Predicting short peptide immunogenic B cell epitopes distinct in RHDV1 and RHDV2 of *Oryctolagus cuniculus*

[1,2]John S. B. Nas

[1] Department of Biology, College of Arts and Sciences, University of the Philippines Manila, Manila, Philippines; [2] Department of Medical Technology, Institute of Arts and Sciences, Far Eastern University, Manila, Philippines. Corresponding author: J. S. B. Nas, jbnas@up.edu.ph

Abstract. Rabbit haemorrhagic disease virus (RHDV) can be lethal to *Oryctolagus cuniculus*. There are 2 types of RHDV, namely RHDV1 and RHDV2, which share a similar protein sequence. The protein sequence of these viruses is similar to other caliciviruses, such as the European brown hare syndrome virus (EBHSV) and Australian rabbit calicivirus (RCV-A1). High homology in their protein sequence may lead to the antibody's cross-reactivity to different antigens from these viruses. To design a short peptide synthetic epitope for diagnostic purposes, this study predicts 12 amino acid long immunogenic epitopes unique to RHDV1 and RHDV2. The protein sequences of RHDV1 and RHDV2 were mapped for possible epitopes. The identified epitopes, which were at least 12 amino acid long, were evaluated for antigenicity, hydrophilicity, and surface accessibility. The peptide sequence of the qualified epitopes was aligned with the protein sequence of RHDV1, RHDV2, EBHSV, and RCV-A1. The results show that out of 64 potential epitopes in RHDV1 and 67 varying length epitopes of RHDV2, only 31 and 32 12 amino acid long epitopes in RHDV1 and RHDV2, respectively, have desirable antigenic properties. Only three 12 continuous epitopes from each type of RHDV can distinguish them from each other. Among these 3 candidate short peptide epitopes, only SYPGNNATNVLQ in RHDV1 and VPSNPQPTTTTS in RHDV2 may differ from EBHSV and RCV-A1. These findings suggest that the unique immunogenic epitopes of RHDV1 and RHDV2 may potentially impact the development of diagnostic assays and vaccines specific to the different types of RHDV.

Key Words: haemorrhagic disease, immune system, *in silico*, rabbit, virus.

Introduction. Rabbit hemorrhagic disease mainly affects the liver, leading to a high mortality rate (Abrantes et al 2012). However, some studies recorded hemorrhage in other organs like lungs, resulting in dyspnea and tracheitis (Ueda et al 1992). Additionally, the rabbit's immune response is hindered due to the exhaustion of the lymphocytes in the necrotic hepatic tissues (Marques et al 2010). There was evidence that the sublethal effect of the virus develops the antibody production of *Oryctolagus cuniculus* (Patton 1989), wherein high levels of IgM, IgA, and IgG were detected in individuals who gain resistance (Lavazza & Capucci 2008).

The humoral immune response appears to be an integral factor in the development of diagnostic tools and vaccines. In serological testing, the antigen or antibody serves as the biomarker for infection. There is the possibility of cross-reactivity because of the highly similar epitopes of the RHDV with the other caliciviruses.

RHDV is among the caliciviruses that negatively affect various organisms, but primarily *O. cuniculus* (Abrantes et al 2012). There are 2 RHDV types identified in *O. cuniculus*, RHDV1 and RHDV2 (Capucci et al 2017). These 2 types have high protein sequence homology, as presented in Table 1 (Mahar et al 2016). Notably, the high similarity in their peptide sequence is also observed with other caliciviruses such as EBHSV and RCV-A1 (Table 1). High sequence similarity is problematic in developing infection-associated markers.

Hence, this study aims to predict a short sequence epitope that is unique to RHDV1 and RHDV2, which also has desirable antigenic, surface accessible, and hydrophilic properties.

Table 1

Protein sequence homology of RHDV1 and RHDV2 with RHDV, EBHSV, and RCV-A1 (source: Mahar et al 2016)

Viruses	*%Homology*	
	RHDV1	*RHDV2*
RHDV1	100.0%	93.13%
RHDV2	93.13%	100.0%
EBHSV	77.55%	78.29%
RCV-A1	87.74%	88.42%

Material and Method

Epitope prediction and selection. The amino acid sequence of RHDV1 (GenBank Accession No.: ATN96667.1) and RHDV2 (GenBank Accession No.: QJR96952.1) were retrieved from the protein database of the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/protein). The B cell epitopes were predicted using Bepipred Linear Epitope Prediction 2.0, which is available in the Immune Epitope Database.

Assessment of antigenicity, surface accessibility, and hydrophilicity. A 12 amino acid continuous epitope was mapped based on the various length of epitopes recently identified in RHDV1 and RHDV2. The epitopes generated were assessed for antigenicity using the method of Kolaskar & Tongaonkar (1990). These epitopes were further characterized based on the method of predicting hydrophilicity of Parker (1986) and the predictive method for surface accessibility (Emini et al., 1985). These predictive tools were also available in the Immune Epitope Database.

Sequence similarity of RHDV1 and RHDV2 epitopes with RHDV1 and RHDV2 protein sequence. The 12 amino acid continuous epitopes of RHDV1 and RHDV2, which qualified for antigenicity, surface accessibility, and hydrophilicity were aligned using pairwise alignment in BlastP with the protein sequence of either of the 2 RHDV types to determine the unique epitopes for each RHDV type. The top 3 epitopes with the lowest identity percentage were qualified for the next evaluation.

Sequence similarity of RHDV1 and RHDV2 with the protein sequence of other caliciviruses. The qualified RHDV1 and RHDV2 epitopes were aligned with protein sequences from other caliciviruses, such as EBHSV (GenBank Accession No.: ATU74513.1) and RCV-A1 (GenBank Accession No.: AOC59456.1) to validate whether the unique epitopes of RHDV1 and RHDV2 were exclusive. The lowest identity percentage was chosen as the unique immunogenic epitope of RHDV1 and RHDV2.

Results and Discussion

Predicted immunogenic epitopes in RHDV1 and RHDV2. The B cell epitope prediction shows that there were 64 possible epitopes with varying lengths present in RHDV1 and 67 epitopes in RHDV2, as presented in Tables 2 and 3. However, to design an antigenic epitope for detection, a short sequence, 12 amino acids long, is desired. Hence, from the respective number of epitopes from RHDV1 and RHDV2, only 27 and 28 epitopes, respectively, were evaluated for antigenicity, surface accessibility, and hydrophilicity.

The B cell epitope predictor determines the epitope and non-epitope regions in a protein sequence through a random forest algorithm (Larsen et al 2006). The predicted

linear B cell epitope's antigenic propensity is expected to elicit B cell response (Blythe & Flower 2005). In this study, the predicted linear epitopes were continuous and in varying lengths, ranging from 1 to 89. However, a previous study suggests that the average length of a conformational epitope is 15 amino acid residues (Kringelum et al 2013). Typically, synthetic peptides range from 5 to 20 amino acids (Carter & Loomis-Price 2004). Hence, the average length of synthetic peptides considered in this study was 12 amino acid long.

Table 2

Predicted immunogenic epitopes of RHDV1

Start	*End*	*Peptide sequence*	*Length*
1742	1830	ADRKRVVSVVPDDEFVNVMEGKTRAAPQGEAAGTATTASVPGTTT DGMDPGVVATTSVVTADNSSASIATAGIGGPPQQVDQQETWRTN	89
1238	1310	TITKGVYETSNFFCGEPIDYRGITAHRLVGAEPRPPVSGTRYAKVPGV PDEYKTGYRPANLGRGDPDSDKSLM	73
632	694	ARNKAVESWQATRHGSKPGKSCYNKDMSHLTFQVYPHNMPAPGFV FVGDKLVKSQVTPREYKY	63
118	174	NKVIPCAYQFNEGFPNPAFEGEADDLFVELGAPTNMGFMDKKLLKK GKKLMDKFVDV	57
719	772	FVYPDAQYDQALLMWKQYFVMYGCVARLAKNFVDDIPYNQVHISRA SDPKIEGC	54
984	1033	KAFQGVKGKTKHGRGARVNLGNDEYDEWQAARREFINAHDMTAEE YLAMK	50
2044	2092	YGWSSPRFADIDHRKGSASYPGNNATNVLQFWHANAGSAIDNPIS QVAP	49
1347	1390	LTKGERQANLNFKAAFNTLDLSTSCGPFVPGKKIDHVKDGVMDQ	44
1149	1188	LVKGEAIRSVAQIAEGTPVCDWKKSPISTYGIKKTLSDST	40
1071	1106	GVRNEVIRTRARQAPRGPKTLDDGGFYDNDYEGLPG	36
1986	2018	RAPSSKTVDSISPAGLITTPVLTGVGNDNRWNG	33
2184	2214	IISTPNANAITYTPQPDRIVTTPGTPAAAPV	31
179	201	LTSRDASLLDSIASDSTIRAKLE	23
929	948	VDRGDQGIDVFTDPNLISGF	20
8	25	ILPEKKPLSFFLDLRDKT	18
537	553	HPSTVNFGLDHFDSYTG	17
2228	2244	RTGDVNATAGSTSGTQY	17
583	597	PCPLNCDKVENKNKV	15
2097	2111	DMSFVPFNGPGIPAA	15
2144	2158	PGNLQPTTNTSGAQT	15
819	832	VTVTTVNNILPFHS	14
611	623	MILNATHPRAGAF	13
705	717	HPDVASFEGANKF	13
1629	1641	AADKTEFIDVCPL	13
2322	2334	STLVFNLGGAANG	13
441	452	KMANEAATLDQL	12
1318	1329	QVYQQEPKLDKV	12

Table 3

Predicted immunogenic epitopes of RHDV2

Start	*End*	*Peptide*	*Length*
1748	1834	ADRKRVVSVVPDDEFVNVMEGKARATPQGETAGTATTASVPGTTTD GMDPGVVATTSVVTTENASTSIATAGIGGPPQQVDQQETWR	87
1244	1317	TVTKGVYETSNFFCGEPIDYRGITAHRLVGAEPRPPVSGTRYARVPG VPDEYKTGYRPANLGRGDPDSDKSLMN	74
641	699	KAVEHWQNTRHGSKPGKSCFSKDMSHLSFQVYPHNMPAPGFVFVG EKLVKSQVAPREYK	59
1059	1114	TRRQLRPDEDQVTIVGKGGVRNEVIRTRARQTPKGPKNLDDGGFYD NDYEGLPGFM	56
1993	2038	APSSKTVDSISPADLLTTPVLTGVGTDNRWNGEIVGLQPVPGGFST	46
1353	1395	LTKGERQSNLNFKAAFNTLDLSTSCGPFVPGKKIDHVKDGVMD	43
991	1031	AFQGVKGKTKRGRGARVNLGNDEYDEWQAARREFVNAHDMT	41
2042	2075	HWNLNGSTYGWSSPRFAAIDHDRGNASFPGSSSS	34
2192	2221	STPNSSATTYTPQPNRIVNAPGTPAAAPIG	30
122	146	ALNKVIPCAYRFDKRIPNPIFEGEV	25
740	762	KQYFVMYGCVARLAKSFVEDIPY	23
2233	2255	RRTGDINAEAGSTNGTQYGAGSQ	23
1158	1179	GEAIRSVAQIAEGTPVSDWKKS	22
149	168	LFVELGAPTSMGFMDKKLLK	20
933	952	DNVDRGDQGVDVFTDPNLIS	20
2143	2162	LGFATGVPSNPQPTTTTSGA	20
171	189	KKLMDKFVDIDEPCLTSKD	19
5	22	SRLTGMTTAILPAKKPLS	18
2103	2120	DMSFVPFSGTTIPTAGWV	18
544	559	PSTINFGLDHFDSYTG	16
824	839	GVTVTTVNNILPFHSQ	16
2085	2099	AGSAADNPISQIAPD	15
590	603	CPLNCDKAENKNKV	14
1323	1335	LQVYQQEPKLDKV	13
1635	1647	ATDKTEYIDVCPL	13
2328	2340	STLVYNLGGTTNG	13
88	99	KQQHKFGPTCLC	12
2172	2183	VANGINQTTAGL	12

Antigenicity, surface accessibility, and hydrophilicity of the candidate epitopes of RHDV1 and RHDV2. The generated varying lengths of peptide sequences were trimmed down to 12 continuous epitopes of RHDV1 and RHDV2 and assessed for antigenicity, surface accessibility, and hydrophilicity. Each continuous epitope should pass the threshold value for the different parameters. The threshold values were based on the threshold output of the prediction tool used. The 12 continuous epitopes with higher values than the threshold are presented in Table 4 for RHDV1 and in Table 5 for RHDV2. The 73 amino acid long continuous epitope positioned at 1238-1310 in RHDV1 generated the most significant number of 12 continuous epitopes, which exceeds the cut-off value. There were seven 12 continuous epitopes identified to have antigenicity, surface accessibility, and hydrophilicity scores of 1.016 to 1.04, 1.182 to 2.495, and 2.858 to 3.397, respectively.

Similarly, the 74 sequence long peptide located at 1244 to 1317 positions in RHVD2 generated the highest number of 12 continuous epitopes with antigenicity, surface accessibility, and hydrophilicity values greater than the threshold. The scores of the 7 qualified epitopes from this position varied from 1.016 to 1.03, 1.137 to 2.452, and 2.733 to 3.267, respectively. Overall, 31 twelve continuous epitopes qualified from RHDV1 and 34 from RHDV2.

The antigenic propensity of peptide sequences is influenced by its physicochemical properties, such as hydrophilicity, surface accessibility, and flexibility (Kolaskar &

Tongaonkar 1990). In nature, the antigenic sites are recognized by antibodies. Thus, it only makes sense that the predicted epitope should be accessible on the protein (Emini et al 1985). These peptide fragments located on the surface of the protein are more exposed to bulk water; hence, assumptions are drawn that antigenic sites are hydrophilic (Parker et al 1986).

Table 4

Antigenicity, surface accessibility, and hydrophilicity scores of putative antigenic epitopes of RHDV1

Start	*End*	*Sequence*	*Antigenicity* ≥0.974	*Surface accessibility* ≥1	*Hydrophilicity* ≥1.322
1813	1824	GIGGPPQQVDQQ	1.018	1.031	3.633
1814	1825	IGGPPQQVDQQE	1.016	1.805	3.808
1281	1292	KVPGVPDEYKTG	1.024	1.182	3.392
1285	1296	VPDEYKTGYRPA	1.017	2.495	3.117
1280	1291	AKVPGVPDEYKT	1.04	1.207	3.092
1268	1279	AEPRPPVSGTRY	1.016	1.935	3.033
1269	1280	EPRPPVSGTRYA	1.016	1.935	3.033
1277	1288	TRYAKVPGVPDE	1.035	1.182	2.967
1270	1281	PRPPVSGTRYAK	1.023	2.234	2.858
680	691	DKLVKSQVTPRE	1.039	2.082	3.05
679	690	GDKLVKSQVTPR	1.041	1.19	2.875
683	694	VKSQVTPREYKY	1.056	3.712	2.667
681	692	KLVKSQVTPREY	1.063	1.953	2.058
682	693	LVKSQVTPREYK	1.063	1.953	2.058
719	730	FVYPDAQYDQAL	1.083	1.399	1.033
720	731	VYPDAQYDQALL	1.097	1.332	1.033
2062	2073	SYPGNNATNVLQ	1.005	1.089	2.817
1375	1386	VPGKKIDHVKDG	1.03	1.377	3.108
1376	1387	PGKKIDHVKDGV	1.03	1.377	3.108
1081	1092	ARQAPRGPKTLD	0.987	1.258	3.35
1986	1997	RAPSSKTVDSIS	1.024	3.208	3.633
1987	1998	APSSKTVDSISP	1.04	2.533	3.458
1988	1999	PSSKTVDSISPA	1.04	2.533	3.458
1989	2000	SSKTVDSISPAG	1.024	1.621	3.758
2184	2195	TYTPQPDRIVTT	1.018	1.786	2.633
182	193	RDASLLDSIASD	1.024	1.178	2.625
933	944	DQGIDVFTDPNL	1.009	1.214	2.158
1629	1640	AADKTEFIDVCP	1.054	1.101	2.125
441	452	KMANEAATLDQL	0.989	1.000	2.117

Table 5

Antigenicity, surface accessibility, and hydrophilicity scores of putative antigenic epitopes of RHDV2

Start	*End*	*Sequence*	*Antigenicity* ≥0.966	*Surface accessibility* ≥1	*Hydrophilicity* ≥0.674
1819	1830	GIGGPPQQVDQQ	1.018	1.108	3.633
1820	1831	IGGPPQQVDQQE	1.016	1.939	3.808
1287	1298	RVPGVPDEYKTG	1.019	1.138	3.267
1291	1302	VPDEYKTGYRPA	1.017	2.452	3.117
1274	1285	AEPRPPVSGTRY	1.016	1.901	3.033
1275	1286	EPRPPVSGTRYA	1.016	1.901	3.033
1285	1296	ARVPGVPDEYKT	1.035	1.161	2.967
1283	1294	TRYARVPGVPDE	1.03	1.137	2.842
1276	1287	PRPPVSGTRYAR	1.018	2.15	2.733
686	697	EKLVKSQVAPRE	1.05	1.793	2.608
685	696	GEKLVKSQVAPR	1.052	1.025	2.433
687	698	KLVKSQVAPREY	1.076	1.623	1.8
688	699	LVKSQVAPREYK	1.076	1.623	1.8
1059	1070	TRRQLRPDEDQV	0.987	2.892	3.9
1060	1071	RRQLRPDEDQVT	0.987	2.892	3.9
1993	2004	APSSKTVDSISP	1.04	2.377	3.458
1994	2005	PSSKTVDSISPA	1.04	2.377	3.458
1996	2007	SKTVDSISPADL	1.043	1.58	2.808
2000	2011	DSISPADLLTTP	1.035	1.462	1.942
1381	1392	VPGKKIDHVKDG	1.03	1.189	3.108
1382	1393	PGKKIDHVKDGV	1.03	1.189	3.108
2053	2064	SSPRFAAIDHDR	1.004	1.514	2.717
2054	2065	SPRFAAIDHDRG	0.992	1.118	2.65
740	751	KQYFVMYGCVAR	1.098	1.087	0.042
2244	2255	STNGTQYGAGSQ	0.958	1.46	4.975
2240	2251	AEAGSTNGTQYG	0.949	1.101	4.758
2241	2252	EAGSTNGTQYGA	0.949	1.101	4.758
2149	2160	VPSNPQPTTTTS	1.002	1.543	4.117
178	189	VDIDEPCLTSKD	1.047	1.087	3.15
11	22	TTAILPAKKPLS	1.05	1.532	0.858
2107	2118	VPFSGTTIPTAG	1.025	1.031	1.575
2105	2116	SFVPFSGTTIPT	1.039	1.197	0.7
2088	2099	AADNPISQIAPD	1.013	1.414	2.833
88	99	KQQHKFGPTCLC	1.084	1	1.908

The similarity of the predicted epitopes with the different types of RHDV. The 12 continuous epitopes predicted in RHDV1 were aligned with the protein sequence of RHDV2 to determine the uniqueness of the candidate epitopes to RHDV1, as shown in Table 6. Contrastingly, the predicted epitopes in RHDV2 were aligned with the protein sequence of RHDV1, as shown in Table 7. Typically, the identity the majority of identified epitopes from RHDV1 has 83 to 100% similarity with RHDV2. Only three 12 continuous epitopes have at most 75% homology. These continuous epitopes were between 2062 and 2073, 1081 and 1092, and 2184 and 2195 positions. Similarly, most of the predicted continuous epitopes of RHDV2 had 83 to 100% similarity with RHDV1. Only three 12 continuous epitopes of RHDV2 were identified to have less than 73% similarity with RHDV1. These epitopes are found from between 2149 and 2160, 2107 and 2118, and 2105 and 2116 positions in RHDV2.

Sequence similarity analysis identifies epitopes that are distinct to RHDV1 and RHDV2. The unique epitopes may distinguish *O. cuniculus* infected with RHDV1 from those infected with RHDV2, whose symptoms are relatively similar. Ideally, unique

synthetic peptides may pave the way for developing screening tests, immunization, and vaccines specific to the two types of RHDV (Potocnakova et al 2016).

Table 6

Sequence similarity of the predicted immunogenic epitopes of RHDV1 with RHDV2

Start	*End*	*Sequence*	*RHDV2*	
			Identity (%)	*Alignment*
1813	1824	GIGGPPQQVDQQ	100	-
1814	1825	IGGPPQQVDQQE	100	-
1281	1292	KVPGVPDEYKTG	100	*VPGVPDEYKTG
1285	1296	VPDEYKTGYRPA	100	-
1280	1291	AKVPGVPDEYKT	91.67	A*VPGVPDEYKT
1268	1279	AEPRPPVSGTRY	100	-
1269	1280	EPRPPVSGTRYA	100	-
1277	1288	TRYAKVPGVPDE	91.67	TRYA*VPGVPDE
1270	1281	PRPPVSGTRYAK	100	PRPPVSGTRYA*
680	691	DKLVKSQVTPRE	83.33	*KLVKSQV*PRE
679	690	GDKLVKSQVTPR	83.33	G*KLVKSQV*PR
683	694	VKSQVTPREYKY	91.67	VKSQV*PREYKY
681	692	KLVKSQVTPREY	91.67	KLVKSQV*PREY
682	693	LVKSQVTPREYK	91.67	LVKSQV*PREYK
719	730	FVYPDAQYDQAL	83.33	F*YPDAQY*QAL
720	731	VYPDAQYDQALL	83.33	*YPDAQY*QALL
2062	2073	SYPGNNATNVLQ	50	S*PG****NVL*
1375	1386	VPGKKIDHVKDG	100	-
1376	1387	PGKKIDHVKDGV	100	-
1081	1092	ARQAPRGPKTLD	75	ARQ*P*GPK*LD
1986	1997	RAPSSKTVDSIS	100	-
1987	1998	APSSKTVDSISP	100	-
1988	1999	PSSKTVDSISPA	100	-
1989	2000	SSKTVDSISPAG	100	-
2184	2195	TYTPQPDRIVTT	75	TYTPQP*RIV**
182	193	RDASLLDSIASD	100	*DASLLDSIASD
933	944	DQGIDVFTDPNL	91.67	DQG*DVFTDPNL
1629	1640	AADKTEFIDVCP	83.33	A*DKTE*IDVCP
441	452	KMANEAATLDQL	100	-

Table 7

Sequence similarity of the predicted immunogenic epitopes of RHDV2 with RHDV1

Start	*End*	*Sequence*	*RHDV1*	
			Identity (%)	*Alignment*
1819	1830	GIGGPPQQVDQQ	100	-
1820	1831	IGGPPQQVDQQE	100	-
1287	1298	RVPGVPDEYKTG	100	-
1291	1302	VPDEYKTGYRPA	100	-
1274	1285	AEPRPPVSGTRY	100	-
1275	1286	EPRPPVSGTRYA	100	-
1285	1296	ARVPGVPDEYKT	91.67	A*VPGVPDEYKT
1283	1294	TRYARVPGVPDE	91.67	TRYA*VPGVPDE
1276	1287	PRPPVSGTRYAR	100	PRPPVSGTRYA*
686	697	EKLVKSQVAPRE	83.33	*KLVKSQV*PRE
685	696	GEKLVKSQVAPR	83.33	G*KLVKSQV*PR
687	698	KLVKSQVAPREY	91.67	KLVKSQV*PREY
688	699	LVKSQVAPREYK	91.67	LVKSQV*PREYK
1059	1070	TRRQLRPDEDQV	100	-
1060	1071	RRQLRPDEDQVT	100	-
1993	2004	APSSKTVDSISP	100	-
1994	2005	PSSKTVDSISPA	100	-
1996	2007	SKTVDSISPADL	91.67	SKTVDSISPA*L
2000	2011	DSISPADLLTTP	83.33	DSISPA*L*TTP
1381	1392	VPGKKIDHVKDG	100	-
1382	1393	PGKKIDHVKDGV	100	-
2053	2064	SSPRFAAIDHDR	90	SSPRFA*IDH***
2054	2065	SPRFAAIDHDRG	88.89	*SPRFA*IDH***
740	751	KQYFVMYGCVAR	100	-
2244	2255	STNGTQYGAGSQ	83.33	ST*GTQYG*GSQ
2240	2251	AEAGSTNGTQYG	90	**AGST*GTQYG
2241	2252	EAGSTNGTQYGA	90	*AGST*GTQYG*
2149	2160	VPSNPQPTTTTS	72.73	*P*N*QPTT*TS
178	189	VDIDEPCLTSKD	83.33	VD*DEPCLTS*D
11	22	TTAILPAKKPLS	83.33	T*AILP*KKPLS
2107	2118	VPFSGTTIPTAG	66.67	VPF*G**IP*AG
2105	2116	SFVPFSGTTIPT	72.73	SFVPF*G**IP*
2088	2099	AADNPISQIAPD	83.33	A*DNPISQ*APD
88	99	KQQHKFGPTCLC	83.33	QQ*HKFGP*CLC

The similarity of the predicted epitopes of RHDV with other caliciviruses. There are only two species of *Lagovirus* known, which are RHDV and EBHSV. These species of *Lagovirus* are present in Lagomorpha. However, they are distinct to a genus of Leporidae. RHDV is distinct to *Oryctolagus* (Capucci et al 2017), whereas EBHSV to *Lepus* (Lopes et al 2014). Another notable Caliciviridae virus that also poses detrimental effects on the health of *O. cuniculus* is RCV-A1 (Le Gall-Reculé et al 2013). These candidate epitopes underwent sequence similarity analysis with EBHSV and RCV-A1 (Table 8) to validate whether unique RHDV epitopes that can distinguish between RHDV1 and RHDV2 are also distinct to RHDV.

The top 3 unique epitopes in RHDV1 and RHDV2 were aligned with the protein sequence of another *Lagovirus*, EBHSV. Two out of the three 12 continuous epitopes in RHDV1 have greater than 80% homology with EBHS. Only the SYPGNNATNVLQ from the 2061 to 2073 amino acid position in RHDV1 has an identity lower than 70% with EBHSV. Two candidate epitopes from RHDV2 have greater than 80% similarity with the EBHSV protein sequence. The best peptide sequence in RHDV2 is VPSNPQPTTTTS, from the 2149 to 2160 position, 70% homology with EBHS.

Among the three candidate epitopes of RHDV1, only SYPGNNATNVLQ, from the 2061 to 2073 position is distinct to RHDV1, whereas the other two have greater than 80% similarity with RCV-A1. Contrastingly, all the three putative epitopes from positions 2105 to 2160 in RHDV2 are incomparable with RCV-A1, having less than 70% homology.

Considering that RHDV1 and RHDV2 share about 75% to 90% protein sequence with EHBSV and RCV-A1, problems may arise with the possibility of the cross-reactivity of antibodies with the other caliciviruses (Robinson et al 2002). Identifying the unique immunogenic epitopes in RHDV1 and RHDV2 may distinguish the RHDV1 from RHDV2 and other caliciviruses.

Table 8

Sequence similarity of the unique immunogenic epitopes of RHDV1 and RHDV2 with other *Caliciviridae*

ID	*Start*	*End*	*Sequence*	*EBHS*		*RCV-A1*	
				Identity (%)	*Alignment*	*Identity (%)*	*Alignment*
RHDV1	2062	2073	SYPGNNATNVLQ	66.67	SYP***ATN***	66.67	SYPG***TNVL*
RHDV1	1081	1092	ARQAPRGPKTLD	81.82	*R*AP*GPKTLD	81.82	*RQAP*G*KTLD
RHDV1	2184	2195	TYTPQPDRIVTT	83.33	TYTPQP**IVTT	90	TYTPQP+RIV**
RHDV2	2149	2160	VPSNPQPTTTTS	70	*PSN**P*TTT*	63.64	*P*N**PTT*TS
RHDV2	2107	2118	VPFSGTTIPTAG	80	*PFSG*TIPT**	58.33	VPF*G***PT*G
RHDV2	2105	2116	SFVPFSGTTIPT	80	***PFSG*TIPT	66.67	SFVPF*G***PT

Conclusions. The high similarity of the RHDV protein sequence with the other caliciviruses may pose a possible concern on cross-reactivity, impeding diagnostic, and vaccine development in *O. cuniculus*. This study identified one 12 continuous epitopes from RHDV1 and RHDV2, which qualify for the antigenicity, surface accessibility, and hydrophilicity threshold values. These epitopes were SYPGNNATNVLQ, from the 2061 to 2073 amino acid of RHDV1 and VPSNPQPTTTTS, from the 2149 to 2160 amino acid of RHDV2. They may distinguish each respective RHDV virus from each other and other caliciviruses, like EBHSV and RCV-A1.

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Authors:
John Sylvester Brusola Nas, Department of Biology, College of Arts and Sciences, University of the Philippines Manila, P. Faura St., 1000 Manila, Philippines; Department of Medical Technology, Institute of Arts and Sciences, Far Eastern University, Nicanor Reyes St., 1006 Manila, Philippines, e-mail: jbnas@up.edu.ph

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