OFFLU Technical Meeting
Coordinating world-wide surveillance for influenza in swine

OIE headquarters, Paris, France
April 6-7, 2011
Current SIV surveillance networks, state of the art in South America – Brazil

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Embrapa Swine and Poultry Research Center
Sustainability of surveillance in Swine in Brazil

• The National Program of Swine Health (PNSS) - Efforts are concentrated on the eradication of major diseases affecting pigs, such as Classical Swine Fever, Aujeszky's disease (AD), the African Swine Fever (ASF)**, Swine Vesicular Disease (SVD)*, the trichinellosis*, the Porcine Reproductive and Respiratory Syndrome (PRS)*, swine brucellosis, the Transmissible Gastroenteritis (TGE) and vesicular stomatitis (VS).
• * exotic
• ** eradicated since 1984.

In Brazil, until recently, influenza infection in pigs was not considered a problem.
Influenza in Swine – Brazil - VI

• 1938 a 1943 – Bueno cases of bronchopneumonia in pigs, where it describes an illness compatible with SIV [reviewed by (Cunha et al., 1978)], however, failed to isolate filterable agent due to poor knowledge about the virus at the time.

• 1978 - first SIV was isolated from a 3 months old pig in MG State (Cunha et al., 1978). The agent was isolated in embryonated chicken eggs and characterized from chicken hyperimmune serum, produced against A/Swine/Illinois/1/63. The Brazilian isolate produced HI neutralizing activity >1:320, but had no cross-reaction against the virus A2/Hong Kong/1/68. However, no studies with more detailed molecular genetic to ensure the nature of the strain or subtype which belongs to this virus.

• 2006 - Mancini et al. reported to have isolated the swine influenza virus (SIV) oro-nasal samples from pigs in the state of Sao Paulo. However, the isolates were not characterized.
Influenza in Swine – Brazil - Serology

• 2002 – Brentano et al. Serological studies examining serum collected from pigs in 10 Brazilian states for the period 1996 to 1999 indicated the presence of antibodies against viral subtypes H1N1/Texas/1/77 (2.2%) and H3N2/New Jersey/76 (16.7%) (Brentano, 2002).

• 2006 - Mancini et al. found antibodies against the H1N1 subtype (85.29%), H3N2 (85.29%) and against influenza type B (91.17%).

• 2010 - Caron et al. (2010) analyzed the serum of pigs in the state of Parana reported that 46% of the farms examined were positive for influenza virus, with a prevalence of antibodies against swine H3N2 subtype from 20%.
The big challenge...

- 8.514.876 km²
- 189.612.814 inhabitants
- 27 States
- 5.565 cities
- 36.819.017 pigs
- 22 million pigs slaughter / year
- 1.496.107 pig farms
- 5.335 Official Veterinarians
- 180 countries import Brazilian pork
Approach and Organization for our project

• Project funded by CNPq/MAPA: “Diagnostic, molecular characterization and pathogenesis studies of infectious agents economically important for the Brazilian Swine Production”. 02/2009 – 02/2012

• The objective of this work is to perform and implement diagnostic methods for economical important infectious agents for swine production. The election of these pathogens was based in several reasons, mainly on the difficulty to do an investigation of agents considered up this date exotic in Brazil, such as the virus of porcine reproductive and respiratory syndrome (PRRSV) and the swine influenza virus (SIV). Other agents include the Aujeszky’s disease virus or Pseudorabies virus (PRV), knowingly present in domestic swine herds in some Brazilian states, but of unknown epidemiology and virulence in wild pig population.
## Accomplishments to date

<table>
<thead>
<tr>
<th>ID</th>
<th>Sample Origen</th>
<th>Year (sampling)</th>
<th>Viral Isolation</th>
<th>RT-PCR gene M</th>
<th>Sequencing M, HA and NA genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>*71/10</td>
<td>Toledo, PR</td>
<td>2008</td>
<td>2</td>
<td>+</td>
<td>H3N8</td>
</tr>
<tr>
<td>132/09</td>
<td>Ituporanga-SC</td>
<td>2009</td>
<td>2</td>
<td>+</td>
<td>pH1N1</td>
</tr>
<tr>
<td>12a/10</td>
<td>CNPSA-SC</td>
<td>2010</td>
<td>1</td>
<td>+</td>
<td>pH1N1</td>
</tr>
<tr>
<td>83c/10</td>
<td>Braço do Norte-SC</td>
<td>2010</td>
<td>1</td>
<td>+</td>
<td>pH1N1</td>
</tr>
<tr>
<td>98c/10</td>
<td>Curitibanos-SC</td>
<td>2010</td>
<td>1</td>
<td>+</td>
<td>pH1N1</td>
</tr>
<tr>
<td>107/10</td>
<td>Grão Pará-SC</td>
<td>2010</td>
<td>1</td>
<td>+</td>
<td>pH1N1</td>
</tr>
<tr>
<td>*131/10</td>
<td>Rio do Sul-SC</td>
<td>2010</td>
<td>2</td>
<td>+</td>
<td>pH1N1</td>
</tr>
</tbody>
</table>

* Samples sent to the lab.

| Total of isolated samples | 10 |
| Total of sequenced samples | 10 (genes HA, M, NA and PB1) |
DETECTION OF A HORSE-DERIVED H3N8 INFLUENZA VIRUS IN PIGS IN BRAZIL

Schaefer, Rejane; Zanella, Janice Reis Ciacci.; Brentano, Liana; Ritterbusch, Giseli Aparecida; Silva, Alessandra D’avila; Silveira, Simone; Schiochet, Marisete Fracasso; Caron, Luizinho; Caron, Luis Felipe.

<table>
<thead>
<tr>
<th>ID</th>
<th>71/10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples Origin</td>
<td>2 herds from Toledo, PR, Brazil</td>
</tr>
<tr>
<td>Year</td>
<td>2008</td>
</tr>
<tr>
<td>Sampling</td>
<td>A total of 70 nasal swabs and lung tissue samples from finishing pigs showing pulmonary lesions resembling SIV infection were collected at slaughter.</td>
</tr>
<tr>
<td>VI</td>
<td>2/70 (SPF embryonated eggs) (-EP e FBP).</td>
</tr>
</tbody>
</table>

Table 1. Blast analysis of Brazilian H3N8 isolates

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Ident. (%)</th>
<th>E-value</th>
<th>Virus designation</th>
<th>Access No</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>Primers HA (Hgga &amp; Ht7 – NVSL/USDA)</td>
<td>98.49</td>
<td>0.0</td>
<td>A/eq/Miami/1/1963</td>
<td>CY028836</td>
</tr>
<tr>
<td>M</td>
<td>Chan et al., 2006</td>
<td>100.0</td>
<td>0.0</td>
<td>A/eq/Miami/1/1963</td>
<td>CY028837</td>
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<tr>
<td>NA</td>
<td>Chan et al., 2006</td>
<td>100.0</td>
<td>0.0</td>
<td>A/equ/Swit./2225/1979</td>
<td>CY033491</td>
</tr>
</tbody>
</table>
DETECTION OF A HORSE-DERIVED H3N8 INFLUENZA VIRUS IN PIGS IN BRAZIL

Schaefer, Rejane; Zanella, Janice Reis Ciacci.; Brentano, Liana; Ritterbusch, Giseli Aparecida; Silva, Alessandra D’avila; Silveira, Simone; Schiochet, Marisete Fracasso; Caron, Luizinho; Caron, Luis Felipe.

- Phylogenetic analysis (Mega 5.01 software, Neighbor joining, number differences model with 500 bootstraps) of the HA gene (nt918-1604) of the H3N8 SIV isolates with GenBank sequences from equine, avian and swine viruses from the Americas and Eurasia indicated that the Brazilian swine H3N8 virus grouped with a prototype H3N8 EIV, A/eq/Miami/1/1963/H3N8 (CY028836) and the only other available H3N8 sequences of equine viruses from Brazil (A/eq/SP/6/1963, CY032293; A/eq/SP/1/1969, CY032397) and Uruguay (A/eq/Uruguay/1/1963, M24718). The Brazilian SIV isolates did not clustered with avian or avian-derived H3N8 EIV, neither with H3N2 SIVs. The only other reported horse-derived H3N8 SIV isolated from pigs in China (4) is more closely related with later European H3N8 EIV from the 1990s.
ISOLATION AND CHARACTERIZATION OF PANDEMIC H1N1 INFLUENZA VIRUSES FROM PIGS IN BRAZIL

Schaefer, Rejane; Zanella, Janice Reis Ciacci.; Ritterbusch, Giseli Aparecida; Brentano, Liana; Silva, Alessandra D’avila; Silveira, Simone; Schiochet, Marisete Fracasso; Mores, Nelson; Caron, Luizinho.

- Pig farm consisting of a 175-sow farrowing- nursery operation with 754 animals
- 29% of the pigs were affected (5 sows and 213 nursery pigs) showing clinical signs of fever, cough and loss of appetite, which lasted about 10 days. No clinical signs were observed in piglets and no animal had died.
- VI: nasal swabs and lungs (SPF embryonated chicken eggs and in MDCK cells).
- Histopathology and IHC
- RT-PCR and sequencing (HA gene -WHO, CDC, Atlanta, M and NA genes - Chan et al. 2006.

Table 1. Blast analysis of Brazilian H1N1 isolate

<table>
<thead>
<tr>
<th>Gene</th>
<th>Ident(%)</th>
<th>E-value</th>
<th>Virus designation</th>
<th>Access No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>99</td>
<td>0.0</td>
<td>(A/Guandg/55/2009/H1N1)</td>
<td>HQ011423</td>
</tr>
<tr>
<td>M</td>
<td>99</td>
<td>0.0</td>
<td>(A/Kenya/0026/2009 H1N1)</td>
<td>HQ214452</td>
</tr>
<tr>
<td>NA</td>
<td>99</td>
<td>0.0</td>
<td>A/Guandg/45/2009/H1N1</td>
<td>HQ011420</td>
</tr>
</tbody>
</table>
CURRENT AND RETROSPECTIVE SEROLOGY STUDY OF INFLUENZA A VIRUSES ANTIBODIES IN BRAZILIAN PIG POPULATIONS

Janice Reis Ciacci Zanella, Rejane Schaefer, Marisete Fracasso Schiochet, Simone Silveira, Luizinho Caron, Ubiratan Piovezan

- 09 commercial farms and 01 feral swine herd (176/09/2009) from Pantanal wetland in Brazil.
- 359 serum samples collected between 2006 – 2010.
- HI assays: classic H1N1-A/sw/IA/31(AAF6/19/92) or H1N1, H3N2-A/sw/IA/8548-2 or H3N2, both purchased from NVSL-ARS-USDA and pH1N1/107b/10-3A (H1N1) or pH1N1 isolated from Embrapa.
- Lack of specific antibodies to the pH1N1, which suggests Brazilian pigs were not fully protected against the pH1N1 from previous exposure.
- Besides commercial swine herds, feral swine population (176/09/2009) resulted positive to IAV antibodies by Elisa (5/31) and HI.
Current Opportunities or Gaps

• No official surveillance program.
• Lack of official diagnostic labs.
• Few resources (and researchers) in influenza in swine research.
• Funding for training.
• Need training – twining program / project.
Contributions we can make to the OFFLU working group

• Collaborator center - reference lab in South America – training in influenza in swine to other SA countries

• Can provide viruses data to OFFLU and resources, technical expertise, etc. to SA.
Thanks for your attention!