Detection methodology for the UK OsHV-1 surveillance programme.

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Overview

- Commission Decision amending Decision 2010/221/EU approving national measures for limiting the impact of Ostreid herpesvirus 1 µvar (OsHV-1 µvar) (SANCO/7206/2010)
- Comparison of PCR-based methods
- Surveillance programme
- Findings
- Conclusions

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SANCO/7206/2010

Definition of OsHV-1 uVar

For the purposes of this Regulation, OsHV-1 µvar means a genotype of the virus Ostreid herpesvirus-1 (OsHV-1) which is defined on the basis of partial sequence data exhibiting a systematic deletion of 12 base pairs in ORF 4 of the genome in comparison with OsHV-1 (GenBank # AY509253).

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Comparison of partial sequence of OsHV-1 wild type and OsHV-1 µVar

```
OsHV-wt          GAGACGAGCT CTTTACAAAT GTCGCATGTT AACCTCGTCT GGTTGATGAA  [ 50]
OsHV_µVar        .......... .......... .......... .......... ..........  [ 50]

OsHV-wt          AAAGTCGTTG CTGGCTGATG TGATGGCTTT GGTCAAGGTG CAAAATTCTG  [100]
OsHV_µVar        .......... .......... .......... .......... ..........  [100]

OsHV-wt          TGAAAGGCTG CATTTTTTCA GTAGTAGTAG TAGTAGTAGT AGTAGATGCT  [150]
OsHV_µVar        .......... .......-.. .......... ...------- -----.....  [150]

OsHV-wt          GTTAATTTAG CAGTTGTGGT ATACTCGAGA TTGAGTGTGT GGGTATGATT  [200]
OsHV_µVar        .......... ......... .......... .........- -.........  [200]

OsHV-wt          CTAAACTGAC AAATCGCGCC TATTTATACT TTTTCCCCGG GG--TTTAAA  [250]
OsHV_µVar        .......... .......... .......... ....T...C. ..GC......  [250]

OsHV-wt          TTCCTTGGCC CCCATGTGGT TTTTTTAATT TTAGATTGTG GAACCCATTT  [300]
OsHV_µVar        .......... .........G .....-.... .......... ..........  [300]

OsHV-wt          TC
OsHV_µVar        ..
```

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Comparison of partial sequence of OsHV-1 wild type and OsHV-1 µVar

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Sampling

A sample of Pacific oysters should consist of the following:

(i) In the case of larvae, 3 pools of 50 mg of whole oysters (including shell), which should be collected between 4 and 8 days after fecundation.

(i) In the case of spat smaller than or of 6 mm, 30 pools of 300 mg of whole oysters (including the shell).

(ii) In the case of oysters bigger that 6 mm, 150 oysters which should be tested individually. Oysters could be tested in pools provided that an equal sensitivity and specificity of the diagnosis can be demonstrated.

Prior to test oysters in pools the EU-RL for mollusc diseases should be consulted and the Commission and other Member States should be informed.

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The Diagnostic Test

The diagnostic test that should to be used for detection and identification of OsHV-1 µvar is a combination of real-time Polymerase Chain Reaction (real-time-PCR) and conventional PCR (PCR).

- Real-time PCR should be used to screen the samples for the detection of OsHV-1.
- If the real-time PCR used is not able to distinguish between OsHV-1 µvar and other strains of OsHV-1, a conventional PCR, which allows for such distinction should be used for confirmation of OsHV-1 µvar.

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In the case of a positive detection of OsHV-1 by a RT PCR method that does not distinguish between OsHV-1 µvar and other strains combined with a negative result by conventional PCR, the following procedure should be followed:

- The samples should be sent to the EURL for mollusc diseases for possible confirmation of OsHV-1 µvar, and

- The sampling point from which the real-time PCR positive sample originates should be sampled again and the oysters kept alive in water holding at least 16 °C for at least two weeks, before being tested again.

Comparison of molecular assays for oyster herpes virus

5 assays compared

- Sybr green real-time (Webb *et al.,* 2007)
- CF/CR conventional PCR (Segarra *et al*., 2010)
- C2/C6 Conventional PCR (Arzul et al 2001) which targets the region that can also be used to discriminate between OsHV-1 wt and OsHV-1 uVar
- Nested PCR using C2/C6 as a template (Cefas unpublished)
- Taqman quantitative real-time PCR (Martenot *et al.*, 2010)

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Comparison of molecular assays for oyster herpes virus

Dilution

Sample no.	Neat				10-1				10-2				10-3				10-4			
	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 &CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 &CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 &CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 &CFCR Conventional	Nested	Taqman	Sybr Green
2	+++++	+++++	+++ (28.7)	++++ (24.2)	+++	+++++	++ (32.2)	+++ (27.2)	-	+++++	+ (36.5)	++ (31.4)	-	+++++	-	-	-	+/- (1/3)	-	-
17	++++	+++++	+++ (29.5)	++++ (24.3)	++	+++++	33.0	+++ (27.7)	-	+++++	+ (36.6)	-	-	+/- (1/3)	+/- (2/3)	-	-	-	+/- (1/3)	-
1	+++	+++++	+++ (29.4)	+++ (25.3)	+	+++++	33.3	+++ (28.0)	-	+++++	+ (35.3)	++ (31.7)	-	-	-	-	-	-	-	-
3	+++	+++++	++ (30.9)	26.3	-	+++++	34.1	+++ (29.0)	-	1/3	+ (37.2)	++ (32.0)	-	-	+/- (1/3)	-	-	-	-	-
5	+	+++++	++ (33.8)	29.7	-	1/3	37.4	++ (31.6)	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+++++	++ (34.5)	29.4	-	+++++	38.2	-	-	-	-	-	-	-	-	-	-	-	-	-

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Comparison of molecular assays for oyster herpes virus

Dilution

Sample no.	Neat				10-1				10-2				10-3				10-4			
	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green
2	+++++	+++++	+++ (28.7)	++++ (24.2)	+++	+++++	++ (32.2)	+++ (27.2)	-	+++++	+ (36.5)	++ (31.4)	-	+++++	-	-	-	+/- (1/3)	-	-
17	++++	+++++	+++ (29.5)	++++ (24.3)	++	+++++	33.0	+++ (27.7)	-	+++++	+ (36.6)	-	-	+/- (1/3)	+/- (2/3)	-	-	-	+/- (1/3)	-
1	+++	+++++	+++ (29.4)	+++ (25.3)	+	+++++	33.3	+++ (28.0)	-	+++++	+ (35.3)	++ (31.7)	-	-	-	-	-	-	-	-
3	+++	+++++	++ (30.9)	26.3	-	+++++	34.1	+++ (29.0)	-	1/3	+ (37.2)	++ (32.0)	-	-	+/- (1/3)	-	-	-	-	-
5	+	+++++	++ (33.8)	29.7	-	1/3	37.4	++ (31.6)	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+++++	++ (34.5)	29.4	-	+++++	38.2	-	-	-	-	-	-	-	-	-	-	-	-	-

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Comparison of molecular assays for oyster herpes virus

Dilution

Sample no.	Neat				10-1				10-2				10-3				10-4			
	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 &CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green	C2C6 & CFCR Conventional	Nested	Taqman	Sybr Green
2	+++++	+++++	+++ (28.7)	++++ (24.2)	+++	+++++	++ (32.2)	+++ (27.2)	-	+++++	+ (36.5)	++ (31.4)	-	+++++	-	-	-	+/- (1/3)	-	-
17	++++	+++++	+++ (29.5)	++++ (24.3)	++	+++++	33.0	+++ (27.7)	-	+++++	+ (36.6)	-	-	+/- (1/3)	+/- (2/3)	-	-	-	+/- (1/3)	-
1	+++	+++++	+++ (29.4)	+++ (25.3)	+	+++++	33.3	+++ (28.0)	-	+++++	+ (35.3)	++ (31.7)	-	-	-	-	-	-	-	-
3	+++	+++++	++ (30.9)	26.3	-	+++++	34.1	+++ (29.0)	-	1/3	+ (37.2)	++ (32.0)	-	-	+/- (1/3)	-	-	-	-	-
5	+	+++++	++ (33.8)	29.7	-	1/3	37.4	++ (31.6)	-	-	-	-	-	-	-	-	-	-	-	-
7	+	+++++	++ (34.5)	29.4	-	+++++	38.2	-	-	-	-	-	-	-	-	-	-	-	-	-

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Report entitled : **An evaluation of the suitability of four PCR-based assays for use in surveillance programmes for ostreid herpesvirus-1 (OsHV-1)**

Order of Sensivitivity

Martenot real-time and C2/C6 nested PCR > Webb Sybr green real-time > Conventional CF/CR and C2/C6

Based on these results the UK proposed the use of the Martenot real-time PCR assay for the surveillance programme and the C2/C6 nested PCR assay to discriminate between the wild type virus and the uVar by sequence analysis. This was agreed with the Commission and the EURL.

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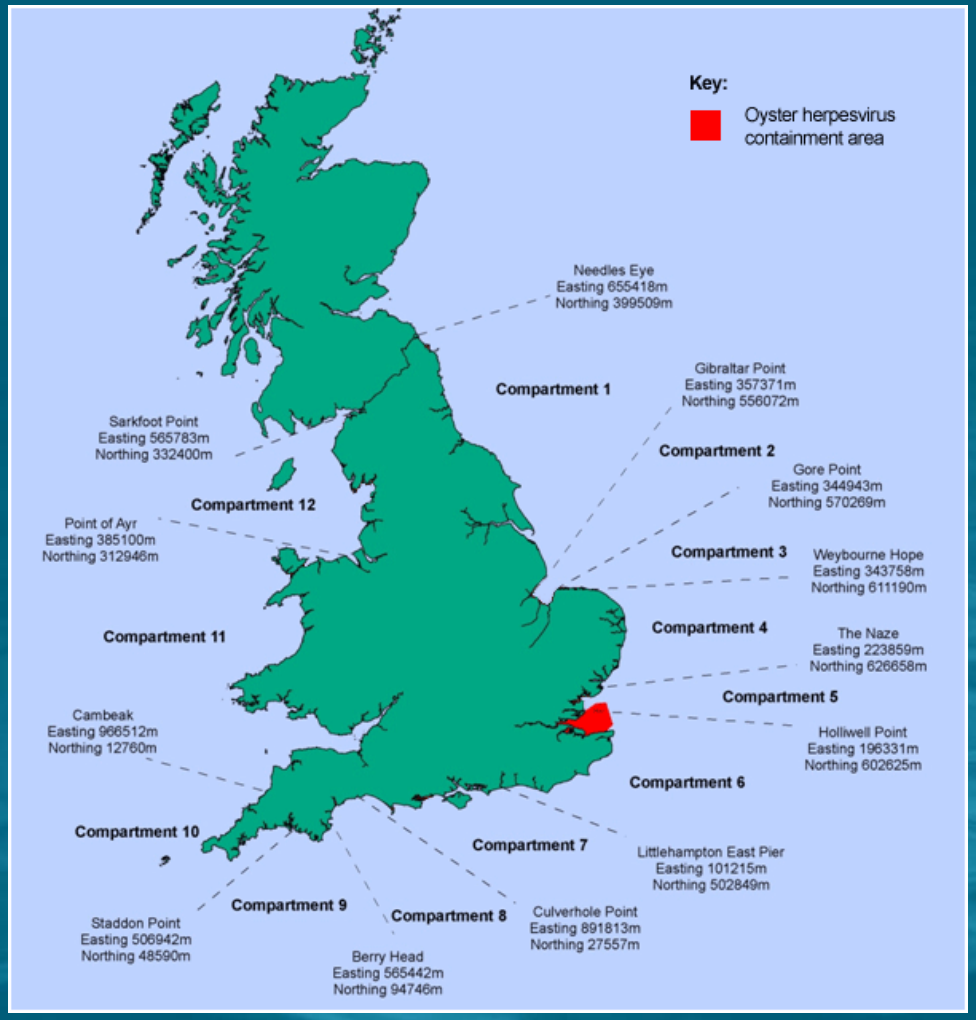
The Survey

Map of compartments used in the surveillance programme for oyster herpesvirus in England and Wales 2011

- 34 sites
- 5400 animals



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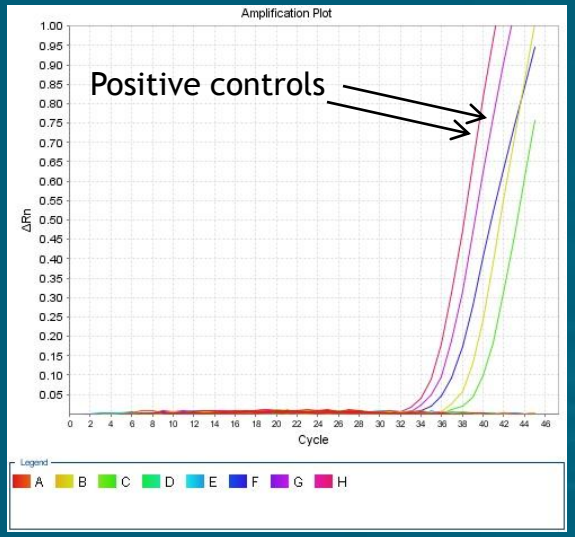
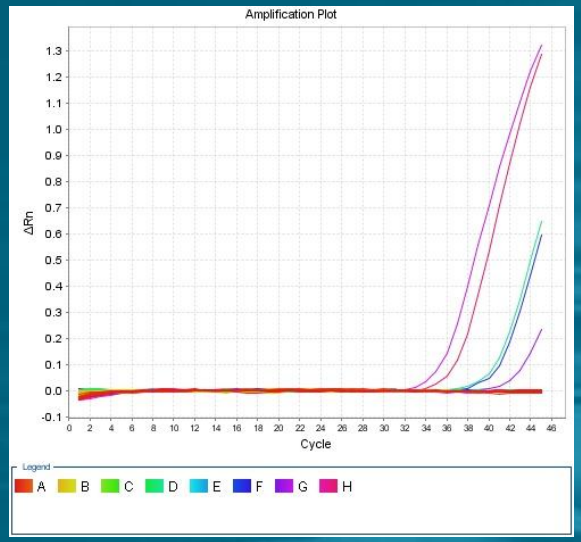
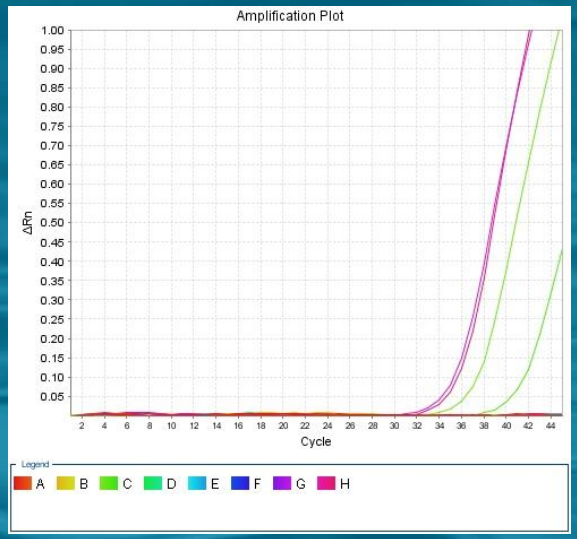
Findings

- Whitstable site was positive
- All negative for OsHV-1 with the exception of three sites from two different compartments
- Positive using the Taqman real-time assay and confirmed using the Sybr green assay







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Characterisation of a novel OsHV-1

- C2/C6 assay was negative for two of the samples and therefore these could not be characterised further.

  Lack of amplicon with the C2/C6 nested assay suggests that they are not OsHV-1 wild type or OsHV-1 uVar, however, further work is required to confirm this.

- C2/C6 assay was positive for the third sample.

  Sequence data indicates that it is an OsHV-1, but is neither the OsHV-1 wt or OsHV-1 uVar

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Comparison of partial sequence of OsHV-1 wild type, OsHV-1 µVar and a novel OsHV-1

```
OsHV-wt                 GAGACGAGCT CTTTACAAAT GTCGCATGTT AACCTCGTCT GGTTGATGAA  [ 50]
OsHV_PM21008-97         .......... .......... .......... .......... ..........  [ 50]
OsHV_microVar           .......... .......... .......... .......... ..........  [ 50]

OsHV-wt                 AAAGTCGTTG CTGGCTGATG TGATGGCTTT GGTCAAGGTG CAAAATTCTG  [100]
OsHV_PM21008-97         .......... .......... .......... .......... ..........  [100]
OsHV_microVar           .......... .......... .......... .......... ..........  [100]

OsHV-wt                 TGAAAGGCTG CATTTTTTCA GTAGTAGTAG TAGTAGTAGT ------AGTA  [150]
OsHV_PM21008-97         .......... .......... .......... .......... AGTAGT....  [150]
OsHV_microVar           .......... .......-.. .......... ..------- ----------  [150]

OsHV-wt                 GATGCTGTTA ATTTAGCAGT TGTGGTATAC TCGAGATTGA GTGTGTGGGT  [200]
OsHV_PM21008-97         .......... .......... .......... .......... ..........  [200]
OsHV_microVar           -......... .......... .......... .......... .....--...  [200]

OsHV-wt                 ATGATTCTAA ACTGACAAAT CGCGCCTATT TATACTTTTT CCCCGGGG--  [250]
OsHV_PM21008-97         .......... .......... .......... .......... ........--  [250]
OsHV_microVar           .......... .......... .......... .......... T...C...GC  [250]

OsHV-wt                 TTTAAATTCC TTGGCCCCCA TGTGGTTTTT TTAATTTTAG ATTGTGGAAC  [300]
OsHV_PM21008-97         .......... .......... .......... .......... ..........  [300]
OsHV_microVar           .......... .......... .....G.... .-........ ..........  [300]

OsHV-wt                 CCATTTTC [308]
OsHV_PM21008-97         ........ [308]
OsHV_microVar           ........ [308]
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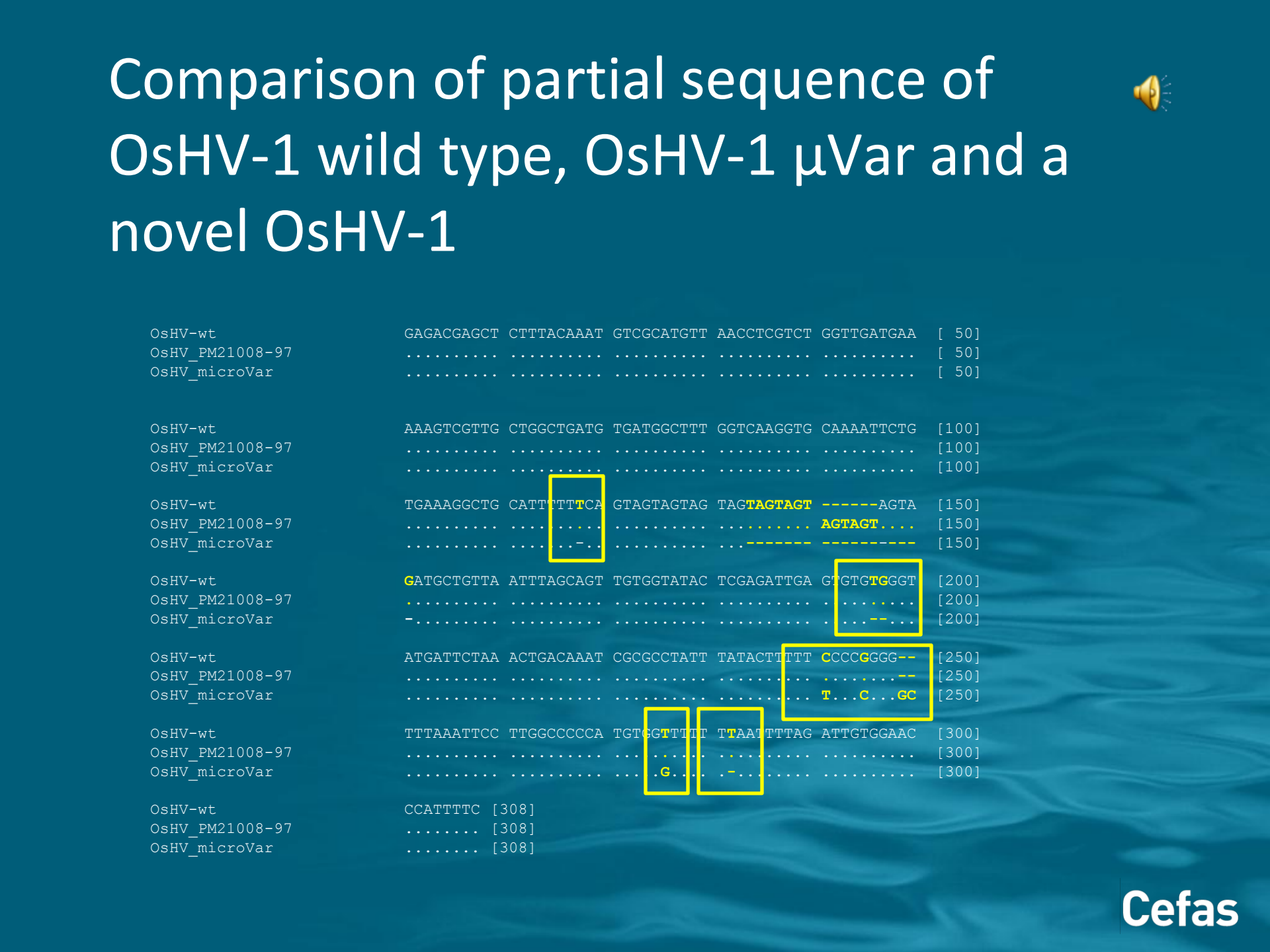
Comparison of partial sequence of OsHV-1 wild type, OsHV-1 µVar and a novel OsHV-1



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Conclusions

- Determined the most appropriate tests to use for the OSHV-1 surveillance programme and these were agreed with the Commission and the EURL
- All samples were negative by real-time PCR with the exception of three sites.
- Characterisation of the OsHV-1 on two of the sites is on going
- Sequence analysis revealed a novel OsHV-1 on the third site. This virus is more closely related to the OsHV-1 wt.

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Thank you

Mike Gubbins will field the questions?

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