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Short Communication

Immunologic cross-reactivity between Muscovy duck parvovirus and goose parvovirus on the basis of epitope prediction

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Abstract

Through bioinformatic prediction, between Muscovy duck parvovirus (MDPV) and goose parvovirus (GPV), there were one epitope AA503-509 (RANEPKE) on non-structural protein and three epitopes AA426-430 (SQDLD), 540-544 (DPYRS), 685-691 (KENSKRW) on structural protein might cross-react with each other. Furthermore, the four epitops were expressed in *Escherichia coli*. All the four recombinant proteins could react with GPV-antisera and MDPV-antisera in Western blot.

Key words: MDPV, GPV, cross-reactivity, prediction, epitope.

Muscovy duck parvovirus (MDPV) and goose parvovirus (GPV) usually cause high morbidity and mortality in young Muscovy ducklings. The infections of MDPV and GPV are an imminent threat to the commercial Muscovy duck industry (zhang *et al.*, 2010)

The genome of MDPV or GPV contains two major open reading frames. The first, encoding the non-structural (NS) protein, is involved in viral replication and regulatory functions. NS protein includes two proteins NS1 and NS2 which are derived from the same gene. The entire amino acid sequence of NS2 is contained within the carboxyl terminal portion of NS1. The second includes three capsid proteins VP1, VP2 and VP3. The proteins VP1, VP2 and VP3 are derived from the same gene, and the entire amino acid sequences of VP2 and VP3 are contained within the carboxyl terminal portion of VP1 (Zadori *et al.*, 1995; Tsai *et al.*, 2004; Yu *et al.*, 2012). Because of GPV and MDPV share highly amino acid sequence identity in NS proteins and VP proteins (Tatár-Kis *et al.*, 2004; Tsai *et al.*, 2004), there are immunizing cross-reactivity between MDPV and GPV (Poonia *et al.*, 2006; Palya *et al.*, 2009).

In order to explain immunizing cross-reactivity between MDPV and GPV, nucleotide sequence of MDPV FM strain NS1 and VP1 gene (GenBank number: NC006147) were translated into amino acid sequence by EditSeq, one module of DNAStar.

According to Jameson-Wolf method (Jameson and Wolf, 1988) and secondary structural indexes, the potential epitopes located at AA248-252, 503-509, 545-554 on MDPV NS1 and AA27-33, 39-50, 67-75, 111-115, 149-155, 260-264, 426-430, 448-452, 485-489, 521-525, 540-544, 685-691 on MDPV VP1(Table 1). Some regions showed antigenicity by Jameson-Wolf method only, but other epitope-related secondary structural indexes were lower. Such regions were questionable.

The NS1 linear B-cell epitopes of GPV were located on the C-terminal, AA485-627 (Yu *et al.*, 2011). Sequence alignment by DNAMAN showed the duplicate epitope was AA503-509. The VP1 linear B-cell epitopes of GPV were located on the AA35-71, 123-198, 423-444, 474-491, 531-566, 616-669 and 678-732 (Yu *et al.*, 2012). There were three epitopes might cross-react with each other, AA426-430, 540-544, 685-691. The possibility of cross-reaction in these epitopes was up to 100%.

Furthermore, we constructed four peptides, NS (503-509) (the numbers in bracket mean the location of amino acids), VP (426-430), VP (540-544) and VP (685-691) (The cross-reaction corresponds to that region of MDPV.). Annealing products were cloned into the expression vector pGEX-6P-1 (Amersham Pharmacia Biotech, Sweden) and sequenced by Sangon (China) to confirm the integrity of the inserted sequence. Each fragment was expressed as

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Table 1 - Prediction of secondary structural and antigenic index of MDPV by DNAStar.

Protein	Predictable parameter (value)	Domain (AA)
NS	Hydrophilicity plot (> 1)	76-81, 145-150, 243-253, 324-328, 502-509, 544-555
	Surface probability plot (> 1)	247-251, 503-509, 545-554
	Antigenic index (> 2)	10-15, 21-30, 79-84, 119-124, 128-32, 142-152, 163-169, 221-225, 248-252, 258-65, 287-292, 324-330, 339-343, 381-387, 403-410, 489-512, 516-522, 540-57
VP	Hydrophilicity plot (> 1)	27-50, 66-74, 84-94, 149-157, 223-229, 261-269, 288-297, 319-327, 341-345, 376-388, 427-431, 447-455, 486-491, 521-525, 540-544, 580-585, 620-626, 657-666, 682-694, 700-704
	Surface probability plot (> 1)	27-34, 40-49, 148-152, 166-176, 262-266, 319-324, 521-525, 683-691
	Antigenic index (> 2)	27-33, 39-50, 67-75, 111-115, 149-155, 260-264, 426-430, 448-452, 485-489, 521-525, 540-544, 685-691

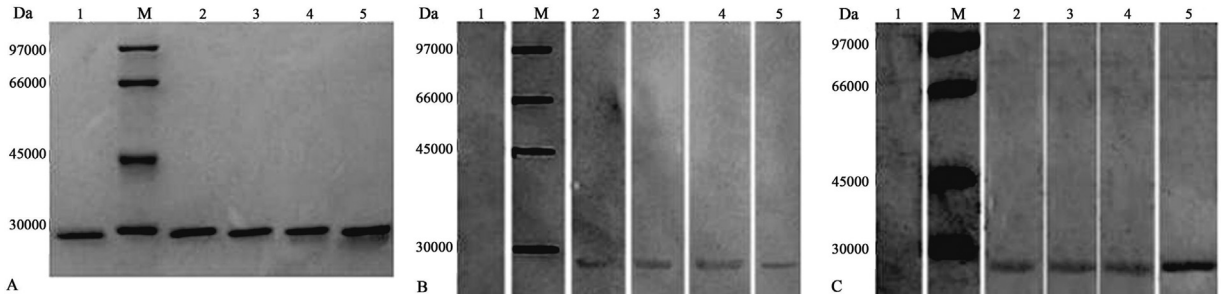

Figure 1 - Identification of predicted epitopes inducing cross-reactivity between MDPV and GPV. A. Identification of recombinant GST fusion epitopes by Western blot. Lane 1: GST control. Lane M: protein marker. Lane 2: NS (503-509). Lane 3: VP (426-430). Lane 4: VP (540-544). Lane 5: VP (685-691). B. The reactivity of recombinant GST fusion epitopes to GPV-antisera by Western blot. Lane 1: GST control. Lane M: protein marker. Lane 2: NS (503-509). Lane 3: VP (426-430). Lane 4: VP (540-544). Lane 5: VP (685-691). C. The reactivity of recombinant GST fusion epitopes to MDPV-antisera by Western blot. Lane 1: GST control. Lane M: protein marker. Lane 2: NS (503-509). Lane 3: VP (426-430). Lane 4: VP (540-544). Lane 5: VP (685-691).

glutathione S-transferase (GST) fusion proteins in *Escherichia coli* Rosetta(DE3)pLysS (Novagen, USA) and the recombinant proteins were purified by eluting from SDS-PAGE as described previously (Shien *et al.*, 2000; Chang *et al.*, 2002).

Western blots were also performed as described (Chang *et al.*, 2002). Anti-GST monoclonal antibody (MoAb) (EarthOx, USA) was used to identify the purified protein (Figure 1. A) and then GPV-infected goose sera or MDPV-infected goose sera were diluted 500-fold and used as the primary antibody while horseradish peroxidase (HRP)-conjugated rabbit anti-goose antibodies or (HRP)-conjugated rabbit anti-duck antibodies (prepared in our lab) were diluted 1000-fold prior to use as secondary antibodies. The fluorescent dye-indicator for peroxidase activity 4-CN (4-chloro-1-napthol; Amresco, USA), was used for color development. All the four recombinant proteins were positive to GPV-antisera (Figure 1. B) or MDPV-antisera (Figure 1. C).

In this article, the predicted epitopes are linear. Almost all the theories of predictable methods were based on protein primary structure, the intermolecular force was neglected. Therefore, the prediction of conformational epitopes was not suitable by these methods.

Viruses are major factors of human and animal infectious diseases. In virology research, virus-related databases and bioinformatic analysis tools are essential for discerning relationships within complex datasets about viruses and host-virus interactions (Persson 2000). Understanding of the immunizing cross-reactivity between MDPV and GPV could provide the theoretical support for development of specific diagnosis reagent for detecting MDPV or GPV infection of Muscovy duck.

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