Acta virologica 53: 287 – 289, 2009 doi:10:4149/av_2009_04_287

Letter to the Editor

Comparative study of various cell lines susceptibility to cytopathic and non-cytopathic strains of Bovine viral diarrhea virus 1 and 2

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Received March 12, 2009; accepted September 9, 2009

Keywords: Bovine viral diarrhea virus 1 and 2; cell line susceptibility; virus recovery

Bovine viral diarrhea virus 1 and 2 (BVDV 1 and 2) (the genus Pestivirus, the family Flaviviridae) is distributed worldwide and causes severe economical losses due to the decreased fertility, abortions, diarrhea, respiratory symptoms, and persistent infection in intrauterinary infected calves. So far, two species BVDV-1 and BVDV-2 are known to infect cattle (*1*). Most of the BVDV isolates appear to be BVDV-1 genotype that comprises 12 different genetic groups (*2*). Strains of BVDV- 2 genotype were initially recognized by their higher virulence, but the isolates of lower virulence have also been assigned to this genotype (*3*). According to the cultivation characteristics and pathogenic properties of BVDV-1, 2 we differentiate between cytopathic (cp) and non-cytopathic (non-cp) BVDV strains (*4*). Non-cp BVDV strains are capable of producing persistent infection and consequently, this is the reason why most of the field isolates are of the non-cp type. Persistently infected animals are life-long virus shedders and are potential candidates for the development of mucosal disease especially after superinfection with antigenically homologous cp BVDV strains or by mutants of cp BVDV derived from non-cp BVDV in persistently infected animals. Although many rapid and less laborious diagnostic techniques are used to detect BVDV, up till now the virus isolation is considered as a gold standard. Besides other factors, the success rate of virus isolation depends on the susceptibility of cell line used to detect and isolate the virus.

Thus, the aim of this study was to compare the susceptibility of different cell lines derived from various tissues to non-cp and cp strains of BVDV. In our experiments we examined four cell lines derived from various bovine tissues and one cell line derived from sheep fetal thymus cells for the inoculation with BVDV strains. Bovine embryonic lungs (BEL), bovine turbinated (BT), Madin-Darby bovine kidney (MDBK), calf oesopharyngeal (KOP-R), and sheep fetal thymus (SFT-R) cell lines were obtained from the Veterinary Faculty, Munich, Germany. All cell cultures were BVDV-free. Cell cultures were grown in Eagle's MEM (Gibco) supplemented with 7% fetal calf serum (Biochrom), penicillin 100 IU/ml, streptomycin 100 µg/ml (Gibco), and 1% non-essential amino acids (Biochrom). The cells were maintained at 37°C in a humidified 5% CO_2 atmosphere.

Cell lines were inoculated with two cp BVDV strains (NADL and Oregon C24V) and two non-cp BVDV strains (PT810 and CS8644) obtained from the Veterinary Faculty, Munich, Germany. Three viral strains tested (NADL, Oregon C24V, PT810) were BVDV-1, whereas fourth strain (CS844) was BVDV-2. Each cell culture was inoculated with 50 µl of viral suspension ($10^{4.0}$ $TCID_{50}$/ml per 25 cm^2 of confluent

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Abbreviations: BEL = bovine embryonic lungs; BVDV-1 and 2 = Bovine viral diarrhea virus 1 and 2; BT = bovine turbinated; cp = cytopathic; KOP-R = calf oesopharyngeal; MDBK = Madin-Darby bovine kidney; ML = mink lungs; non-cp = non-cytopathic; SFT-R = sheep foetal thymus

Table. BVDV strains recovery using BEL, BT, MDBK, KOP-R, and SFT-R cell lines

Cell line/BVDV strain	Titer ± SD (log 10)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. BEL/NADL	5.01 ± 0.04																				
2 BEL/Oregon	5.75 ± 0.22	.**																			
3 BEL/PT810	6.00 ± 0.45	.***	ns																		
4 BEL/CS8644	5.33 ± 0.26	.*	ns	*.																	
5 BT/NADL	5.33 ± 0.26	ns	ns	.*	ns																
6 BT/Oregon	5.67 ± 0.26	*.	ns	ns	ns	ns															
7 BT/PT810	5.58 ± 0.13	ns	ns	ns	ns	ns	ns														
8 BT/CS8644	5.16 ± 0.26	ns	ns	.***.	ns	ns	ns	ns													
9 MDBK/NADL	2.92 ± 0.13	.***	***.	.***	***.	.***	***.	.***	.***												
10 MDBK/Oregon	3.58 ± 0.13	.***	***.	.***	***.	.***	***.	.***	.***	*.											
11 MDBK/PT810	4.50 ± 0.45	ns	.***.	.***	***.	.***	.***	***.	.*	***.	.***										
12 MDBK/CS8644	3.67 ± 0.26	.***	***.	.***	***.	.***	.***	.***	.***	**.	ns	.***									
13 KOP-R/NADL	6.67 ± 0.26	.***	***.	.***	***.	.***	***.	.***	***.	.***	***.	.***	***.								
14 KOP-R/Oregon	6.17 ± 0.26	.***	ns	ns	.***.	.***.	ns	ns	***.	***.	***.	***.	***.	ns							
15 KOP-R/PT810	5.25 ± 0.22	ns	ns	.**	ns	ns	ns	ns	ns	.***	.***.	**.	***.	***.	***.						
16 KOP-R/CS8644	5.00 ± 0.45	ns	.**	***.	ns	ns	.*	ns	ns	***.	***.	ns	***.	***.	***.	ns					
17 SFT-R/NADL	4.83 ± 0.26	ns	.***	***.	ns	ns	.***.	**.	ns	***.	***.	ns	.***.	***.	***.	ns	ns				
18 SFT-R/Oregon	5.25 ± 0.39	ns	ns	.**	ns	ns	ns	ns	ns	.***	***.	.**	.***	.***	.***	ns	ns	ns			
19 SFT-R/PT810	5.50 ± 0.04	ns	ns	ns	ns	ns	ns	ns	ns	.***	***.	***.	***.	***.	*.	ns	ns	.*	**.		
20 SFT-R/CS8644	6.08 ± 0.46	.***	ns	ns	.**	**.	ns	ns	.***	.***.	***.	***.	.***	ns	ns	.***	***.	.***	***.	ns	

(*) P <0.05; (**) P <0.01; (***) P <0.001; ns = non-significant.

monolayer) and the cells were incubated for 4 days at 37°C. After the incubation we carried out a virus titration. The cells were first examined for the characteristic cytopathic effect (cp strains) and viral antigen of cp BVDV strains and non-cp BVDV strains was detected by immunofluorescence assay. Primary mouse monoclonal antibody WB 103/105 (1:500; Weybridge) and secondary antibody against mouse immunoglobulins (ALEXA Fluor®, 1:1000; Invitrogen) were used in the assay. Titres of individual BVDV strains growing in different cell lines were calculated by using Reed-Muench method and statistical differences were evaluated using ANOVA GraphPad Prism test (*5*).

The cell line BEL enabled significantly higher (P <0.001) recovery of non-cp strain PT810 ($10^{6.0}$ $TCID_{50}$/ml) than other strains, while the recovery of cp strain NADL was the least effective (Table). Significant differences were also observed between titers of non-cp strains PT810, CS8644 (P <0.05) and cp strains NADL, Oregon (P <0.01) and NADL vs. CS8644 (P <0.05) (Table). Similar results were also observed in MDBK cell line, however, the virus titers were generally lower and differences in virus titers between the strains were noticeable (Table). No significant difference in the recovery among BVDV strains was observed when the BT cell line was used. Nevertheless, the significant difference in the viral recovery was observed, when BT and MDBK cell lines were compared. NADL strain showed 100-fold higher virus titer ($10^{5.3}$ $TCID_{50}$/ml) in BT than in MDBK cell line (Table).

The KOP-R cell line appeared as the most suitable for cultivation of the NADL strain resulting in $10^{6.6}$ $TCID_{50}$/ml. In this cell line we observed relatively large differences between titers of cp and non-cp viral strains NADL, PT810, CS8644 (P <0.001) and Oregon, PT810, CS8644 (P <0.001, Table).

Surprisingly, the highest mean harvest of the both non-cp BVDV strains (PT810, CS8644) was observed on the cell line derived from non-bovine tissue (SFT-R from sheep fetal thymus). Titer of CS8644 strain ($10^{6.1}$$TCID_{50}$/ml) was

significantly higher than of the strains NADL and Oregon ($P < 0.001$). Significant differences were also observed between titers of the strains PT810 and NADL ($P < 0.05$, Table).

Comparison of the examined types of cell cultures in relation to cultivation rates of cp strains of BVDV (NADL and Oregon) allowed us to confirm that KOP-R was the most susceptible cell line that yielded in $TCID_{50}$ /ml of both cp viral strains higher than $10^{6.0}$, while MDBK cell line was at least susceptible to the above strains (Table). On the other hand, examination of the non-cp BVDV strains showed that BEL (strain PT810 with $10^{6.0}TCID_{50}$/ml) and SFT-R cell lines were the most susceptible (strain CS8644 with $10^{6.1}$ $TCID_{50}$/ml), while MDBK ranked as less sensitive with the lowest titers (Table). The difference in recovery of the cp (mean titer $10^{6.4}$ $TCID_{50}$/ml) and non-cp (mean titer $10^{5.1}$ $TCID_{50}$/ml) viral strains was highest ($P < 0.01$), when the KOP-R cell line was used.

Onyekaba et al. compared susceptibility of ST (swine testicle), ML (mink lungs), BT (bovine turbinate), PK15 (porcine kidney), and ED (equine dermal) cell lines to BVDV and found that BT and ML cell lines were the most susceptible to BVDV as well as suitable for its isolation (*6*). The authors observed that BVDV titer reached up to $10^{4.1}$ $TCID_{50}$ /ml in BT and $10^{3.2}$ $TCID_{50}$ /ml in ML cell lines. Consistently, we found higher titers in BT cell line for both cp and non-cp viral strains. Another study has reported evident susceptibility of other cell lines like low-passaged bovine primary kidney, turbinate and testicular cells to BVDV (*7*). Ferrari investigated susceptibility of different cell lines (primary bovine embryonal kidney, tracheal, calf testicle, and buffalo lungs) to the cp BVDV virus and observed no significant difference in the viral recovery (*8*).

Titer comparison of the individual strains used in our study allowed us to conclude that the KOP-R cell line was the most susceptible for the isolation of cp BVDV strains and SFT-R and BEL cell lines for non-cp BVDV strains. However, any of the cell cultures tested was universal for all BVDV strains. Paradoxically, the lowest harvest of all BVDV strains tested was found in the cell line MDBK, which is one of the most frequently used cell line for the isolation of BVDV (*9, 10, 11*).

Acknowledgements. This study was supported by the contract APVV-20-019605 from the Slovak Research and Development Agency. Our special thank belongs to DVM G. Wolf from the Veterinary Faculty, Munich, Germany, who kindly provided us with the cell cultures and BVDV strains. His professional advice is highly appreciated.

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