OFFLU teleconference - laboratory diagnosis of Pandemic H1N1 2009 (new A/H1N1) in swine

Thursday 14th May 2009, 3.00-4.30 pm (Paris time, GMT+2)

Chair: Steve Edwards (OFFLU Chairman). OIE, FAO and WHO representatives as well as 12 laboratories/universities attended the teleconference.

Comments on existing protocols

There is a chapter on swine influenza in the OIE *Manual of Diagnostic Tests and Vaccines in Terrestrial Animals*; this needs updating to account for molecular and immuno diagnostics.

Action: OIE to take forward update of the swine influenza chapter with the OIE Biological Standards Commission.

In the WHO Information for Laboratory Diagnosis of New Influenza A (H1N1) in humans[1], guidance is provided on tests that differentiate between human seasonal influenza strains and new A/H1N1. Veterinary diagnostic tests will also need to differentiate between new A/H1N1 and others circulating in pigs.

Human and animal testing protocols should be harmonised as much as possible, acknowledging that there are reasons for some differences.

Action: OFFLU to develop a paper providing guidance on diagnostic protocols and tests to detect new A/H1N1 (and to differentiate it from other influenza A H1N1 viruses) from animal samples.

Molecular tools for detection and discrimination

M gene amplification

Several OFFLU laboratories found that the Spackman RT PCR protocol[2] has reduced sensitivity for new A/H1N1 owing to 4 nucleotide substitutions mismatches in the reverse primer region. A variety of protocols have been developed, based around synthesis of new reverse primers (perfectly matching or degenerate); these have all improved sensitivity to new A/H1N1 significantly, but required further validation.

The newly developed Winnipeg protocol has comparable sensitivity for existing avian influenza and swine influenza viruses (SIV). Several other labs are developing protocols for M gene; most of these laboratories have designed a new reverse primer with perfect match to the novel H1N1 and have are either replaced the primer or used them mixed with the Spackman primer. Promising results have been obtained but further validation is required. FLI are working on a PCR to target the nucleoprotein (NP) gene, which also requires further validation. IZSVe has tested the conventional RT-PCR protocol developed by Fouchier et al. (2000)[3] for the detection of influenza type A from swine, human and avian. Primers are matching 100% with this novel H1N1. However some problems with specificity have been noted; primers amplify ribosomal RNA.

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[1] http://www.who.int/csr/resources/publications/swineflu/WHO_Diagnostic_RecommendationsH1N1_20090521.pdf
[2] Spackman et al. Clin. Microbiol. , 2002, (40 : 3256-3260.)
[3] Fouchier et al. J Clin Microbiol. 2000 (38::4096-101)

Clear recommendations on primers for the detection of H1N1 are needed as soon as possible for purchase of new primers in veterinary diagnostic laboratories.

Action: For OFFLU laboratories to share notes on their approaches to M and NP gene amplification for detection of new A/H1N1 and other SIV strains.

H1 gene

Several laboratories are developing RRT-PCR to H1 gene with potential to differentiate between different H1 subtype viruses (classical swine H1, new A/H1N1 and human-like H1 viruses).

For conventional and real-time RT-PCR, veterinary laboratories apply the same protocol as in human health (CDC), and use the CDC recommended primers for HA sequencing. However, it should be noted that the routine CDC protocol for H1 gene amplification may not be able to detect some endemic swine H1 viruses and they will most likely not be able to discriminate between the novel H1 and other swine H1 viruses. Only sequencing would allow making this discrimination.

N1 gene

SEPRL have developed and are evaluating a real-time RT-PCR protocol that should enable to differentiate novel A/H1N1 viruses from classical swine H1N1 viruses. They have developed two separate tests with the idea to multiplex them.

Overall Actions: OFFLU labs to share protocols and available validation data on RT-PCR methods for all genes and send them to OFFLU secretariat. OFFLU to collate and distribute the information.

Virus Isolation

Most laboratories have tried growing new A/H1N1 in embryonated eggs and MDCK cells. Results have been good in tissue culture and variable in eggs (some laboratories have found it difficult to grow virus in eggs); there has also been variation in results between different lines/passage number of MDCK cells but more data is needed.

New A/H1N1 growth in embryonated chicken eggs has not always resulted in embryo deaths. There is a need to test allantoic fluid by HA and/or RT-PCR.

Until more information is available there is a case for using both MDCK cells and embryonated chicken eggs to attempt virus isolation of new A/H1N1.

Some laboratories have found that the new A/H1N1 does not haemagglutinate chicken red blood cells; others have observed haemagglutination of red blood cells from turkeys and chickens. Therefore mixed experienced is again noted.

Action: For OFFLU laboratories to share information on virus isolation in eggs versus cell cultures, and on HA activity of different isolates.

Serology

Different strategies can be applied (seroneutralisation, HI, ELISA). Mixed results have been obtained with ELISA: some laboratories note high levels of false positive results, others consider the type A ELISA assays as appropriate assays.

Some laboratories have demonstrated little to weak cross reactivity between different H1 swine viruses and new A/H1N1 – there may therefore be potential to discriminate exposure to different H1 viruses in swine on serology. Although the EU have a bank of reference sera and in the US,

laboratories have been working to develop a panel of reference sera; overall there is still a deficiency in well-defined reference sera.

Action: OFFLU to facilitate an exchange of sera and antigens to assist with developing global/regional reference panels for H1 subtyping and HI tests.

Antigen Rapid Detection Kits

These may have a role to play, although sensitivity cannot compare to RT-PCR. The ARS-USDA had previously tested an antigen rapid detection test and had shown that it could detect 3 log of SIV.

Jane Cunningham from WHO is looking for several human and animal influenza laboratories to collaborate on validating some rapid antigen detection kits.

Action: Any OFFLU laboratories interested in collaborating in this validation should contact Jane (cunninghamj@who.int). Any other validation data should be shared with OFFLU.

Sequence data

SIV sequences are lacking in some geographical areas and many sequences are only 200 bases long. Full genome sequences are more useful than partial genome sequences.

Action: OFFLU laboratories should check if viruses held in repositories have been sequenced; they should continue to deposit sequence data in publicly available databases. Sequencing of longer fragments -and even the whole genome- would be more useful. If GISAID database is used, OFFLU laboratories are requested to push the sequence data to Genbank when depositing their sequences on GISAID. Laboratories should contact OFFLU if some funding is needed for sequencing SIV viruses.

Sample collection/shipment

From live pigs, deep nasal swabs are better than nasopharyngeal swabs and it is important that they are inserted deep into the nasal cavity.

Shipment of RNA samples only (as opposed to swabs, serum, body fluids) can avoid problems with foot and mouth disease import restrictions (from countries where this diseases occurs in ruminants and swine), as well as simplifying packaging requirements. However shipment of isolates/samples is preferred, if possible.

Action: FLI and FAO to circulate – to OFFLU who will further distribute - recommendations on preparation/shipment of RNA.

Any Other Business

Action: If any OFFLU experts have access to information relevant to public health, please contact OFFLU (offlu@oie.int) who will inform WHO. Confidentiality will be maintained and there will be no repercussions on publications. WHO is currently working with journals to renew agreements from past emergencies which ensured that that future publication of public health-relevant information supplied to WHO would not be affected.