EVIDENCE OF CLOSE RELATEDNESS BETWEEN TURKEY AND BOVINE CORONAVIRUSES

Peter Tijssen, Arnold J. Verbeek and Serge Dea

CRMC - Institute Armand-Frappier, University of Quebec

Laval-des-Rapides, Quebec, Canada, H7N 4Z3

INTRODUCTION

Immunoelectron microscopy and indirect immunofluorescence studies in 1973 showed that turkey enteric coronavirus (TCV or "Bluecomb agent") is unrelated to another avian coronavirus IBV, and to mammalian coronaviruses (1, 2). Coronaviruses have thus been classified, according to antigenic cross-reactivity, into four distinct subgroups within the family, two subgroups of avian viruses and two subgroups of non-avian viruses (3, 4, 5).

TCV is one of the major causative agents of epidemic diarrhea in turkey poults (6, 7). Studies on TCV have long been hampered by the failure to obtain sufficient virus and by the lack of a suitable tissue culture system (6, 8). Recently, however, we have been able to adapt this virus to HRT-18 cells, an established human rectal tumor cell line (9, 10). Studies on tissue culture- and egg-adapted TCV demonstrated that this virus shares many morphological, biological and molecular properties with bovine coronavirus (BCV; 11, 12), such as (i) the presence of additional short granular surface projections (hemagglutinating glycoprotein), (13, 14); (ii) antigenic relatedness as demonstrated by ELISA, (15); and (iii) intestinal tropism (in respective animals and replication "in vitro" in HRT-18 cells) (9, 16).

Here, we report studies undertaken to establish the extent of relatedness between BCV and TCV. These studies led us to conclude that these viruses are very closely related and that they should be reclassified.

RESULTS

TCV and BCV readily adapted to HRT-18 cells. Although the yield of infective viral particles were comparable for the two viruses, the cytopathic effect of TCV was considerably more pronounced (polykaryocytosis).

Monoclonal antibodies were raised against both TCV (12) and BCV (manuscript in preparation). About 30 MAbs were obtained against the five major structural proteins of each virus. These MAbs were used in Western immunoblotting, neutralisation, and hemagglutination inhibition studies.

The structural polypeptide profiles after electrophoresis under denaturing conditions were comparable for the two viruses. In the presence of 2-mercapto-ethanol, the 140K polypeptide of TCV was split into two monomers (65K), as

Table 1. Characteristics of MAbs distinguishing BCV and TCV

MAb	Immunizing Virus	Polypeptide Specificity	ELISA		Reactivity by SN			HAI	
			TCV	BCV	TCV	BCV	TCV	TCV	BCV
M23	TCV	gp200/100	5.6 ^a	3.8	-	-	-	-	-
43C3	TCV	p52	4.0	-	-	-	-	-	-
M31	TCV	gp140	5.0	5.0	5120 ^b	-	1280 ^c	-	-
M33	TCV	gp200/100	6.2	6.2	10230	-	-	-	-
VIF4E6	BCV	gp200/100	3.8	4.7	-	320	-	-	-
VIF4E5	BCV	gp200/100	3.8	4.7	40	640	-	-	-
VIA5	BCV	gp200/100	3.8	6.2	40	160	-	-	-
VIF3A	BCV	gp140	3.8	4.7	-	-	-	-	-
VIF3B	BCV	gp140	2.6	4.7	-	-	-	-	80
314C	BCV	p52	2.6	5.3	-	-	-	-	-
V27	BCV	p52	2.6	5.9	-	-	-	-	-

^a Log 10 of the highest dilution of ascitic fluid giving an A492 value 2.5 X A492 value of a buffer control.

^b Seroneutralization titers were expressed as the reciprocal of the highest dilution of ascitic fluid neutralizing CPE produced by 100 TCID₅₀ of virus.

^c Hemagglutination-inhibition titers were expressed as the reciprocal of the highest dilution ascitic fluid neutralizing 8 HA units of virus.

previously described for BCV (13, 17). Western immunoblotting of these proteins was used to establish the protein specificity of 29 anti-TCV MAb and 20 anti-BCV MAb. Neutralising antibodies were directed to gp 200, gp 120 and gp 100 or gp 140. MAb inhibiting hemagglutinating activity were all directed to gp 140. The gp 200, gp 120 and gp 100 were shown by MAb to be interrelated. Polyclonal antisera to TCV and BCV cross-reacted strongly with the proteins of the heterologous virus. Anti-BCV and anti-TCV MAb revealed also an extensive heterologous activity. Most MAb reacted similarly against both viruses by ELISA, SN, and HAI tests. At least four anti-TCV and at least seven anti-BCV MAb could distinguish the two viruses (Table 1). On the other hand, we have shown recently that even among TCV strains, the variation in reactivity exists among the anti-TCV MAb towards their homologous virus (12).

Different radio-isotopically labelled recombinant plasmids, containing both the matrix and the nucleocapsid gene, and other sequences obtained by cloning random-primed cDNA, were independently able to detect TCV under rather stringent hybridization conditions, optimized for BCV detection (17). TCV could also be detected in clinical specimens, using BCV-specific cDNA probes either labeled by nicktranslation or by amplification of BCV specific fragments using the polymerase chain reaction. These data and sequencing studies in progress demonstrated the presence of strong homologies between both viral genomes.

Experimental inoculation studies of turkey poult with TCV revealed that egg-adapted and tissue culture-adapted TCV isolates still caused the typical symptoms of enteritis (9, 19). Infection of poult with BCV (either from diarrheic fecal samples or from tissue culture strains) did not cause diarrhea, and poult killed after one week p.i. did not show any histologic lesions. However, coronavirus particles were detected by EM and by hybridization with cDNA probes in the clarified intestinal contents from poult from two different groups of seronegative poult.

It can be concluded from these studies that TCV and BCV are, on a molecular basis, almost indistinguishable, although they infect different animal species. Therefore, these viruses should be reclassified into a single subgroup.

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REFERENCES

1. K.A. Pomeroy, B.L. Patel, C.T. Larsen, and B. S. Pomeroy. Combined immunofluorescence and transmission electron microscopic studies of sequential intestinal samples from turkey embryos and poult infected with turkey enteritis coronavirus. Am. J. Vet. Res. 39:1348 (1978).
2. A.E. Ritchie, 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 (1973).

3. N.C. Pedersen, I. Wark and W.L. Mengeling. Antigenic relationships of the feline infectious peritonitis virus to coronaviruses of other species. Arch. Virol. 58:45 (1978).
4. M.C. Horzinek, H. Lutz, and N.C. Pedersen. Antigenic relationships among homologous structural polypeptides of porcine, feline and canine coronaviruses. Infect. Immun. 37:1148 (1982).
5. S. Siddell, H. Wege and V. Ter Meulen. The biology of coronaviruses. J. Gen. Virol. 64:761 (1983).
6. B.S. Pomeroy. Coronaviral enteritis of turkeys, In M.S. Holstad, H.J. Barnes, B.W. Calnek, W.M. Reid, and H.W. Yoder (eds), "Diseases of poultry", 8th ed. Iowa's State Univ. Press, Ames (1984).
7. S. Dea and P. Tijssen. Viral agents associated with outbreaks of diarrhea in turkey flocks in Quebec. Can. J. Vet. Res. 52:53 (1988).
8. D.R. Deshmukh, C.T. Larsen, and B.S. Pomeroy. Survival of bluecomb agent in embryonating turkey eggs and cell cultures. Am. J. Vet. Res. 34:673 (1973).
9. S. Dea and P. Tijssen. Isolation and trypsin-enhanced propagation of turkey enteric (bluecomb) coronaviruses in a continuous human rectal tumor (HRT-18) cell line. Am. J. Vet. Rec. (in press)
10. W.A.F. Tompkins, A.W. Watrach, J.D. Schmale, R.M. Schultz, and J.A. Harris. Cultural and antigenic properties of newly established cell strains derived from adenocarcinomas of the human colon and rectum. J. Natl. Cancer Inst. 52:101 (1974).
11. S. Dea and P. Tijssen. Identification of the structural proteins of turkey enteric coronavirus. Arch. Virol. 99:173 (1988).
12. S. Dea and P. Tijssen. Antigenic and polypeptide structure of turkey enteric coronaviruses as defined by monoclonal antibodies. J. Gen. Virol. (in press).
13. B. King, B.J. Potts, and D.A. Brian. Bovine coronavirus hemagglutinin protein. Virus Res. 2:53: (1985).
14. B.G. Hogue, B. King, and D.A. Brian. Antigenic relationships among proteins of bovine coronavirus, human respiratory coronavirus OC43, and mouse hepatitis coronavirus A59. J. Virol. 51:384 (1984).
15. J. Laporte and P. Bobulesco. Polypeptide structure of bovine enteric coronavirus: comparison between a wild strain purified from feces and a HRT-18 cell-adapted strain. Adv. Exp. Med. Biol. 142:171 (1981).
16. W. Lapps, B.G. Hogue, and D.A. Brian. Sequence analysis of the bovine coronavirus nucleocapsid and matrix protein genes. Virology 157:47 (1987).
17. A. Verbeek, and P. Tijssen. Biotinylated and radioactive cDNA probes in the detection by hybridization of bovine enteric coronavirus. Molecular Probes 2:209 (1988).
18. N.R. Adams, R.A. Ball, and M.S. Hofstad. Intestinal lesions in transmissible enteritis of turkeys. Avian Dis. 14:392 (1970).