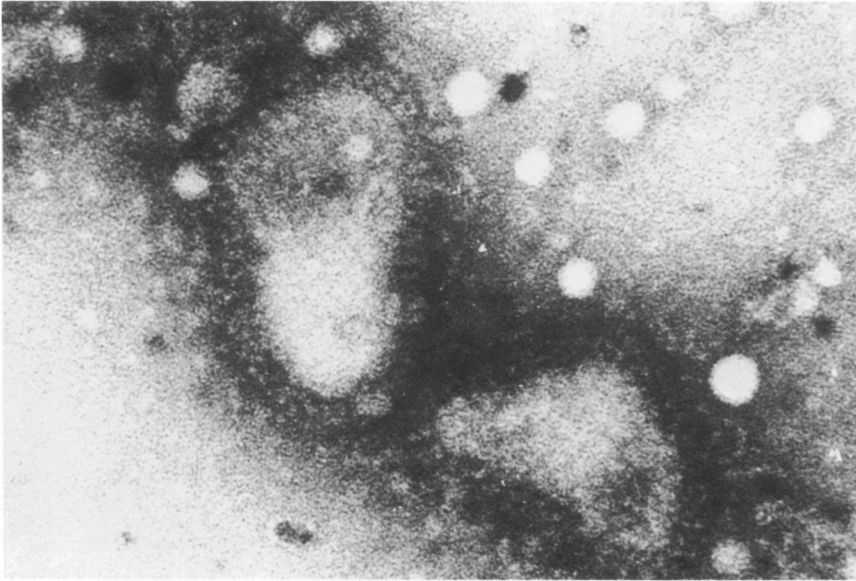


Fig. 5. Electron micrograph of viral particles obtained from the fifth passage of the Quebec turkey coronavirus isolated in primary chicken-embryo kidney cells. 260,000 $\times$ .

The HA titers varied from 32 to 1024, depending on the number of egg passages. The incubation temperature (4, 22, or 37 C) did not influence the hemagglutinating activity. Hemagglutinin was associated only with particles corresponding to sucrose density of 1.18–1.20 g/ml.

Guinea pig hyperimmune serum produced against the Minnesota strain inhibited the hemagglutinating activity of the homologous virus; the antiserum had an HI titer of 1:128 to 1:256. Serum from preimmunized guinea pigs did not inhibit the hemagglutinating activity of the virus. Hemagglutination was also not inhibited following pre-incubation of the viral preparation with antisera against TGEV, IBV, calf corona, and murine MHV-3 viruses. The anti-Minnesota coronavirus serum reacted similarly against the Quebec isolates, and anti-Quebec coronavirus serum reacted against the Minnesota isolate.

Twenty-five percent of the convalescent sera obtained from one of the Quebec flocks had HI antibodies for bluecomb virus. Positive sera (HI titers of 1:64 to 1:256) could not protect embryonated chicken eggs inoculated with IBV (Beaudette strain) and could not neutralize MHV-3 and TGEV cultivated on secondary porcine testicle cells and continuous mouse fibroblast (L2) cells, respectively.

Attempts to propagate the American strain in primary and secondary cell lines of chicken and

turkey origin were unsuccessful. Three weekly blind passages were made, and no evidence of viral replication in these cell cultures was obtained, as suggested by the absence of a cytopathic effect (CPE) and absence of viral particles in cell extracts examined by EM. However, syncytia formation could be observed on the third passage of one of the Quebec isolates (85-403) in primary chicken-embryo kidney cells. On the fourth passage, small clusters of rounded and translucent cells were noted as soon as 24 hr postinoculation (Fig. 4), followed by progressive production of syncytia containing up to 10 nuclei. The number of multinucleated cells peaked after 72 hr. As the infection progressed, the syncytia detached and floated free in the culture fluid. The monolayers were never completely destroyed; the infected cells were continuously replaced by normal cells, and the monolayers looked normal for at least 7 days. Similar degenerative effects were observed after the fifth passage. Typical coronavirus particles were seen by EM in infected cell extracts prepared from the third and fifth passages (Fig. 5). However, there was a concomitant infection with few reoviruses detected by EM only on the fifth passage.

No well-defined CPE was produced after three successive passages of the Quebec isolate in HRT-18 cells, but a few coronavirus particles could be detected by EM in the infected culture fluids. No

reoviruses were recovered from these cell cultures.

Hemagglutinating activity was obtained from infected culture fluids of both cell lines. HA titers varied from 1:16 to 1:256, and HA activity was inhibited by the guinea pig hyperimmune serum produced against the Minnesota strain. Hemagglutination was not inhibited following incubation with a pool of antisera produced in rabbits against different isolates of reoviruses from chicken and turkey origins.

## DISCUSSION

The ultrastructural properties and the sucrose-gradient ultracentrifugation profile of the viruses characterized in the present study are in agreement with previous reports that the etiologic agent of bluecomb in turkeys is a member of the coronaviridae family (13,14,22).

A previously unreported hemagglutinating activity has been demonstrated and was shown to be specific for the bluecomb coronaviral agent, since it could be neutralized by specific antiserum. Cross-reaction studies by hemagglutination-inhibition supported the finding that the turkey coronavirus is antigenically unrelated to other animal coronaviruses involved in enteric problems and to IBV of chickens (20).

At present, a serum-virus-neutralization test using day-old poult is the only serologic test routinely used for diagnostic purposes (2,8). This test is costly and has limited application in most diagnostic laboratories. More recently, an indirect fluorescent-antibody test, using frozen sections of infected intestines as a source of antigen, has also been described to detect antibodies of turkey flocks that have recovered from the disease (15,16). Although this test is faster and more specific than the poult infectivity assay, the test does not appear to be sensitive enough to allow detection of chronic carriers (15). The HI test provided a means more easily applicable to serologic diagnosis. Further work is needed to optimize and standardize this test and to compare it with other methods.

Field and laboratory studies indicated that turkey flocks recovering from transmissible (coronaviral) enteritis are immune for the normal life of the flock (18). The immunizing ability of killed vaccines was disappointing (D. R. Deshmukh and B. S. Pomeroy, unpublished data, 1973). The use of non-attenuated virus to produce active

immunity in turkey flocks under field conditions has also been evaluated; it was shown to have value in a "controlled exposure" program (10,20). However, the non-attenuated vaccine has limitations, because vaccinated flocks perpetuate the disease and abnormal losses occasionally occur. An attenuated virus vaccine is thus needed that would stimulate an immune response and would remove the risk and loss now associated with "controlled exposure" with virulent virus. At present, such a vaccine is not available owing to failure to cultivate the virus in cell cultures.

In this study, a Quebec isolate could be propagated for at least five passages in primary and secondary chicken-embryo kidney cells. Evidence of virus growth included demonstration of the virus in infected culture fluids and production of cytopathic changes comparable to what was previously described for other coronaviruses (11,12,23,25). The hemagglutinating activity could also be recovered from cell-culture supernatants. However, a concomitant infection with reoviruses was also presented in the infected cell-culture fluids. Attempts to clone the turkey coronavirus by plaque technique are in progress.

## REFERENCES

1. Adams, N. R., R. A. Ball, and M. S. Hofstad. Intestinal lesions in transmissible enteritis of turkeys. *Avian Dis.* 14:392-399. 1970.
2. Adams, N. R., and M. S. Hofstad. Isolation of transmissible enteritis agent of turkeys in avian embryos. *Avian Dis.* 15:426-433. 1971.
3. Dea, S., R. S. Roy, and M. E. Begin. Counterimmunoelectrophoresis for detection of neonatal calf diarrhea coronavirus: methodology and comparison with electron microscopy. *J. Clin. Microbiol.* 10:240-244. 1979.
4. Dea, S., R. S. Roy, and M. E. Begin. Bovine coronavirus isolation and cultivation in continuous cell lines. *Am. J. Vet. Res.* 41:30-38. 1980.
5. Deshmukh, D. R., C. T. Larsen, and B. S. Pomeroy. Survival of bluecomb agent in embryonating turkey eggs and cell cultures. *Am. J. Vet. Res.* 34:673-675. 1973.
6. Erlandson, V. A., and D. O. Mesick. Minnesota's turkey industry—1961. *Bull. State-Federal Crop and Livestock Reporting Service*, Minnesota, June 1962.
7. Ferguson, A. E. Bluecomb-transmissible enteritis in turkeys. *Can. Poult. Rev.* 85:74-76. 1961.
8. King, D. J. Comments on the etiology and immunity of transmissible (coronaviral) enteritis of turkeys (bluecomb). *Am. J. Vet. Res.* 36:555-556. 1975.
9. Laporte, J., P. Bobulesco, and F. Rossi. Une



- lignée cellulaire particulièrement sensible à la réplique du coronavirus entérique bovin: les cellules HRT-18. *C. R. Acad. Sc. Paris* 290, série D:623–626. 1980.
10. Larsen, C. T. Progress in research on bluecomb disease and long range research needs. National Turkey Federation Annual Meeting. 1968.
  11. Mebus, C. A., E. L. Stair, M. B. Rhodes, and M. J. Twiehaus. Neonatal calf diarrhea: propagation, attenuation, and characteristics of a coronavirus-like agent. *Am. J. Vet. Res.* 34:145–150. 1973.
  12. Mengeling, W. L., A. D. Boothe, and A. E. Ritchie. Characteristics of a coronavirus (Strain 67N) of pigs. *Am. J. Vet. Res.* 33:297–308. 1972.
  13. Naqi, S. A., B. Panigrahy, and C. F. Hall. Purification and concentration of viruses associated with transmissible (coronaviral) enteritis of turkeys (bluecomb). *Am. J. Vet. Res.* 36:548–552. 1975.
  14. Panigrahy, B., S. A. Naqi, and C. F. Hall. Isolation and characterization of viruses associated with transmissible enteritis (bluecomb) of turkeys. *Avian Dis.* 17:430–438. 1973.
  15. Patel, B. L., D. R. Deshmukh, and B. S. Pomeroy. Fluorescent antibody test for rapid diagnosis of coronaviral enteritis of turkeys (bluecomb). *Am. J. Vet. Res.* 36:1265–1267. 1975.
  16. Patel, B. L., E. Gonder, and B. S. Pomeroy. Detection of turkey coronaviral enteritis (bluecomb) in field epidemiology, using the direct and indirect fluorescent antibody tests. *Am. J. Vet. Res.* 38:1407–1411. 1977.
  17. Peterson, E. H., and T. A. Hymas. Antibiotics in the treatment of an unfamiliar turkey disease. *Poult. Sci.* 30:466–468. 1951.
  18. Pomeroy, B. S., and J. M. Sieburth. Bluecomb disease of turkeys. *Proc. 90th Ann. Meeting, Am. Vet. Med. Assoc.* pp. 321–328. 1954.
  19. Pomeroy, B. S. Transmissible enteritis of turkeys (bluecomb) and hemorrhagic enteritis. In: *Diseases of poultry*, 6th ed. M. S. Hofstad, B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder, Jr., eds. Iowa State University Press, Ames. pp. 745–752. 1972.
  20. Pomeroy, B. S., G. T. Larsen, D. R. Deshmukh, and B. L. Patel. Immunity to transmissible (coronaviral) enteritis of turkeys (bluecomb). *Am. J. Vet. Res.* 36:553–555. 1975.
  21. Pomeroy, K. A., B. L. Patel, G. T. Larsen, and B. S. Pomeroy. Combined immunofluorescence and transmission electron microscopic studies of sequential intestinal samples from turkey embryos and poults infected with turkey enteritis virus. *Am. J. Vet. Res.* 39:1348–1354. 1978.
  22. Ritchie, A. E., D. R. Deshmukh, C. T. Larsen, and B. S. Pomeroy. Electron microscopy of coronavirus-like particles characteristic of turkey bluecomb disease. *Avian Dis.* 17:546–558. 1973.
  23. Robb, J. A., and C. W. Bond. Pathogenic murine coronaviruses: I. Characterization of biological behavior in vitro and virus-specific intracellular RNA of strongly neurotropic JHMV and weakly neurotropic A59V viruses. *Virology* 94:352–372. 1979.
  24. Silim, A., M. A. S. Y. El Azhary, and R. S. Roy. A simple technique for preparation of chicken-embryo-skin cell cultures. *Avian Dis.* 26:182–185. 1982.
  25. Tyrrell, A. J., D. J. Alexander, J. D. Almeida, C. H. Cunningham, B. C. Easterday, D. J. Garwes, J. C. Hierholzer, A. Kapikian, M. R. MacNaughton, and K. McIntosh. *Coronaviridae*, 2nd report. *Intervirology* 10:321–328. 1978.

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