Swine Influenza Viruses, a zoonotic threat with pandemic potential - Viet Nam, 2013
(EPT Plus Program)

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Overview

• The EPT Plus project document was approved by the Government of Vietnam (MARD) on 06 November 2012.

✓ Project title: Surveillance and Characterization of Influenza Viruses posing Risk as the Next Global Pandemic.
✓ Duration: September 2012 – September 2013
✓ Budget: USD 200.000 funded by USAID
• **Study Design:** Cross-sectional Study

✓ **Overall hypothesis:** Greater genetic diversity of influenza viruses exists in low bio-secure, open intensive swine systems where contact among birds, people and pigs is possible (multiple introductions) and large numbers of swine will be exposed to, amplify, and spread the virus
✓ **Objectives:** Characterize the influenza virus pool in Vietnam from **two swine production systems** and determine if greater genetic diversity exists in any of these production systems.

✓ **Study locations (12 provinces):**
  - Six (06) provinces in the Red River Delta: Bac Ninh, Hai Duong, Hoa Binh, Nam Dinh, Ha Nam, Hai Phong
  - Six (06) provinces in the Mekong River Delta: Vinh Long, Ben Tre, Binh Duong, Dong Nai, Soc Trang, Dong Thap
✓ Production systems for this study:

- Low bio-secure, medium sized **farrow-to-weaning** farms with 50-200 sows and about 500 pigs aged 4-12 weeks old (farm with 100-200 sows is preferable)

- Low bio-secure, medium sized **fattening** farms with 100-4000 pigs. These farm must have different age groups and not being all-in/ all-out production system.
✓ Samples:

- For each province (n=12), 02 farrow-to-weaning farms and 03 fattening farms will be sampled to give a total of 05 farms to be sampled.
- Pigs aged 4-12 weeks, sows and 8-16 weeks will be randomly sampled. Serum and nasal swabs will be collected.
- The single random sampling technique is used to select pigs in each farm.

- Sampling in 4 rounds:
  - The first 3 rounds: sampled as designed (60 farms)
  - The forth round: re-sampled positive farms, and the negative farms will be replaced by the new farms.
✓ Sample size for the first 3 rounds (60 farms, 3600 swabs, 720 bloods) and repeated in 4\textsuperscript{th} round

✓ For farrow-to-weaning farm (n=24):
  ➢ Pigs aged 4-12 weeks: 60 nasal swabs and 10 bloods
  ➢ Sows: 5 bloods

Total: 1440 swabs; 240 piglet bloods, 120 sow bloods

✓ For fattening farm (n=36):
  ➢ Pigs aged 8-16 weeks: 60 nasal swabs and 10 bloods

Total: 2160 swabs; 360 bloods

Remark: Calculation of 60 samples per farm would allow us with 95\% confidence to detect at least 1 positive pig if the Al virus presented in the herds mentioned above (based on the assumption of virus prevalence at farm level is 5\%)
Testing

- NCVD (in Ha Noi) and RAHO6 (in Ho Chi Minh)
- All samples were sent to Labs in the same day of collection for RRT-PCR analysis/viruses isolation. Isolated viruses are sent to NCVD for further analysis of genetic characteristics.
- Serum samples: ELISA
- Swab samples: 5 swab samples will be pooled for M gene testing, RRT – PCR applied for single swab in (+) pool, then follow by virus isolation.

Questionnaires: applied to all selected farms. All detailed information related to farms and farms’ owners will be collected. Face-to-face interview is applied to the farm owners/technicians.
**Laboratory Testing Flowchart**

- **Sample receipt**: 24 hours
- **Inoculation**: 2-4 days
- **Isolation**

**Nasal Swab Samples**
- Screen by real-time PCR for Influenza A virus
  - Positive
  - Virus isolation on floating MDCK
    - Negative
    - Positive
    - Passage on MDCK
      - Positive
      - Isolated viruses
        - HA and NA sub-typing by PCR
        - HA and NA gene sequencing
          - Full characterization (genetic/antigenic) at Reference Laboratories

**Sera Samples**
- Antibodies to influenza virus by ELISA
  - Positive
  - HA sub-typing of antibodies by HI test
Study location

- 12 Provinces:
  - 06 in the North: Bac Ninh, Hai Duong, Hai Phong, Hoa Binh, Ha Nam, Nam Dinh
  - 06 in the South: Dong Thap, Vinh Long, Dong Nai, Ben Tre, Binh Duong, Soc Trang
Results (1)

• A total of 7,200 nasal swab samples and 1,434 serum samples were collected at random from 88 pig farms (32 breeding farms and 56 fattening farms) over one year period.

• 5 swab samples will be pooled for M gene testing, therefore 1,440 swab pools and 1,434 serum samples tested.
Results (2)

• The overall prevalence of influenza type A virus infection in pigs was 6.94% (95% CI 5.69 - 8.38).
• Fifteen out of 120 (12.5%) study farms had at least one pig was positive with influenza type A virus.
• The prevalence of influenza type A virus infection in pigs at breeding farms is 10.28% (95% CI 7.90 - 13.09), significant higher than the one recorded for fattening farm ($\chi^2$ test statistic = 13.7754, df = 1, p-value = 0.0002).
• The prevalence of influenza type A virus infection in pigs at fattening farms 4.79% (95% CI 3.48 - 6.43).
The proportion (%) of nasal swab samples that were positive with influenza type A virus stratified by production type of pigs (breeding and fattening).
Results (3)

• 12.5% study farms had at least one pig positive with influenza type A virus. The highest prevalence of influenza virus infection at farm level is 30.83% (95% CI 22.73 - 39.91).

• 75% study provinces had influenza type A virus circulated in their pig population.
Results (4)

• The sero prevalence of influenza in fattening and weaned pigs was 26.76% (95% CI 24.26 - 29.36), the highest sero prevalence was 63.00% (95% CI 52.76 - 72.44). All 12 study provinces had pigs with sero positive.

• The sero prevalence of influenza in weaned pigs was 29.91% (95% CI 25.80 - 34.29), slightly higher than that of 24.73% (95% CI 21.63 - 28.03) recorded for pigs at fattening farms ($\chi^2$ test statistic = 2.2428, df = 1, p-value = 0.1342).

• The sero prevalence of influenza in sows was 58.80% (95% CI 52.30 - 65.10), much higher than that of fattening pigs ($\chi^2$ test statistic = 41.7478, df = 1, p-value < 0.0001).
The proportion (%) of serum samples of fattening pigs and weaned pigs that were positive with influenza type A virus stratified by breeding and fattening farms.
Results (5)

• All swab samples (n = 106) that were positive or suspect with influenza type A viruses were used for virus isolation and identification.

• A total of 6 samples in 5 provinces were positive with H1N2 and H3N2 viruses

• All positive samples were also sent to Macrogen lab (Korea) for sequencing.

• Phylogenetic analyses indicated that swine influenza viruses in Viet Nam belong to the North American strain.
Phylogenetic relationship of Influenza A virus subtype H3 isolated from mammalians (Based on HA1 sequences)
20/01/2013

- Swine Asia
- Swine America
- Swine Europe
- Human

SIV isolated in North Vietnam in EPT on 08 Jan 2013
SIV isolated in South Vietnam in 2010
Conclusion (1)

• High proportion of pigs with sero-conversion to SIVs in Viet Nam (26.76% sero-positive) indicates that many pigs exposed to SIVs which circulated previously on the pig farms.

• Lower proportion of pigs shedding the SIVs (6.9% RRT-PCR positive) would be explained that those pigs might get a new infection in the farm.

• The infected pigs would continue to shed the SIVs to others and pose a risk to infect humans, especially those in close contact with pigs.
Conclusion (2)

• H3N2 and H1N2 were found in 06 farms from 05 provinces would directly pose the risk to pigs and human that in contact within/ between farms

• Possible concurrent infection with seasonal influenza viruses or Highly Pathogenic Avian Influenza (H5N1, H5N6) may result in new virulent strains with human pandemic potential.

• Swine influenza virus monitoring should continue to timely detect emerging viruses with potential pandemic potential and prevent such events
Thank you for your attention