



UPDATE ON SWINE INFLUENZA DIAGNOSIS

Update on Swine Influenza Diagnosis

Swine influenza viruses are among the main agents of pneumonia in pigs of all ages.

Infections associated with these viruses can result in major economic losses (growth retardation, mortality, abortion).

In addition, they represent a significant danger to human health (some viruses are zoonotic agents).

The epidemiological context of swine influenza can change rapidly, as illustrated by the recent emergence of a new strain in Ontario.

We therefore thought it would be useful to present an update on the various diagnostic options available.

| Main tests offered at Biovet | Code | Turnaround Time |
|---|------------|-----------------|
| PCR influenza type A | DPOR-40138 | 1-2 days |
| PCR influenza H1 (alpha, beta and pandemic) and H3 (IV-B, IV, IV-C and H3N2 2010.1) | DPOR-40211 | 1-2 days |
| Influenza HA Sequencing (Biovet) | DPOR-40210 | 10-15 days |
| Influenza HA Sequencing (Winnipeg) * | DPOR-70110 | Up to 1 month |

* In the coming months, all H3 cases will be submitted to the CFIA laboratory in Winnipeg for sequencing with a short turnaround time.

Virus detection

The vast majority of influenza strains affecting swine belong to type A (in Canada, type B and C strains appear to be extremely rare).

The detection of these influenza strains usually relies on the use of real-time PCR that targets the matrix gene (M).

Indeed, this gene is well conserved among all strains of influenza A virus, which allows it to be used to detect all strains, regardless of subtype.

As a reminder, the main samples used to detect the virus are nasal swabs, nasal "wipes", oral fluids or lungs.

Subtyping

Once the influenza diagnosis is established, it is interesting to determine the virus subtype involved.

In Canada, the main subtypes encountered are H1N1, H3N2 and H1N2.

This last subtype, which appeared relatively recently in Quebec, is increasingly encountered.

Subtyping can be done using multiple real-time PCRs that target the hemagglutinin (H1 and H3) and neuraminidase (N1 and N2) genes.

Due to the constant evolution of these genes, subtyping PCRs need to be regularly updated to detect new circulating variants.

The PCRs used at Biovet identify the main subtypes currently circulating in Canada (including the new Ontario strain H3N2 2010.1).

Phylogenetic groups

A first step in characterizing strains may be to determine to which phylogenetic groups (clades) they belong.

In Canada, these are mainly alpha, beta and pandemic clades for H1 strains and clades IV, IV-B and IV-C for H3 strains.

The phylogenetic group (clade) is usually determined by sequencing the hemagglutinin gene (see below).

However, at Biovet, we have developed PCRs that can identify major clades faster and more cheaply than sequencing.

We are also developing a PCR that would specifically identify the clade to which the new Ontario strain belongs (H3N2 2010.1).

Sequencing

Further characterization of the strains relies on genome sequencing of the virus.

As a reminder, it consists of 8 strands of RNA that encode 11 proteins.

For economic reasons, routine sequencing is usually limited to the hemagglutinin (HA) gene.

However, under surveillance programs, the NA gene or even the whole genome of the virus is sometimes also sequenced.

Whole genome sequencing is obviously more complex, time-consuming and expensive than HA sequencing alone.

HA gene sequences can be used for different purposes.

They make it possible to determine precisely to which phylogenetic groups (clades) the strains belong (see above).



They are essential for the development of autogenous vaccines such as SEQUIVITY.

They also make it possible to verify the homology of strains with those included in inactivated vaccines (regional vaccine, for example).

Finally, they can allow monitoring of the epidemiology of the virus (appearance of new strains, success or failure of control or eradication protocols, etc.).

It should be noted that HA gene sequencing must be preceded by determining the H1 or H3 subtype (see above).

In addition, successful sequencing depends on viral loads (these must be quite high, we speak of Ct < 30).

Finally, with an equivalent viral load, sequencing is easier by using nasal swabs or lungs rather than oral fluids.

Feel free to contact us for further information if required.

[André Broes](#), D.V.M., Ph.D., Technical Support Manager, swine and ruminants

[Christian Savard](#), Ph.D., Director, Molecular Biology R&D

