Northern blot hybridization法によるオルビウイルス各株のブルータングウイルス血清型17に対する反応性

誌名	Japanese journal of veterinary science
ISSN	00215295
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巻/号	51巻1号
掲載ページ	p. 187-190
発行年月	1989年2月

Reactivity of Orbivirus Strains to the Cloned L3 Gene of Blue tongue Virus Serotype 17 (BTV-17) by Northern Blot Hybridization

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(Received 20 June 1988/Accepted 26 August 1988)

Jpn. J. Vet. Sci. 51 (1): 187–190, 1989

KEY WORDS: blue tongue virus, northern blot hybridization, orbivirus.

Orbiviruses have a ten-segmented, double-stranded (ds) RNA genome. They have been physicochemically [1] and morphologically [11] characterized and divided into 13 groups on the basis of their serological reactions. Blue tongue virus (BTV), the prototype virus of the *Orbivirus* genus, causes a degenerative and sometimes fatal disease of sheep [6]. In areas where the disease is enzootic, infection of cattle with BTV is mostly mild or inapparent, but outbreaks of clinical infection in cattle have been reported [6].

Many orbivirus strains which share common antigens detectable by complement-fixation (CF) test, immunodiffusion test, and fluorescent-antibody test have been isolated in different parts of the world [6]. There has been reluctance to include the viruses of both Eubenangee and Epizootic haemorrhagic disease of deer (EHDV) group within the BTV serogroup despite many reports of distinct serologic relationships among these orbivirus strains [4, 9, 10, 14]. Such cross-reaction between diverse orbivirus strains has led to problems in the taxonomy of these groups.

Recently, Purdy *et al.* [12] cloned the BTV-17 segment 3 gene into the pBR322 plasmid and showed that the cDNA hybridized not only to homologous RNA but also to the corresponding segments of 3 other BTV serotypes in USA. More recently, Roy *et al.*[13] showed that 19 BTV serotypes tested, cross-reacted with BTV-17 segment 3 cDNA by northern hybridization and *in situ* hybridization.

In this communication, we try to clarify relationships between BTV and other orbivirus strains belonging to different serogroups by using a cDNA probe of BTV-17 segment 3.

Baby hamster kidney (BHK-21) cells were cultured by standard methods in Eagle's minimum essential medium (EMEM) supplemented with 10% calf serum and 10% tryptose phosphate broth (TPB). Confluent cell monolayers in tissue culture flasks were washed three times with Earl's balanced salt solution before inoculation with various orbivirus strains at a multiplicity of infection of 0.1 to 1.0. After adsorption for 1 hr at 37°C, without washing, 25 m*l* of serum-free EMEM containing 10% TPB, 1% glucose, and 5% yeast extract was added. The cells were then incubated at 37°C for 2 to 3 days. In total, 16 strains belonging to 6 different serogroups of the genus *Orbivirus* were employed in the present study (Table 1).

The cDNA probe used in this study was previously described [12]. The cDNA was radiolabelled with [α-^{32}P] dATP (3000 Ci/mM, New England Nuclear, Boston, Mass, U.S.A.) by nick-translation.

The extracted RNA (5 to 10 μg) was electrophoresed on a 1% agarose gel [13]. The gel was soaked in 50 mM NaOH containing 1 μg of ethidium bromide per m*l* for 20 min. The gel was washed four times (5 min intervals) in 25 mM sodium phosphate buffer (pH 6.5) and osmotically blotted overnight to Genescreen hybridization transfer membrane (NEN). After blotting, the membrane was air-dried and baked in an over at 80°C for 2 hrs. RNA blots were prehybridized at 42°C for 2 hrs in 50% formamide in 5 x SSC (1 x SSC equals 0.15 M NaCl, 0.15 M sodium citrate), 1% SDS, 100 μg of denatured salmon sperm DNA per m*l*, and 0.04% each of polyvinylpyrrolidone, Ficoll, and bovine serum albumin. The membrane was then hybridized for 16 hrs at 42°C to the nick-translated, [^{32}P]-labelled cDNA probe of BTV-17 segment 3 gene [12] in the same mixture. After hybridization, the membrane was washed twice at 25°C for 15 min in 2 x SSC, twice at 65°C for 45 min, each in 2 x SSC containing 0.5% SDS, and twice for 30 min each at 25°C in 0.1 x SSC. After washing, the membrane was air-dried and autoradiographed.

Rabbit antiserum against each orbivirus strain was prepared by two intramuscular injections of virus mixed with Freund's complete adjuvant and

Table 1. Orbivirus strains used in the present study

Serogroup	Strain	Isolation	
		Year	Country
Blue tongue	BT62–45S (serotype 17)	1973	United States
Epizootic haemorrhagic disease of deer	New Jersey	1955	United States
	Alberta	1962	Canada
	Ibaraki-2	1959	Japan
	CSIRO 753	1982	Australia
	CSIRO 439	1982	Australia
	CSIRO 775	1982	Australia
	DPP 59	1982	Australia
Eubenangee	In 1074	1968	Australia
Palyam	B8112 (D'Aguilar)	1972	Australia
	CSIRO 11	1974	Australia
	CSIRO 58	1979	Australia
	CSIRO 82	1975	Australia
Warrego	Warrego	1969	Australia
	Mitchell River	1969	Australia
Corriparta	Corriparta	1963	Australia

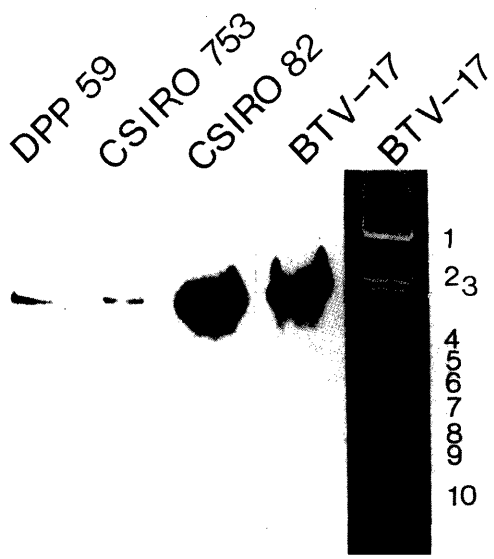

Fig. 1. Hybridization by northern blotting of segment 3 RNA of strains to the cDNA probe of BTV-17: The right hand is the electrophoretic pattern of BTV-17 on a 1% agarose gel, other lanes are autoradiograms of nick-translated, [^{32}P]-labelled cDNA hybridized to genome RNA of four orbivirus strains blotted in Genescreen paper transfered from one agarose gel.

one intravenous injection (two weeks intervals). Serum was collected two to three weeks after the last injection, inactivated at 56°C for 30 min and stored at −20°C until use.

CF tests were performed by a microtiter method using mouse brain antigen prepared by the method of Clarke and Casals [3].

Fig. 1 shows the results of hybridization of the blotted viral RNA segments to [^{32}P]-labelled cDNA of segment 3 of BTV-17. The probe hybridized with the corresponding segments of strains CSIRO 82 (Palyam serogroup), CSIRO 753 and DPP 59 (EHDV serogroup) as well as BTV-17, although low level hybridization of strains CSIRO 753 and DPP 59 signals have been observed. On the other hand, the probe completely lacked the ability to react with the RNAs of the remaining 12 orbivirus strains tested. The present data showed that the strains BTV-17, CSIRO 82, CSIRO 753, and DPP 59, although belonging to different serogroups, share homologous sequences in segment 3. Therefore it seems that CSIRO 753 and DPP 59 are genetically closer to BTV-17 than other EHDV strains tested here. Strains of CSIRO 753, CSIRO 439, CSIRO 775, and DPP 59 have been distinguished

Table 2. Complement-fixation tests among virus strains belonging to blue tongue, palyam, and epizootic haemorrhagic disease of deer serogroup

Antigen	Antisera prepared with		
	BTV-17	D'Aguilar	CSIRO82
BTV-17	32	NT[a)]	8
D'Aguilar	<4	64	32
CSIRO 82	16	16	32
CSIRO 753	<4	NT	16
DPP 59	8	NT	16

a) Not tested

from each other by a cross-neutralization test [15]. Recently, Roy *et al.* [13] have reported that all BTV serotypes reacted with the cDNA of a cloned BTV-17 segment 3 gene. This result suggests that the strain CSIRO 82 belonging to marakai serotype in palyam serogroup [8] is closely related to the BTV serogroup because of a strong signal in hybridization.

In the CF test anti-complementary activity of rabbit sera was less than 1:4. Strains CSIRO 82 and DPP 59 reacted with antiserum against BTV-17 but strain CSIRO 753 did not. In addition, BTV-17 also reacted with antiserum against strain CSIRO 82 (Table 2). The results also suggest that strain CSIRO 82 is related to the BTV serogroup. Of the three strains reacting with BTV-17 segment 3 cDNA probe by hybridization, two strains, CSIRO 82 and DPP 59, also reacted with antiserum against BTV-17. These results indicate that the results of hybridization are consistent with those of CF tests except for strain CSIRO 753.

The presence of a segmented genome, the demonstration of genetic reassortment among orbiviruses [7] and many factors concerning serological reactions suggests that the division of orbivirus strains into clearly distinct serogroups may be impossible. Antigenic determinants giving rise to low cross-reaction between two virus strains in different serogroups may not be shared by all members of each group. The results of the present study indicate that cross-reactions among strains belonging to different serogroups observed here by a hybridization technique may cause confusion in classification by serological reactions because of low level of cross-reaction. Strain CSIRO 753 did not react with antiserum against BTV-17 by CF test although the strain had same intensity as strain DPP 59 by northern hybridization, which may mean that there are some viral polypeptides, involving CF reaction, other than those encoded by L3 gene. Recently, it was indicated that there was weak cross-reaction by RNA-RNA blot hybridization in some segments between BTV and EHDV [2, 5], suggesting that the segments code some proteins responsible for the cross-reactive antigen. It seems that more extensive examination is needed to assess the interaction among virus strains belonging to different serogroups.

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要　約

Northern blot hybridization 法によるオルビウイルス各株のブルータングウイルス血清型17に対する反応性（短報）：長野秀樹・明石博臣[1)]・徳井忠史[1)]・稲葉右二[1)]（北里研究所附属家畜衛生研究所，[1)]農林水産省家畜衛生試験場）――ブルータングウイルス（BTV）と他の血清群に属するウイルス株との関係を BTV L3 遺伝子の cDNA を用いた northern blot hybridization 法で調べた．その結果，パリアム群に属する1株（CSIRO　82株）及び EHD 群に属する2株（CSIRO　753及び DPP　59株）の計3株が BTV L3 遺伝子と反応した．また，3株中2株が補体結合反応で BTV と反応した．これらの結果から，L3 遺伝子の相同性が類属反応の一因となり得ることが示唆された．