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FOI Number: FOI2016/62

Date: 20 December 2016

Request: All "diagnostic test results" as described in the following web page: http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=18075

All results corresponding to "virus sequencing" as described in the following web page: http://www.oie.int/wahis_2/public/wahid.php/Reviewreport/Review?page_refer=MapFullEventReport&reportid=20384"

Document(s): 1 and 2

For more information, please refer to CSIRO's FOI disclosure log at www.csiro.au/FOILog

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NATA Accreditation - 13546

Document 1

Diagnostic Testing Report

Final Report (Copy)

Chief Veterinary Officer ACT
Attn: Wendy Townsend
Territory and Municipal Services
GPO Box 158
Canberra ACT 2601 AUSTRALIA

SAN: 15-01893
Collection Date: 13-May-2015
Received at AAHL: 23-Jun-2015
Report Date: 01-Jul-2015

Sender: Tanja Strive
CSIRO Black Mountain

Ref: Not Advised

Owner: Tanja Strive
CSIRO Black Mountain
Black Mountain ACT 2601 AUSTRALIA

Examination Requested: Calicivirus sequencing
Sample Information provided: 4 x Fresh tissue (Liver)

Molecular Diagnostics

Test: General Sequencing Analysis

No.	Specimen ID	Species	Specimen Type	Test Result
1	BLCK MT 1 Liver Rabbit RHDV 13/05/15 (RNA)	Rabbit	Liver	Positive
2	BLCK MT 1 Liver Rabbit RHDV 13/05/15	Rabbit	Liver	Positive
3	BLCK MT 2 Liver Rabbit RHDV 09/06/15	Rabbit	Liver	Positive
4	BLCK MT 2 Liver Rabbit RHDV 09/06/15 (RNA)	Rabbit	Liver	Positive

Date Tested: 25-Jun-2015

Comment: NATA accreditation does not cover the performance of this service.
Samples #1 and #2 were 100% identical to each other at the nucleotide level across the partial RHDV polyprotein gene sequence. Samples #3 and #4 were also 100% identical to each other across the RHDV polyprotein gene sequence.
Please see appended Sequencing Analysis report for further details of genotyping and phylogenetic analysis.

Summary

Samples #1 and #2 had closest nucleotide and amino acid sequence BLAST identities to RHDV viruses from Portugal, belonging to the RHDV2 genotype cluster. This strain has not previously been detected in Australian rabbits. These results are further described in the sequencing report in Appendix A.
Samples #3 and #4 were from a different rabbit found in a similar geographical location. Sequence from these samples had closest nucleotide BLAST identity to the RHDV Czech prototype (KF594473) and grouped with circulating Australian RHD viruses.

Yours Faithfully

Debbie Eagles - Veterinary Diagnostician

Appendix A - sequence report

Summary Comments:

14-01893-1	BLCK MT 1 Liver Rabbit RHDV 13/05/15
14-01893-2	BLCK MT 1 Liver Rabbit RHDV 13/05/15
14-01893-3	BLCK MT 2 Liver Rabbit RHDV 09/06/15
14-01893-4	BLCK MT 2 Liver Rabbit RHDV 09/06/15

- Sequence data for the RHDV polyprotein gene segment was produced from specimens 15-01893 #1-4 using primers detailed in Kovaliski et al (2014), Wang et al (2013) and Strive et al (2009).

- Samples #1 and #2 were 100% identical to each other at the nucleotide level across the polyprotein region sequence able to be obtained (~1740 nt). Sample #3 and #4 were also 100% identical to each other across the polyprotein gene sequence obtained (~2370 nt).

- Samples #1 and #2 were confirmed to have closest nucleotide (98%) and amino acid (99%) sequence BLAST identities to CBAlgarve14-4 (2014)-like RHD viruses (eg. KM115716) from Portugal, which belong to the RHDV2 (also known as RHDVb) genotype cluster (Figure 1; Lopes et al., 2015).

- Samples #3 and #4 had closest nucleotide BLAST identity (94%) to the RHDV Czech V351 prototype (KF594473) and grouped with the Australian RHD viruses (Figure 1).

- Phylogenetic analysis using the partial VP60 gene sequence (977 nt) confirms that RHDV samples 15-01893 #1 and #2 do not belong to the Australian lineage linked to the Czech V351 prototype inoculum strain, and also differ from the previous CH4 subgroup viruses submitted in 2014 (SAN 14-00313) (Figure 1).

Sequence alignment: SAN 15-01893 #1 and 2 plus reference

Nucleotide Sequence Alignment

```
Reference molecule:  RHDV_CBAlgarve14-4(KM115716) polyprotein cds
Number of sequences to align:  6

Sequence Similarity                       Match   Non-Match      %Match
RHDV_CBAlgarve14-4(KM115716)
15-01893-1 (RHDV2-like)                   1715    26             98
15-01893-2 (RHDV2-like)                   1715    26             98
15-01893-3 (Australian-like)              1480    261            85
15-01893-4 (Australian-like)              1480    261            85
```

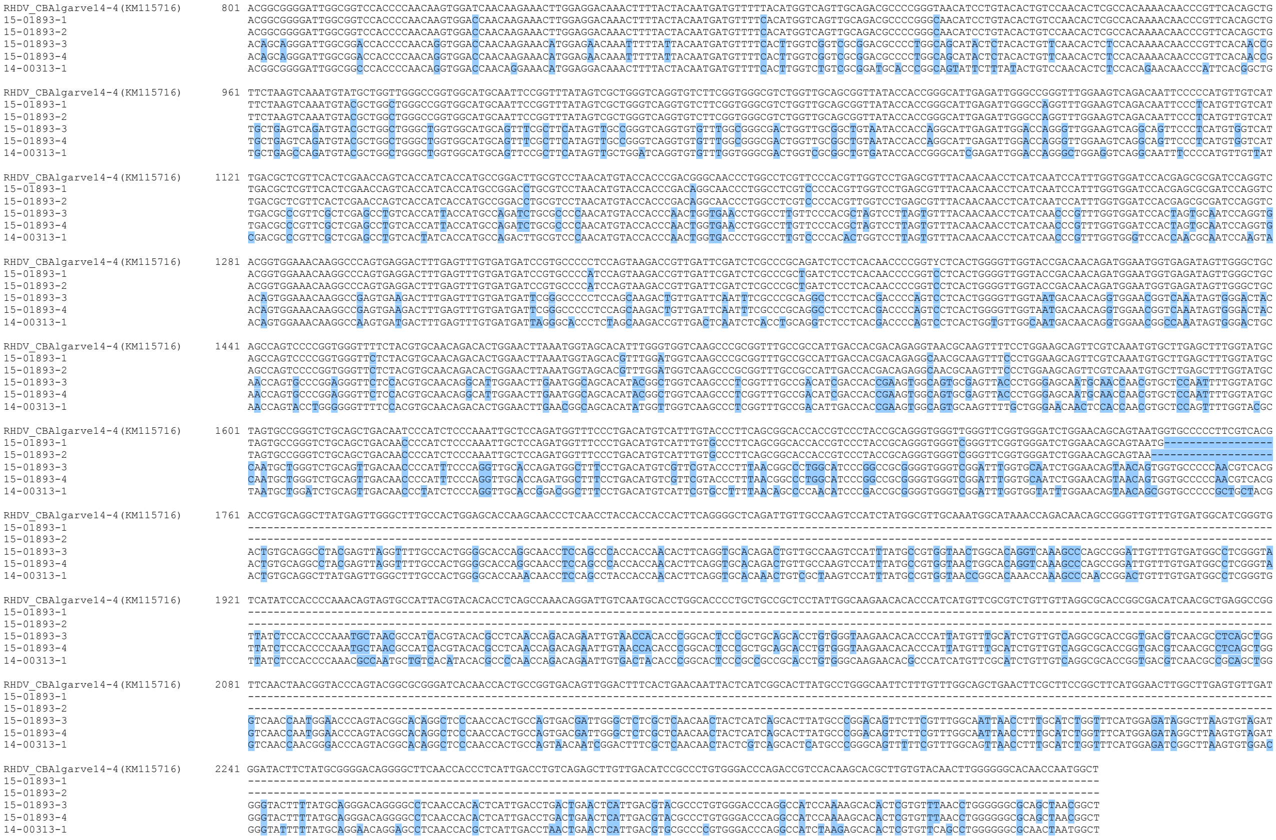
```
RHDV_CBAlgarve14-4(KM115716)    1 TTGTGCAAACTAAGCGTGGCCTTCCAAGCGGCATGCCCTTCACCTCGGTCATTAACTCCATATGCCATTGGTTGCTTTGGTCTGCAGCTGTGTACAAGTCTTGTGCTGAAATTGGTTTACATTGCTCCAACCTGTATGAGGATGCCCCTTTCTACACGTA
15-01893-1                        TTGTGCAAACTAAGCGTGGCCTTCCAAGCGGCATGCCCTTCACCTCGGTCATTAACTCCGTATGCCATTGGTTGCTTTGGTCTGCAGCTGTGTACAAGTCTTGTGCTGAAATTGGTTTGCATTGCTCCAACCTGTATGAGGATGCCCCTTTCTACACGTA
15-01893-2                        TTGTGCAAACTAAGCGTGGCCTTCCAAGCGGCATGCCCTTCACCTCGGTCATTAACTCCGTATGCCATTGGTTGCTTTGGTCTGCAGCTGTGTACAAGTCTTGTGCTGAAATTGGTTTGCATTGCTCCAACCTGTATGAGGATGCCCCTTTCTACACGTA
15-01893-3                        TCGTGCAAACTAAGCGTGGTCTTCCGAGTGGTATGCCCTTCACCTCAGTCATCAACTCCATTTGTCATTGGTTGCTTTGGTCCGCAGCCGTTTACAAGTCTTGTGCTGAAATTGGCTTACACTGCTCCAACTTGTACGAGGACGCCCCATTCTACACTTA
15-01893-4                        TCGTGCAAACTAAGCGTGGTCTTCCGAGTGGTATGCCCTTCACCTCAGTCATCAACTCCATTTGTCATTGGTTGCTTTGGTCCGCAGCCGTTTACAAGTCTTGTGCTGAAATTGGCTTACACTGCTCCAACTTGTACGAGGACGCCCCATTCTACACTTA
14-00313-1                        ----------------------------------------------------------------------------------------------------------------------------------------------------------------

RHDV_CBAlgarve14-4(KM115716)  161 TGGTGATGACGGTGTGTACGCCATGACCCCGATGATGGTGAGTCTATTGCCTGCCATCATAGAGAACCTGAGAGACTACGGGTTGTCACCCACCGCCGCGGACAAAACAGAGTTTATAGACGTCTGCCCATTGAACAAAATCTCGTTTTTGAAGCGCACT
15-01893-1                        TGGTGATGACGGTGTGTACGCCATGACCCCGATGATGGTGAGTCTATTGCCTGCCATCATAGAGAACCTGAGAGACTACGGGTTGTCACCCACCGCCGCGGACAAAACAGAGTTTATAGACGTCTGCCCATTGAACAAAATCTCGTTTTTGAAGCGCACT
15-01893-2                        TGGTGATGACGGTGTGTACGCCATGACCCCGATGATGGTGAGTCTATTGCCTGCCATCATAGAGAACCTGAGAGACTACGGGTTGTCACCCACCGCCGCGGACAAAACAGAGTTTATAGACGTCTGCCCATTGAACAAAATCTCGTTTTTGAAGCGCACT
15-01893-3                        TGGTGATGACGGCGTGTACGCTATGACTCCAATGATGGTGAGCCTTTTACCTGCTATAATAGAAAACTTGAGGGATTATGGTTTGTCACCCACTGCCGCAGACAAAACAGAGTTCATAGATGTTTGCCCACTTAGCAAGATCTCGTTCTTGAAGCGCACC
15-01893-4                        TGGTGATGACGGCGTGTACGCTATGACTCCAATGATGGTGAGCCTTTTACCTGCTATAATAGAAAACTTGAGGGATTATGGTTTGTCACCCACTGCCGCAGACAAAACAGAGTTCATAGATGTTTGCCCACTTAGCAAGATCTCGTTCTTGAAGCGCACC
14-00313-1                        ----------------------------------------------------------------------------------------------------------------------------------------------------------------

RHDV_CBAlgarve14-4(KM115716)  321 TTCGAGCTCACGGACATTGGGTGGGTTTCAAAACTGGACAAGTCCAGCATACTCAGGCAGTTAGAGTGGAGCAAAACAACATCCAGACACATGATAATTGAGGAAACATATGACTTGGCCAAAGAAGAACGTGGTGTGCAGCTAGAGGAACTGCAAGTTG
15-01893-1                        TTCGAGCTCACGGACATTGGGTGGGTTTCAAAACTGGACAAGTCCAGCATACTCAGGCAGTTAGAGTGGAGCAAAACAACATCCAGACACATGATGATTGAGGAAACATATGACTTGGCCAAAGAAGAACGTGGTGTGCAGCTAGAGGAACTGCAAGTTG
15-01893-2                        TTCGAGCTCACGGACATTGGGTGGGTTTCAAAACTGGACAAGTCCAGCATACTCAGGCAGTTAGAGTGGAGCAAAACAACATCCAGACACATGATGATTGAGGAAACATATGACTTGGCCAAAGAAGAACGTGGTGTGCAGCTAGAGGAACTGCAAGTTG
15-01893-3                        TTCGAGCTCACGGACATTGGGTGGGTGTCAAAACTTGACAAGTCCAGCATACTCAGACAGTTAGAGTGGAGTAAAACAACATCTAGACATATGGTGATTGAGGAGACACACGACCTAGCCAAAGAAGAGCGCGGTGTACAACTGGAGGAACTGCAAGTTG
15-01893-4                        TTCGAGCTCACGGACATTGGGTGGGTGTCAAAACTTGACAAGTCCAGCATACTCAGACAGTTAGAGTGGAGTAAAACAACATCTAGACATATGGTGATTGAGGAGACACACGACCTAGCCAAAGAAGAGCGCGGTGTACAACTGGAGGAACTGCAAGTTG
14-00313-1                        ----------------------------------------------------------------------------------------------------------------------------------------------------------------

RHDV_CBAlgarve14-4(KM115716)  481 CTGCTGCCGCACATGGTCAGGAATTTTTCAACTTTGTTCGTAAAGAGCTTGAGCGACAGCAGGCGTACACCCAGTTCAGTGTTTACAGCTACGATGCTGCTAGAAAGATACTTGCAGATCGTAAGAGAGTCGTCTCGGTAGTACCTGACGACGATTTTGT
15-01893-1                        CTGCTGCCGCACATGGTCAGGAATTTTTCAACTTTGTTCGTAAAGAGCTTGAGCGACAGCAGGCGTACACCCAGTTCAGTGTTTACAGCTACGATGCCGCTAGAAAGATACTTGCAGATCGTAAGAGAGTCGTCTCGGTAGTACCTGACGATGATTTTGT
15-01893-2                        CTGCTGCCGCACATGGTCAGGAATTTTTCAACTTTGTTCGTAAAGAGCTTGAGCGACAGCAGGCGTACACCCAGTTCAGTGTTTACAGCTACGATGCCGCTAGAAAGATACTTGCAGATCGTAAGAGAGTCGTCTCGGTAGTACCTGACGATGATTTTGT
15-01893-3                        CTGCCGCTGCACATGGCCAGGAGTTTTTCAACTTTGTTTGCAAGGAGCTTGTGCGGCAACAGGCATACACCCAGTTTAGTGTTTACAGCTATGACGCTGCTAGAAAGATACTTGCAGATCGCAAGAGAGTCGTCTCGGTAGTACCTGACGACGAGTTTGT
15-01893-4                        CTGCCGCTGCACATGGCCAGGAGTTTTTCAACTTTGTTTGCAAGGAGCTTGTGCGGCAACAGGCATACACCCAGTTTAGTGTTTACAGCTATGACGCTGCTAGAAAGATACTTGCAGATCGCAAGAGAGTCGTCTCGGTAGTACCTGACGACGAGTTTGT
14-00313-1                        ----------------------------------------------------------------------------------------------------------------------------------------------------------------

RHDV_CBAlgarve14-4(KM115716)  641 GAATGTTATGGAGGGCAAAGCCCGCGCGGCGCCGCAAGGCGAGACAGCGGGCACTGCTACCACAGCATCGGTCCCTGGAACCACAACCGACGGTATGGACCCTGGTGTTGTGGCCACTACTAGTGTGGTCACCACCGAGAACGCGTCCACGTCGATTGCA
15-01893-1                        GAATGTTATGGAGGGCAAAGCCCGCGCGGCGCCGCAAGGCGAGACAGCGGGCACTGCTACCACAGCATCGGTCCCTGGAACCACAACCGACGGTATGGACCCTGGTGTTGTGGCCACTACTAGTGTGGTCACCACCGAGAACGCGTCCACGTCGATTGCA
15-01893-2                        GAATGTTATGGAGGGCAAAGCCCGCGCGGCGCCGCAAGGCGAGACAGCGGGCACTGCTACCACAGCATCGGTCCCTGGAACCACAACCGACGGTATGGACCCTGGTGTTGTGGCCACTACTAGTGTGGTCACCACCGAGAACGCGTCCACGTCGATTGCA
15-01893-3                        GAATGTTATGGAGGGCAAAGCCCGTGCGGCACCGCAAGGCGAAGCAGTGGGCACTGCCACCACAGCATCAGTTCCCGGGACCACGACTGATGGCATGGACCCTGGTGTTGTGGCCACTACTAGCGTGGTCACTGCAGAAAATTCATCTGCGTCGATTGCA
15-01893-4                        GAATGTTATGGAGGGCAAAGCCCGTGCGGCACCGCAAGGCGAAGCAGTGGGCACTGCCACCACAGCATCAGTTCCCGGGACCACGACTGATGGCATGGACCCTGGTGTTGTGGCCACTACTAGCGTGGTCACTGCAGAAAATTCATCTGCGTCGATTGCA
14-00313-1                        ------------------------------------------------------TGCTACCACAGCATCGGTTCCCGGAACCACAACCGACGGCATGGATCCAGGCGTGGTGGCCGCAACTAGTGTGGTCACTGCAGAAAATTCATCCGCATCGGTTGCA
```

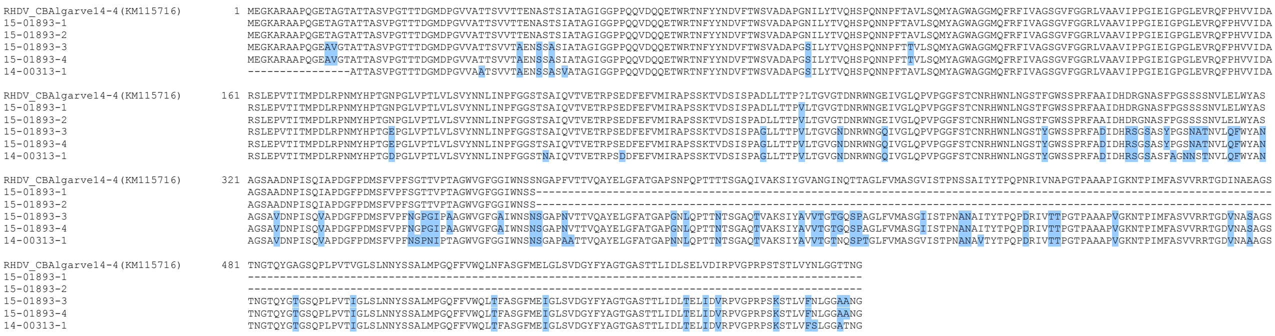


Amino Acid Sequence Alignment

Reference molecule: RHDV_CBAlgarve14-4(KM115716) VP60

Number of sequences to align: 6

Sequence similarity	Match	Non-Match	%Match
RHDV_CBAlgarve14-4(KM115716)			
15-01893-1 (RHDV2-like)	363	1	99
15-01893-2 (RHDV2-like)	363	1	99
15-01893-3 (Australian-like)	332	32	91
15-01893-4 (Australian-like)	332	32	91



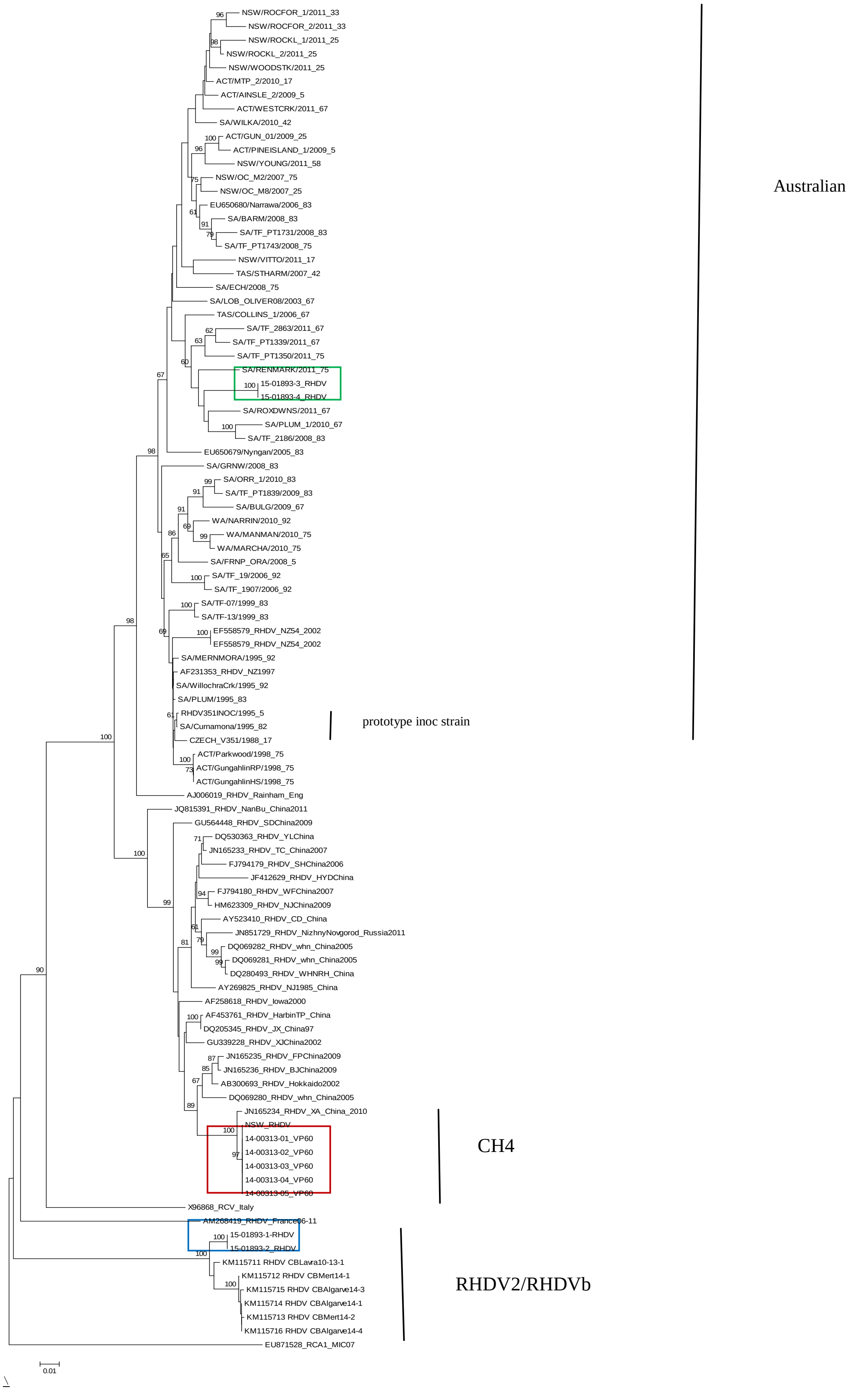


Figure 1: Phylogenetic analysis of RHDV partial VP60 gene (977nt) for 15-01893. Samples 1 and 2 from this submission are in the blue box, samples 3 and 4 are in the green box. RHD CH4 subgroup viruses submitted in 2014 are in the red box.

Project (code) Book No: Cont'd from page 22

Experiment Title Attempt to find hare caliciviruses

8/6/16

Prepare 15µl of Rab1b primer (p106 LN2014/833) at 1.6µM = 2.4µl 10µM stock + 12.6µl dH_2O.
Send Ham-4 11-end for Sanger sequencing with Rab1b primer – order #29409

9/6/16.

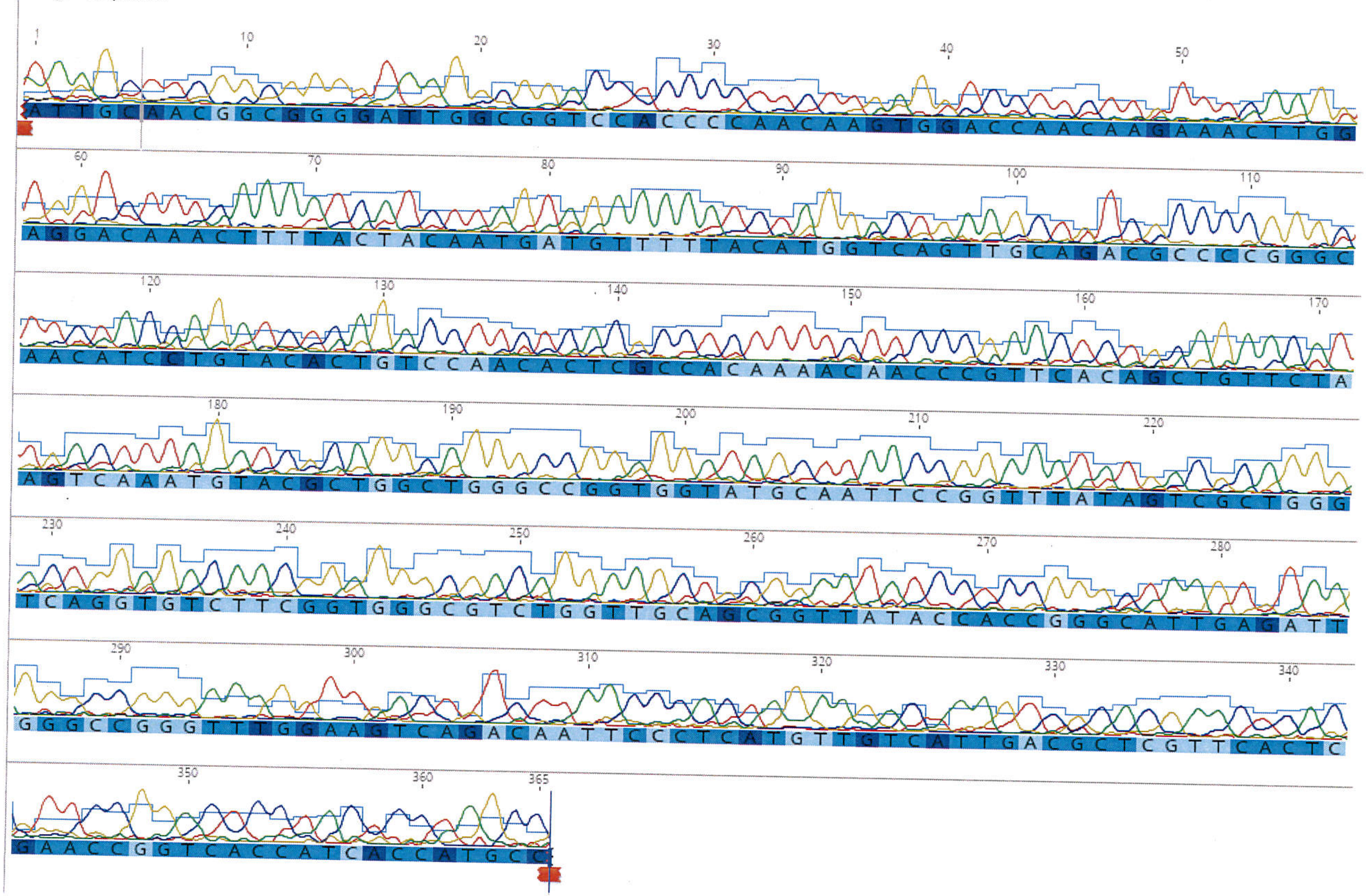
Sequence hare RHDV-2 – Ham-4
Ham-4 is definitely RHDV-2 on Sanger sequencing. 100% to New-1,2,3 based on cleaned sequence (nt 5425-5789). 99.7% blastn to KM979445 (CBVal16 Portuguese RHDV2 Nov 2012).
Need to sequence Ham-3 (collected from wild rabbit at same time as Ham-4) to see how close those sequences are. Could this be a contaminant since the hare and rabbit bodies were in the same bag?

17/6/16.

1. Ham-4_Rab1b.ab1
2. Ham-3_Rab1b.ab1 not cleaned ...

100% match to Ham-4 over region sequenced, despite Ham-3 being from rabbit and Ham-4 being from a hare.

Sanger sequence



Robyn did RT-qPCR of Ham-4

10/6/16

qPCR of field strain samples

Kit: Bioline sensifast SYBR No-Rox One-Step kit
Primers: RHDV all 3 Fw/ 3b Rv (p93 LN2015/780)
Templates: 17/5 Now-3 liver RNA, Mel MF-148 duo RNA, Mel Ham-3 liver RNA, 6/6 –ve RNA, Mel MF-148 liver RNA, Mel MF-149 duo RNA, Mel Ham-4 liver RNA, Mel MF-149 liver RNA, 2/6 –ve RNA, 2/6 Ham-4 liver RNA, 3/6 –ve RNA, 3/6 Gir-1 RNA, 3/6 Ber-1 RNA, 3/6 Gir-2 RNA, 3/6 Ber-2 RNA, Mel Ger-1 RNA, Mel Ger-2 RNA
-ve ctrl: dH_2O **+ve control:** 20/8 K249 control RNA (p98 LN2015/412)
Standards: RHDV full-length prepared 8/2 (p6)

	µl	x54+5%
dH_2O	2.7	153
2x OneStep mix	5	284
10µM primer (each)	0.5	28.4
RNase inhibitor	0.2	11.3
Reverse transcriptase	0.1	5.67
Template	1	-
	10µl	→9µl/tube

→ 100

10/6/16.

Runfile: "H:\QPCR\160610 field strains.pcrd"
Baseline set to 200 for analysis.

Standards are fine above 1e4. Positive control Ct is fine. Std curve efficiency = 99.8%, R^2 0.992. NTCs both negative. 1x MF-148 duo and 1x 6/6 –ve RNA primer dimer – exclude. Poor replication of 1e6 std (evaporation) and MF-149 liver (high Cts). Negative control RNAs all clear.

Unkn-01	17/5 Now3 liver	29.76	29.41	0.493	1.064E+03	1.39E+03	4.66E+02
Unkn-01	17/5 Now3 liver	29.06	29.41	0.493	1.723E+03	1.39E+03	4.66E+02
Unkn-02	MF148 duo	35.41	35.41	0.000	2.126E+01	2.13E+01	0.00E+00
Unkn-03	Ham3 liver	13.78	13.63	0.213	6.760E+07	7.54E+07	1.11E+07
Unkn-03	Ham3 liver	13.48	13.63	0.213	8.328E+07	7.54E+07	1.11E+07
Unkn-04	MF148 liver	35.44	35.41	0.042	2.079E+01	2.12E+01	6.20E-01
Unkn-04	MF148 liver	35.38	35.41	0.042	2.167E+01	2.12E+01	6.20E-01
Unkn-05	MF149 duo	33.38	33.32	0.092	8.626E+01	9.03E+01	5.75E+00
Unkn-05	MF149 duo	33.25	33.32	0.092	9.439E+01	9.03E+01	5.75E+00
Unkn-06	Ham-4 liver	12.36	12.33	0.035	1.810E+08	1.84E+08	4.40E+06
Unkn-06	Ham-4 liver	12.31	12.33	0.035	1.873E+08	1.84E+08	4.40E+06
Unkn-07	MF149 liver	36.88	36.01	1.221	7.699E+00	1.66E+01	1.25E+01
Unkn-07	MF149 liver	35.15	36.01	1.221	2.543E+01	1.66E+01	1.25E+01
Unkn-08	2/6 Ham-4	12.34	12.32	0.028	1.829E+08	1.85E+08	3.60E+06
Unkn-08	2/6 Ham-4	12.30	12.32	0.028	1.880E+08	1.85E+08	3.60E+06

- Now-3 similar to last run (p44 LN2016/352) – try lago PCR just in case there is something there but this is more likely to be contamination since 17/5 –ve RNA was contaminated.
- Hare samples MF-148 and MF-149 all negative. Lago PCR on duo cDNA just in case there is a hare calicivirus not detected by this assay.
- Ham-3, Ham-4, Gir-1, Gir-2, Ber-1, Ber-2, Ger-1, Ger-2 all strong positives. Good replication between RNA extractions (Ber-1/2, Gir-1/2, Ham-4). Ham-4 unlikely to be just a surface contaminant at these high titres.