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Diagnosis of H1N1 (2009) at the CSIRO Australian Animal Health Laboratory

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Prepared for the 2nd OFFLU Technical Meeting

OIE Paris, 15 & 16 September, 2009



AAHL holds the following swine influenza isolates as well as H1N1(2009) isolates

1. Swine Influenza Strains currently held at AAHL

- H1N1 A/New Jersey/8/1976
- H1N1 A/swine/Rachaburi/2000
- H1N2 A/swine/Miyagi/5/2003
- H3N2 A/swine/Nakorn Patham/2002

• 2. H1N1 (2009) strains held at AAHL:

- A/Auckland/1/2009
- A/California/07/2009
- A/Narita/1/2009
- A/New York/18/2009
- A/New York/18/2009
- Australian human isolates
- Australian swine isolates

AAHL's Diagnostic Approach for H1N1 (2009)

Agent Detection and Characterization

- For the purposes of diagnosing infection in swine:
 - (Specimen – nasal swabs from animals showing clinical signs)
- a. *Virus isolation***
- Routinely in embryonated chicken eggs (ECE) or cell culture
 - Agent detected by haemagglutinating activity in allantoic fluid or culture supernatant, followed by characterization by HI and/or qPCR; and /or directly by qPCR
- b. *Real Time PCR (qPCR)***
- New primers were designed based on published sequences
 - Other published or marketed qPCRs are also evaluated
- c. *Sequencing***
- Definitive characterization of any isolate as being H1N1 (2009) Influenza is based on sequencing.

AAHL's Diagnostic Approach for H1N1 (2009)

Serology

- For the purposes of detecting antibodies to 2009 H1N1 Influenza in pigs. If recent exposure is suspected and the purpose of the testing is diagnostic, paired serum samples are recommended. For the purposes of sero-surveillance a single serum sample is adequate.
- **a. Choice of test**
- For avian influenza and equine influenza AAHL screens with the C-ELISA. However this test has not been standardized for porcine sera, which can show a high proportion of false positives.
- It is recommended that the standard serological test for detection of antibodies to the 2009 H1N1 influenza virus in pigs be the HI test.
- The cross reactivity between classic H1 swine influenza and antibodies to the outbreak strain has been investigated (see data slide).
- **b. Availability of reagents**
- AAHL currently holds aliquotted antigen and control antiserum for H1 swine influenza (the H1N1 New Jersey 1976 strain)
- Antiserum to H1N1 (2009) influenza from current animal studies at AAHL will become available in Sept/Oct.
- A strategy to produce H1N1 (2009) antigen is being developed (see later)

Swine influenza cross-HI Testing

Antiserum	Virus				
	Auckland	New Jersey	Rachaburi	Miyagi	Nakorn
Ferret 1510	320	<20	<20	20	<20
Ferret 1513	640	20	<20	<20	<20
Ferret 1514	320	<20	<20	<20	<20
Ferret 1515	1280	40	<20	<20	<20
Swine NJ	320	1280	<20	<20	<20
Normal swine	<20	<20	<20	<20	<20
H3N8	<20	<20	<20	<20	40

- [A/Auckland/1/2009](#)
- [A/swine/Miyagi/5/03 H1N2](#)

- [A/New Jersey/8/76 H1N1](#)
- [A/swine/Nakorn Patham/2002 H3N2](#)

- [A/swine/Rachaburi/2000 H1N1](#)

- These data show a distinct one-way cross reaction between the New Jersey/1976 and Auckland/2009 viruses. Antibodies against the New Jersey/1976 virus will react to significant titres with the Auckland/2009 virus but antibodies against the Auckland/2009 virus do not react well with the New Jersey/1976 virus. It also suggests that the recent H1N1 virus is closer to the older North American swine H1N1 viruses than to the recent Eurasian H1 viruses.

Real Time PCRs for H1N12009 Evaluated at AAHL

- AAHL type A version2 (matrix gene), this is a modified type A targeting swine H1N1 strains, by changing the reverse primer matching (100%) the swine H1N1 pandemic strains.
- Swine H1 HA PCR, TaqMan MGB assay from Applied Biosystems, for detection of swine H1N1 viruses (both current and previous strains, probably some H1N2 strains, based on bioinformatics analysis)
- Swine Flu NP PCR , Taqman MGB assay from Applied Biosystems, for detection of swine H1N1 viruses (both current and previous strains, probably some H1N2 strains, based on bioinformatics analysis of sequences of primers and probe)
- Influenza A (generic), Applied Biosystems, this assay detects all flu A viruses (is not in routine use for H1N1 strain)
- H1N1 NA PCR, modified USDA assay, for detection of NA gene of current H1N1 strains.
- New H1N1 NA gene PCR, In-house Taqman MGB assays specific for current H1N1 strains; waiting for primers probe arrival.

Recent Submissions to AAHL

Detection of H1N1 infection in Australian pigs

- **NSW – first reported H1N1 2009 outbreak in swine in Australia; samples arrived on 31 July 2009**
 - Samples received were 21 sera and 13 nasal swabs
 - Subsequent to this 70 sera from the same farm were submitted for HI testing (H1N1 2009) and more recently 3 samples for isolation (also from the same farm)
- **Multiple farm workers sick ~5 days before clinical signs noted in pigs. Confirmed as H1 flu.**

Results for NSW

- All swabs were tested in 4 qPCR and all sera by HI

	qPCR <i>Flu A</i>	qPCR <i>H1N1</i>	Serology
# Samples	13	13	21
Positive	12	9	21
Indeterminate	1	4	0
Negative	0	0	0

PCR Results from specimens of NSW Pigs (Positive <37, Indeterminate 37-39, Negative >39)

Sample ID	Type A (2)	AB Matrix	AB- HA	AB -NP
09-02535-0021	37.3	34.49	35.995	36.97
09-02535-0022	Undet	37.4	39.385	Undet
09-02535-0023	37.2	36.16	36.66	37.19
09-02535-0024	37.15	36.735	37.405	Undet
09-02535-0025	Undet	Undet	38.54	Undet
09-02535-0026	34.2	32.63	34.56	35.4
09-02535-0027	34.5	33.12	34.515	35.755
09-02535-0028	36.9	35.365	35.61	37.275
09-02535-0029	31.6	31.005	32.66	33.885
09-02535-0030	31.5	30.97	32.89	33.115
09-02535-0031	35.7	34.775	38.06	37.35
09-02535-0032	34.1	32.855	34.01	35.725
09-02535-0033	33.7	32.09	33.69	34.755
Positive (H1N1 NZ)	24.3	23.99	27.115	27.03
Weak Pos (H1N1 NZ)	33.3	33.66	35.67	37.03
CSIRO AAHL: Influenza, Surveillance and Response N/A	Undet	Undet	Undet	Undet

Results for NSW

- **4 animals gave a weak positive HI against Ratchaburi/2000(H1N1), a classical swine flu strain, as well as stronger results against the Auckland/1/2009(H1N1) reference antigen.**
- **Isolation of the virus from these initial samples was not possible but new samples with lower CT values have been recently received.**

Results for NSW

- **Sequencing of the HA gene directly from swabs yielded partial fragments rather than a complete gene sequence. These fragments show 99% homology to the Auckland/1/ 2009 reference strain (3 nucleotide changes).**
- **Other comparisons indicate 88% homology with Ratchaburi/2000 (classical swine H1N1) and 77% homology with Brisbane/59/2007 (human seasonal H1N1).**

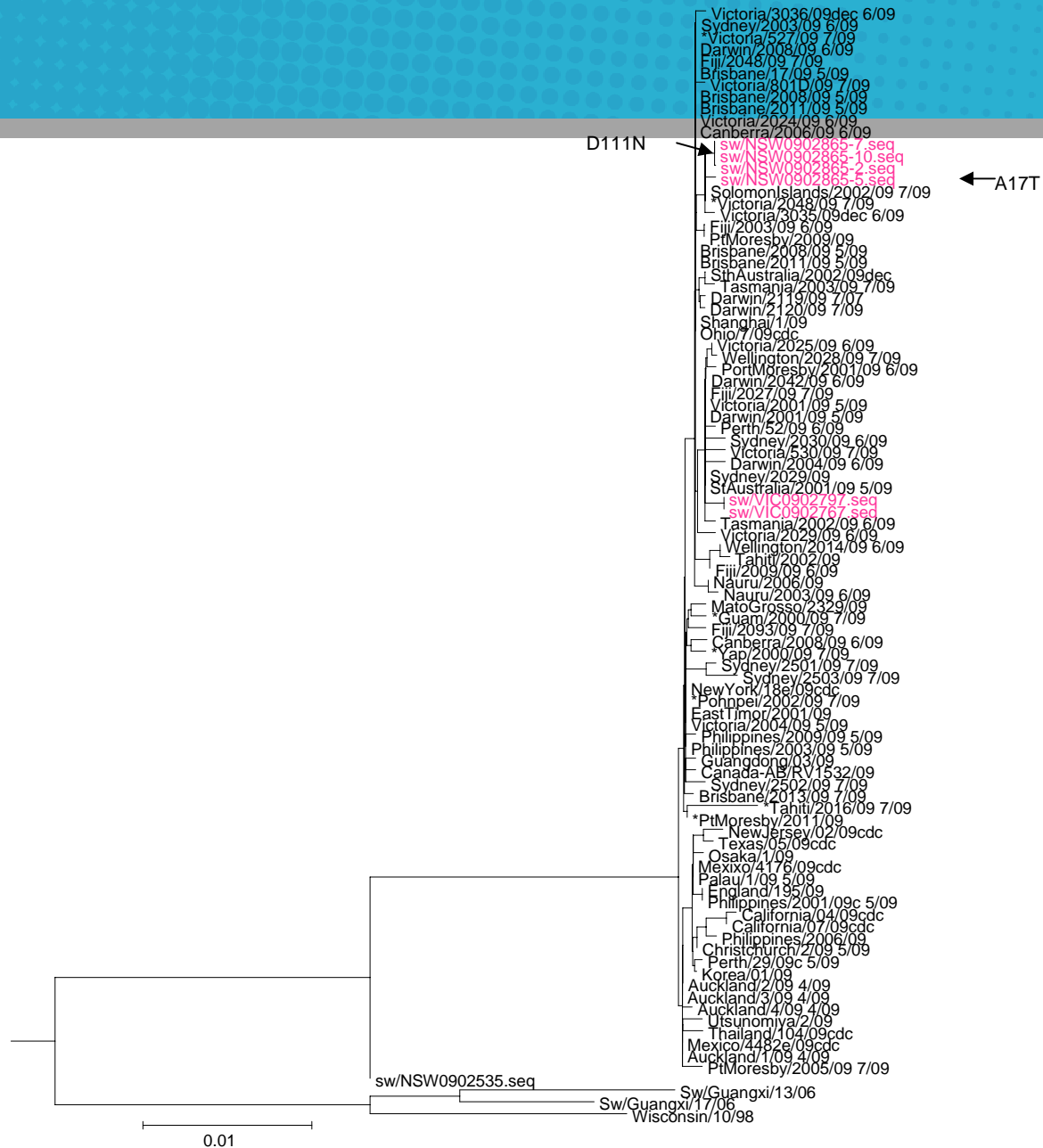
Submissions to AAHL (cont)

- **Victoria – samples received at AAHL on 18 August 2009 as 10 viral transport media.**
 - **Subsequently a further 90 swabs and 56 sera were submitted**

- **5 of 6 employees on the affected property had influenza like illness, with a temporal association supporting their being the source. None were tested for H1**

Victorian Results

- **All 10 initial samples qPCR positive.**
 - HA sequence alignment indicate that samples have highest (99%) sequence homology at both the nucleotide and amino acid level to the 2009 H1N1 pandemic reference strain, A/Auckland/1/2009 (H1N1)
- **H1N1 (2009) was isolated in MDCK cells in the first passage and in eggs in the second passage.**
 - The cells and eggs were inoculated with samples with the best Ct values in the real time RT-PCR .
 - From MDCK cells, it was isolated in 8 of the 9 samples. HA positive range from 2 to 16. 3 samples to PCR to confirm isolation were very strong positives.
- **From eggs, we have about 16 positives, the best titre is 128 (passage 2).**
 - **This will be used to prepare antigen stocks (ie: not Auckland which wasn't growing well with a HA of 2).**



Submissions to AAHL (cont)

- **Queensland also reported an outbreak in August, with tissues and swabs sent to AAHL on 25 August 2009**
- 1 dry sow 'sick'.
- 1 lactating sow 'sick', but is possibly viral pneumonia.
- 1 sucker 'sick'.
- Minor coughing in weaner shed.
- No obvious clinical signs in the rest of the herd.
- **One worker and the vet H1 flu positive**

Queensland Results

- 10 of the 13 swabs positive on qPCR
- HA sequence alignment analyses indicate that the samples have highest (99%) sequence homology at both the nucleotide and amino acid level to the 2009 H1N1 pandemic reference strain, A/Auckland/1/2009(H1N1).
- No isolate yet from this outbreak, it is at passage 2 in eggs and passage 1 in MDCK cells.

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Thank you

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