academicJournals

Vol. 7(41), pp. 4892-4895, 11 October, 2013
DOI: 10.5897/AJMR2013.6193
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

A monoclonal antibody-based antigen-capture enzyme-linked immunosorbent assay (ELISA) for the detection of *bluetongue virus*

Hui-qiong Yin[#], Shu Yang[#], Wen-chao Li, Jun Liu, Rui Wang and Jin-gang Zhang*

Viral Detection Laboratory of National Center of Biomedical Analysis, Institute of Transfusion Medicine, the Academy of Military Medical Sciences, Beijing 100850, China.

Accepted 24 September, 2013

A monoclonal antibody-based antigen-capture enzyme-linked immunosorbent assay (ELISA) was developed for the detection of *bluetongue virus (BTV)* in cell culture lysates and blood samples from sheep. The monoclonal antibody 3E2 and 1C11 specific to BTV VP7 were used as capture antibody and detection antibody, respectively. The assay has detected BTV 1-22 specifically, and had no cross-reactivity with the closely related epizootic hemorrhagic disease virus (EHDV) serotypes 5. The limit of sensitivity of the assay was 9 ng/ml for purified recombinant BTV VP7 and $10^{0.5}$ $TCID_{50}$/ml for BTV-5. The coefficient of variation (CV) of intra-assay and inter-assay range from 3.45 to 6.10%. The developed antigen-capture ELISA showed good coincident rate (100%) with INGEZIM BTV DAS in 5 serotypes BTV and 8 blood samples from sheep. Therefore, the antigen-capture ELISA may be useful for testing large number of samples in a convenient and short time.

Key words: *Bluetongue virus*, antigen-capture enzyme-linked immunosorbent assay (ELISA), serotypes, monoclonal antibody.

INTRODUCTION

Bluetongue (BT) is an insect-borne viral disease of ruminants. Among domestic animals, clinical disease occurs most often in sheep, and can result in significant morbidity. The economic consequences of the outbreak were dramatic. For instance, in the Netherlands, the estimated total net costs of the 2006 and 2007 outbreak were 200 million Euros (Velthuis et al., 2010). It has been included in the World Organization for Animal Health (OIE) list of notifiable diseases (formerly List A) (OIE, 2011). The distribution of BT is determined by the geographic distribution of the arthropod vector and extends globally between latitudes 35°S and 53°N (Martin et al., 2008; Orru et al., 2004). Outbreaks have occurred in many countries in northern and western Europe since 2006 (Saegerman et al., 2008; Carpentera et al., 2009; Kampen and Ortega et al, 2010).

Bluetongue virus (BTV) is the prototype member of the genus *Orbivirus* within the family *Reoviridae*. Thus far 26 serotypes are recognized. BTV is non-enveloped with a double shelled structure and a double-stranded (ds) 10 segment RNA genome. The virus contains 7 structural proteins and its genome encode also for 5 non structural proteins: NS1, NS2, NS3, NS3a and NS4. The outer capsid proteins, VP2 and VP5, are the serotype determinants and are responsible for generation of serotype-specific neutralizing antibody. The antibodies against VP7, the major core protein, will specifically detect the whole BTV serogroup. And the genomic segment 5, encoding NS1, is the most highly conserved of the 10 segments (Roy, 1989).

*Corresponding author. E-mail: zhangjg@nic.bmi.ac.cn.

[#]Authors contributed equally and are co-first authors for this work.

Traditionally, laboratory confirmation of BTV is done by intravenous egg inoculation followed by passages in mammalian cells (World Organization for Animal Health, 2008). Virus isolation is tedious and may take up to 5 weeks for completion. Consequently, alternative methods for virus detection have been sought, which include enzyme-linked immunosorbent assay (ELISA), immuno-electron microscopy, reverse transcription polymerase chain reaction (RT-PCR) (Yin et al., 2008), real-time RT-PCR (Yin et al., 2010), bio-barcode assay (Yin et al., 2011) and so on. ELISA techniques have a number of advantages including being economical, specific and rapid. Additionally, large number of clinical or laboratory samples could be screened by the assay in a very short time during sero-epidemiological studies. In this study, a monoclonal antibody-based antigen-capture ELISA was developed for detecting of VP7 protein.

MATERIALS AND METHODS

Monolonal antibody to VP7 of *bluetongue virus*

The gene of serogroup-specific antigen VP7 was amplified from BTV-5 (China Animal Health and Epidemiology Center, China) and cloned into pET-DsbA (Promega, Madison, U.S.A). Then, the recombinant VP7 was expressed in *Esherichia coli* BL21 (DE3) pLysS and purified using a His-tag affinity chromatography column on Ni^{2+}-nitrilotriacetate (NTA) resin (Qiagen, Hilden, Germany) (Li et al., 2007).

BTV-5 propagated in confluent monolayers of baby hamster kidney (BHK)-21 cells was purified through gradient centrifugation. Balb/c mice were immunized with the purified BTV-5 antigens. Splenocytes from the immunized mice were fused with SP2/0 myeloma cells, and positive hybridoma clones were screened through the expressed recombinant VP7. Monoclonal antibodies (MAb) 3E2 and 1C11 specific to BTV VP7 were prepared and determined to IgG2b (κ) (Yang et al., 2008).

The antigen-capture ELISA

In the antigen-capture ELISA, flat bottom, 96-well plates were incubated with 3E2 MAb and incubated overnight at 4°C. After three washes with PBST, 1% bovine serum albumin (BSA) blocking solution was added to the wells and incubated at 37°C for 60 min and washed three times with PBST. VP7 or BTV samples were added to the wells and incubated at 37°C for 60 min. After three washings, horseradish peroxidase-conjugated 1C11 MAb was added to the wells and incubated at 37°C for 30 min. The solutions were developed by adding 3,3',5,5'-tetramethylbenzidine (TMB) at room temperature. Reactions were stopped after 15 min by the addition of 2 M H_2SO_4. The absorbance in each well was read at 450 nm wavelength on an ELISA reader. A value twice (or more) the mean OD value of the negative antigen control was considered as the positive/negative cut-off value (positive to negative (P/N) ratio≥2).

The working dilutions of the capture and detection antibodies were selected by chequer board titration, and the best and most satisfactory result was obtained at a 1:2000 dilution for the capture antibody and a 1:1000 dilution for the detection antibody, while the dilution of the antigen (recombinant VP7) and negative control was a 1:10 dilution. At this dilution, the protein content of the controls was between 10 and 15 µg per well, and the P/N ratio was 4.0. These dilutions of reagents were followed throughout the study.

Evaluation of the antigen-capture ELISA

The specificity of the antigen-capture ELISA was confirmed by performing it to detect BTV serotypes 1-22 and the closely related orbivirus epizootic hemorrhagic disease virus (EHDV), using reference strains of serotype 5 (China Animal Health and Epidemiology Center, China).

The sensitivity of the assay was evaluated by conducting the antigen-capture ELISA to detect the recombinant VP7 protein at different concentrations and viral 10-fold serial dilutions of a BTV-5 strain cultured in BHK-21 cell, respectively.

The antigen-capture ELISA was carried out to detect strong-positive sample (0.25 µg/mL VP7), weak-positive sample (0.010 µg/ml VP7) and negative sample (distilled water) to determine the reproducibility of the assay. The samples were tested on 5 separate occasions, with 3 identical samples each time. The coefficients of variation (CV) were analyzed according to sample to negative (S/N) ratio (OD_{450} value).

Five serotypes of BTV cultured in BHK-21 cell were 10-fold diluted respectively, and these BTV samples and eight sheep blood samples (a gift from China Animal Health and Epidemiology Center) were processed using the antigen-capture ELISA and INGEZIM BTV DAS (INGENASA, Madrid, Spain) to investigate the coincidence rate.

RESULTS

The specificity

Different serotypes BTV and EHDV-5 were detected using the antigen-capture ELISA, and the results showed that BTV serotypes 1-22 in the infected cell culture supernatant were all detected as positive, whereas EHDV 5 tested as negative (Table 1).

The sensitivity

VP7 of BTV at different concentrations was detected using the antigen-capture ELISA, and the results indicated that the assay could detect VP7 at concentrations as low as 9 ng/ml protein. Then, 10-fold serial diluted BTV-5 was tested using the assay. Results showed that titers as low as 100.5 TCID50/ml BTV-5 were detected positively (Table 2).

The reproducibility

The reproducibility of the antigen-capture ELISA was evaluated by detecting the VP7 protein. And the results showed that the CVs (%) of the strong-positive sample, weak-positive sample and negative sample were 3.79, 4.55 and 3.45 intra-test respectively, and 5.38, 6.10 and 4.88 inter-test, respectively (Table 3).

Coincidence analysis

Five serotypes of 10-fold diluted BTV and eight sheep blood samples were detected using the antigen- capture ELISA and INGEZIM BTV DAS. And the results indicated

Table 1. Detection of BTV and EHDV strains cultured in BHK-21 cell with the antigen-capture ELISA.

Viral sample	BTV serotype																						EHDV serotype
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	5
Results	P[a]	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	N[b]

[a] P: positive; [b] N: negative.

Table 2. Detection of BTV VP7 and BTV at different concentrations with the antigen-capture ELISA.

Sample	The recombinant VP7 of BTV (μg/ml)						BTV-5 ($TCID_{50}$/ml)				
	5	0.5	0.05	0.01	0.009	0.008	10^3	10^2	10^1	$10^{0.5}$	10^0
Results	P[a]	P	P	P	P	N[b]	P	P	P	P	N

[a] P: positive; [b] N: negative.

Table 3. Reproducibility of the antigen-capture ELISA for positive and negative samples.

Sample	CV[a] (%) of S/N[b] of OD_{450} value	
	Intra-test	Inter-test
Strong-positive sample (0.25μg/ml VP7)	3.79	5.38
Weak-positive sample (0.01μg/ml VP7)	4.55	6.10
Negative sample (distilled water)	3.45	4.88

[a]CV: Coefficient of variation; [b]S/N: sample to negative ratio.

that the coincidence rate of the antigen-capture ELISA and INGEZIM BTV DAS was 100% in detecting BTV-5, 14, 16, 21, 22 ($10^{1.57}$, $10^{1.81}$, $10^{2.67}$, $10^{2.93}$, $10^{3.50}$ $TCID_{50}$/ml) and these blood samples (Table 4).

DISCUSSION

Monitoring and controlling of BTV infection in cattle, sheep and goats remains a top priority in BTV-endemic and epidemic countries interested in exporting livestock free of this disease or in restricting the introduction of new serotypes into existing endemic populations. The recent outbreaks of BT in Europe, 2006 to 2010, emphasize the need for surveillance and monitoring of BT, and the clinical and laboratory diagnosis of BT.

Serological assays provide evidence of earlier animal exposure to BTV. VP7 has a highly conserved sequence, displays antigenicity across all serotypes, and is the major group-specific antigen (Mertens et al., 2005). Not surprisingly, VP7 is frequently used in immunoassays designed to detect BTV (Nagesha et al., 2001; Reddington et al., 1991). Many countries use ELISAs that use Abs raised against BTV to detect the virus, even though ELISAs that utilize MAbs that specifically recognize VP7 have greater specificity (Afshar et al., 1992; Reddington et al., 1991).

A recent study demonstrated that the sensitivity of the polyclonal antibody-based sandwich ELISA was estimated to be between $10^{2.4}$ and $10^{2.6}$ $TCID_{50}$/ml with different serotypes of BTV (Chank et al., 2009). In the present study, the analytical detection limit of the antigen-capture ELISA for VP7 protein and BTV-5 was 9 ng/ml VP7 and $10^{0.5}$ $TCID_{50}$/ml BTV-5, respectively. The sensitivity of the assay was compared with the real-time RT-PCR

Table 4. Detection of BTV strains cultured in BHK-21 cell and sheep blood samples with the antigen-capture ELISA and INGEZIM BTV DAS[a].

Sample	BTV serotype					Sheep blood sample							
	5	14	16	21	22	1	2	3	4	5	6	7	8
The antigen-capture ELISA	P[b]	P	P	P	P	P	N[c]	N	N	N	N	P	N
INGEZIM BTV DAS	P	P	P	P	P	P	N	N	N	N	N	P	N

[a] INGEZIM BTV DAS: double antibody sandwich ELISA for detection of BTV VP7 protein purchased from INGENASA (Spain); [b] P: positive; [c] N: negative.

(Yin et al., 2010). As expected, real-time RT-PCR (10^{-1} $TCID_{50}$/ml BTV-1) was found to be at least 10 times more sensitive than the antigen-capture ELISA for detection of BTV in cell culture.

The analytical specificity of the assay has been studied with EHDV, since the virus is closely related to BTV. The results indicate that the 22 serotypes of BTV reference strains in our laboratory tested were detected specifically, with no cross-reactivity against the closely related orbivirus EHDV.

The antigen-capture ELISA developed in this study is based on MAbs specific to BTV VP7, which further improves the specificity of the assay of antibody-based ELISA. The assay is a simple and rapid test for high-throughput detecting BTV in cell culture and blood samples, and will be helpful at least for national purpose at least in China.

ACKNOWLEDGEMENTS

We are grateful to China Animal Health and Epidemiology Center for providing the viruses and the whole blood samples of sheep. This work was partially supported by the National Hi-Tech Research and Development Program (2006AA10Z446) and the National Supporting Program of Science and Technology (2008BAI54B06-21) of China.

REFERENCES

Carpentera S, Wilsona A, Mellora PS (2009). Culicoides and the emergence of *bluetongue virus* in northern Europe. Trends. Microbiol. 17(4):172-178.

Kampen H, Werner D (2010). Three years of bluetongue disease in central Europe with special reference to Germany: what lessons can be learned? Wien. Klin. Wochenschr. 122(3):31-39.

Li WC, Lv MM, Yang S, Yin HQ, Shi LJ, Ma YY, Zhang JG (2007). Molecular cloning and sequence analysis of the S7-ORF of *bluetongue virus 5*. Letters in Biotechnology. 18(3):384-387.

Mertens PPC, Maan S, Samuel A (2005). Oribivirus, Reoviridae. In: Eighth Report of the International Committee on the Taxonomy of Virus. Virus Taxonomy, Classification and Nomenclature of Viruses Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), London. pp.466-483.

Nagesha HS, Wang LF, Shiell B, Beddome G, White JR, Irving RA (2001). A single chain Fv antibody displayed on phage surface recognises conformational group-specific epitope of bluetongue virus. J. Virol. Methods. 91(2):203-207.

Orrù G, Santis PD, Solinas F, Savini G, Piras V, Caporale V (2004). Differentiation of Italian field and South African vaccine strains of *bluetongue virus* serotype 2 using real-time PCR. J. Virol. Methods. 122(1):37-43.

Ortega J, Crossley B, Dechant JE, Drew CP, Maclachlan NJ (2010). Fatal *bluetongue virus* infection in an alpaca (Vicugna pacos) in California. J. Vet. Diagn. Invest. 22(1):134-136.

Reddington JJ, Reddington GM, MacLachlan NJ (1991). A competitive ELISA for detection of antibodies to the group antigen of *bluetongue virus*. J. Vet. Diagn. Invest. 3(4):144-147.

Roy P (1989). *Bluetongue virus* genetics and genome structure. Virus. Res. 13(3):179-206.

Saegerman C, Berkvens D, Mellor PS (2008). Bluetongue epidemiology in the European union. Emerg. Infect. Dis. 14(4):539-544.

Tabachnick WJ, Robertson MA, Murphy KE (1996). Culicoides variipennis and bluetongue disease. Research on arthropod-borne animal diseases for control and prevention in the year 2000. Ann. N. Y. Acad. Sci. 791:219-226.

Velthuis AGJ, Saatkamp HW, Mourits MCM, de Koeijer AA, Elbers ARW (2010). Financial consequences of the Dutch bluetongue serotype 8 epidemics of 2006 and 2007. Prev. Vet. Med. 93(4):294-304.

World Organization for Animal Health (2008). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. pp.158-174.

World Organization for Animal Health (2011). OIE Listed diseases 2011. OIE, Paris, France.

Yang S, Li WC, Lv MM, Yin HQ, Ma YY, Liu M, Zhang JG (2008). Preparation and characterization of group specific monoclonal antibody against *bluetongue virus*. Lett. Biotechnol. 19(6):866-869.

Yin HQ, Zhang H, Shi LJ, Yang S, Zhang GP, Wang SQ, Zhang JG (2010). Detection and quantitation of *bluetongue virus* serotypes by a TaqMan probe-based real-time RT-PCR and differentiation from epizootic hemorrhagic disease virus. J. Virol. Methods. 168(1-2):237-241.

Yin HQ, Jia MX, Shi LJ, Yang S, Zhang LY, Zhang QM, Wang SQ, Li G, Zhang JG (2011). Nanoparticle-based bio-barcode assay for the detection of *bluetongue virus*. J. Virol. Methods. 178(1-2):225-228.

Yin HQ, Zhang GP, Zhang H, Zhang JG (2008). A pair of novel primers for universal detection of the NS1 gene from various *bluetongue virus* serotypes. Virol. Sin. 23(1):68-72.