NOVEL DIAGNOSTICS FOR TRANSBOUNDARY ANIMAL DISEASES

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Animal diseases .... that are of significant economic, trade and /or food security importance for a considerable number of countries; which can easily spread to other countries and reach epidemic proportions; and where control/management, including exclusion, requires cooperation between several countries.
NUMBERS OF KNOWN PATHOGENS

- Humans
- Domestic livestock
- Domestic carnivores
- Wildlife

What diseases to report?

Priority Animal Diseases

1. Foot and Mouth Disease (FMD)
2. Highly Pathogenic Avian Influenza (HPAI)
3. Newcastle Disease (ND)
4. Hog Cholera or Classical Swine Fever (CSF)
5. Hemorrhagic Septicemia (Hemosep)
6. Rabies
7. Anthrax
8. Blackleg
9. Porcine Reproductive and Respiratory Syndrome (PRRS)
10. Caprine Arthritis Encephalitis (CAE)
11. Infectious Laryngotracheitis (ILT)
12. Surra
13. Fasciolosis

2019 AFRICAN SWINE FEVER
Fig. 1 Percentage distribution of production in agriculture

- **Livestock**: 16.2%
- **Fisheries**: 16.2%
- **Poultry**: 17.0%
- **Crops**: 50.6%
### Table 1. Chicken Inventory by Region Philippines: as of 1 January 2017-2019

<table>
<thead>
<tr>
<th>Region</th>
<th>Inventory (number of birds)</th>
<th>Growth Rate</th>
<th>Percent Share to 2019</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHILIPPINES</td>
<td>175,316,918</td>
<td>175,771,740</td>
<td>186,370,297</td>
</tr>
<tr>
<td>CAR</td>
<td>1,470,545</td>
<td>1,735,514</td>
<td>1,903,464</td>
</tr>
<tr>
<td>Ilocos Region</td>
<td>9,263,459</td>
<td>10,009,092</td>
<td>10,041,719</td>
</tr>
<tr>
<td>Cagayan Valley</td>
<td>7,349,890</td>
<td>6,181,378</td>
<td>6,838,770</td>
</tr>
<tr>
<td>Central Luzon</td>
<td>27,461,705</td>
<td>29,477,471</td>
<td>31,764,997</td>
</tr>
<tr>
<td>CALABARZON</td>
<td>26,856,204</td>
<td>26,120,647</td>
<td>26,047,000</td>
</tr>
<tr>
<td><strong>LAYER</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHILIPPINES</td>
<td>34,473,562</td>
<td>35,568,632</td>
<td>38,810,905</td>
</tr>
<tr>
<td>CAR</td>
<td>130,697</td>
<td>136,933</td>
<td>164,970</td>
</tr>
<tr>
<td>Ilocos Region</td>
<td>789,921</td>
<td>823,178</td>
<td>890,305</td>
</tr>
<tr>
<td>Cagayan Valley</td>
<td>656,043</td>
<td>623,953</td>
<td>751,844</td>
</tr>
<tr>
<td>Central Luzon</td>
<td>7,570,899</td>
<td>7,256,756</td>
<td>7,694,462</td>
</tr>
<tr>
<td>CALABARZON</td>
<td>12,614,124</td>
<td>13,127,864</td>
<td>13,982,488</td>
</tr>
</tbody>
</table>
AFRICAN SWINE FEVER ENTRY

International Flights

Food Wastes

Hotels and Restaurants

RIZAL

Landfill
Swill traders
Backyard farmers

ASF Outbreak

Pig/Pork traders

BULACAN

Pig/Pork traders
Stockyard
Backyard farmers

ASF Outbreak

Pig/Pork traders

QUEZON CITY

Landfill
Swill traders
Backyard farmers

ASF Outbreak

Pig/Pork traders

Waterways

Smuggled Pork OR "Co-mingled" imports
### Table 2. Inventory of Swine by Farm Type as of 01 January 2018-2020

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Philippines</td>
<td>12,604,441</td>
<td>12,709,248</td>
<td>12,795,721</td>
<td>0.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Backyard</td>
<td>8,092,940</td>
<td>8,167,864</td>
<td>7,971,400</td>
<td>0.9</td>
<td>-2.4</td>
</tr>
<tr>
<td>Commercial</td>
<td>4,511,501</td>
<td>4,541,384</td>
<td>4,824,321</td>
<td>0.7</td>
<td>6.2</td>
</tr>
</tbody>
</table>

### Table 3. Volume of Hog Production: October - December 2017-2019

<table>
<thead>
<tr>
<th></th>
<th>Production (metric ton, liveweight)</th>
<th>Percent Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Philippines</td>
<td>649,678</td>
<td>662,732</td>
</tr>
</tbody>
</table>
**FIGURE 1** Volume of Hog Production
Philippines: October - December 2017-2019

<table>
<thead>
<tr>
<th>Year</th>
<th>'000 mt, liveweight</th>
<th>Production</th>
<th>Growth Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>649.68</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>2018</td>
<td>662.73</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>2019</td>
<td>597.51</td>
<td>(9.8)</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3. Changes in Swine Inventory by Farm Type**
Philippines: as of 01 January 2018-2020

<table>
<thead>
<tr>
<th>Farm Type</th>
<th>2019/2018</th>
<th>2020/2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Backyard</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Commercial</td>
<td>0.9</td>
<td>6.2</td>
</tr>
</tbody>
</table>

(2.4)
EXTERNAL AGENT

Adaptation to new environmental conditions.

• Intrinsic properties of the pathogens that are associated with this are:
  a. genetic shift as a result of mutation & reassortment, as it occurs in avian influenza virus;
  b. conjugation, transformation, and transduction in bacteria.
  c. adaptation to new vectors and hosts
  d. development of increased virulence or drug resistance
Emerging Infectious Disease Events
1940 to 2012

- 335 events in 72 years (1 every 4-5 years)
- 216 zoonotic events (64.5%)
Earthquakes, volcanic eruptions, tropical cyclones and floods
20 tropical cyclones (TCs) enter PAR/year
Nine TCs make landfall.
Human Population Dynamics

- **2014**: 7.25 Billion people
  - Urban
  - Rural
- **2050**: ~ 9 Billion people
- **2050 Distribution**:
  - Developing Countries: 82.5%
  - Developed Countries: 17.5%
Animal density and biomass.

The optimal density of animals in a specific location is vital to animal health.

Overcrowding or high densities pose high risks and allow faster transfer of parasites and pests.
ENTRY POINTS FOR TADS

85 airports
10 of these are international gateways

429 fishing ports and
821 commercial ports
The New “Super-Vectors”
HOST: Capacity to respond to infectious attack

1. Susceptibility.
The ability to acquire a pathogen and to show a pathological status.

2. Tolerance.
The relative capacity to control the development of a pathogen and to limit its pathological effects.

3. Resistance.
The ability to reduce the growth rate, fecundity, and persistence of a population of pathogens.

4. Resilience.
The ability to grow and be productive despite the presence of a normal pathogen charge.

5. Refractoriness.
The impossibility to acquire an infection because the biological support inhibits the multiplication of the pathogen.
“Surveillance serves as the brain and nervous system for programs to prevent and control disease.”

Henderson, 1976
Components of an effective disease surveillance system.
Evolution of viral detection techniques

- **Molecular**
  - Polymerase chain reaction
  - Dot hybridization
  - Gene chips
  - Nucleic acid sequence-based amplification
  - Loop-mediated isothermal amplification

- **Serological**
  - Enzyme immunoassay
  - Immunofluorescence assay
  - Chemiluminescent immunoassay
  - Radioimmunoassay
  - Immunostaining
  - Hemagglutination-inhibition
  - Immunoblotting assays
  - Complement-fixation
  - Particle agglutination

- **Direct**
  - Cell culture
  - Electron microscopy

Timeline:
- 1910
- 1930
- 1950
- 1970
- 1980
- 1990
- 2000
- 2010
Project Concept

Industry Problem

High mortality and losses due to insufficient disease surveillance & diagnostic systems and services

Intervention

Improved Investigation, Diagnosis and Technical Support for the Control of Animal Diseases in the Philippines

Envisioned Need

- A diagnostic test that minimizes delay between sampling and release of test results
- A disease monitoring and surveillance system even in laboratories with limited resources.
OBJECTIVES OF THE PROJECTS:

1. To **develop and produce laboratory diagnostic kits** for exotic and economically important diseases.

2. To **conduct capacity building activities** for government and private veterinarians, veterinary students and industry stakeholder.

3. To **expose the students to the dynamic animal health management practices and laboratory disease diagnosis** where they could derive practical and relevant experiences on the different activities.
WHO ASSURED GUIDELINES FOR AN IDEAL DIAGNOSTIC TEST

<table>
<thead>
<tr>
<th>Assured</th>
<th>RT-PCR</th>
<th>RT-LAMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affordable</td>
<td>X</td>
<td>/</td>
</tr>
<tr>
<td>Sensitive</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Specific</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>User-friendly</td>
<td>/</td>
<td>/</td>
</tr>
<tr>
<td>Robust and rapid</td>
<td>X</td>
<td>/</td>
</tr>
<tr>
<td>Equipment-free</td>
<td>X</td>
<td>/</td>
</tr>
<tr>
<td>Deliverable to the end user</td>
<td>/</td>
<td>/</td>
</tr>
</tbody>
</table>
RNA EXTRACTION

- Whole blood/tissue/semen = 30 minutes
- Fecal sample = 1 minute

Inoculation of RNA extract into RT-LAMP mixture = 1 minute

Amplification reaction = 30 minutes

Reading of results = 1 minute
REVERSE TRANSCRIPTION - LOOP MEDIATED ISOTHERMAL AMPLIFICATION

WET and DRY FORMATS
WHAT is LAMP?

**Background and Significance**

- **Loop mediated-isothermal amplification**
  - Nucleic acid amplification method
  - Relies on auto-cycling strand displacement DNA synthesis
- **2 phases:**
  - Initial phase
  - LAMP cycling or cyclic amplification
Background and Significance

• **RT-LAMP**
  • a fast, easy, simple, cheap, highly sensitive and efficient diagnostic tool
  • Has potential application in field or bedside diagnosis of diseases
  • **Recommended by the OIE as an alternative technique to RT-PCR**
Dry RT-LAMP

RT-LAMP master mixture components are instilled onto the inner surface of the PCR tube cap for 1 to 2 hours drying before storage until use.
VISUAL ASSESSMENT OF RESULTS

Wet format RT-LAMP – positive = green
  negative = brown to orange
Dry format RT-LAMP – positive = light blue
  negative = dark blue, dark violet or purple

Hydroxy Naphtol Blue (HNB) Salt, dye incorporated in the LAMP premix.

DNA/RNA amplification ➔ dNTPs used up ➔ chelation of Mg²⁺ ions ➔ HNB turns dark blue to light blue (positive)
Vaccine shedding is a term used for the rare release of virus following administration of a live-virus vaccine.

- It’s a mechanism for virus transmission.

- Live attenuated vaccine virus can theoretically infect naïve flocks through viral shedding

- The route of infection = contact with feces, ocular & nasal mucus

- Vaccine virus shedding in stool occurs for up to 28 days.

- Shedding is impossible with killed vaccines or those made using only isolated proteins (recombinase)
The vaccine **genetically and antigenically similar** to the field or wild type strain reduced oral shedding significantly as compared to mismatched strains. Thus, **genotype-matched vaccine** has potential to result in better protection by **limiting the viral shedding**.
Develop a test kit that can differentiate infected from vaccinated animals (DIVA) as a powerful tool for accurate monitoring & surveillance of animal diseases.
INSTRUCTION GUIDE FOR CSF/PRRS RT-LAMP WITH DIVA COMPETENCE

SAMPLES – whole blood, semen, lymphoid organs (tonsils, lungs, spleen)
Analytical specificity of dry format RT-LAMP with DIVA primers. V1- HOVAC, V2 – PESTIVAC, V3 – COGLAPEST, V4 – UNISTRAIN, S1 and S2 are blood samples from Bukidnon. All vaccines (V1-4) were negative. Samples (S1 & S2) were positive for CSF virus.
Analytical specificity test of dry RT-LAMP with DIVA primers. Templates used were as follows: **V1**- Pestivac, **V2**- Coglapest **V3**- Unistrain, **V4**- Ingelvac and **V5**- PED vaccine; **S1-S3**- RNA blood extracts. Only S1 is positive.
INSTRUCTION GUIDE FOR NDV RT-LAMP WITH DIVA COMPETENCE

SAMPLES – cloacal swab, oropharyngeal swab
Analytical Specificity

- field sample
- field sample
- LaSota vaccine
- ILT vaccine
- IB vaccine

POSITIVE POSITIVE
AFRICAN SWINE FEVER
RPA PRODUCTS FROM RE-AMPLIFIED PCR PRODUCT

CLSU-CVSM own primer design based on P72 target gene. (Aug 3, 2018 - China isolate GenBank accession No. MH722351)
TRIAL of RPA protocol using DNA extract from confiscated meat

3/20/2019 at BAI, ADDRLS

Ambient temp (betw 38-43°C) for 20 minutes

Random DNA samples from confiscated products

TRIAL – looking for a positive control from the DNA extracts of confiscated meat
Interpretation of amplified RPA results using Milenia HybriDetect dip sticks-universal test strip.
Concept of the ASFv nanogold biosensor test kit.

RNA/DNA + Premix (primers tagged with modified thiol probes) → Amplification → Gold nanoparticles

- match
- mismatch

Red = negative
Blue = positive
NANODIAGNOSTICS using gold NPs

negative  pos  pos  pos
Let’s promote and patronize our own research technology products with primers designed based on our own Pinoy strain.

TO GOD BE THE GLORY

Maayong hapon kaninyong tanan.