RESEARCH FINDINGS ON ANTIMICROBIAL RESIDUE AND RESISTANCE IN VIETNAM

Present by: Truong Thi Quy Duong, DVM, Msc
Veterinary Hygiene Department,
National Institute of Veterinary Research (Vietnam)
OUTLINE

• Introduction
• Hub programmes
• Biological sampling design
• Antimicrobial residues
• Antimicrobial resistance
INTRODUCTION

• Funded by the UK Research and Innovation through Global Challenge Research Fund

• Lead Organization: Royal Veterinary College

• 27 partners in 10 countries

• Aim: to achieve sustainable intensification of chicken meat and egg production, whilst reducing risks to human and animal health and welfare

• Target health risks: AIV, FBP, and AMR

• Study sites: Bangladesh, India, Sri Lanka, Vietnam

• Study period: March 2019 – February 2024
HUB PROGRAMMES AND PARTNERS IN VIETNAM

• Map chicken networks; Assess chicken flow and dynamics; Investigate roles, relationships, behaviors and practices that have epidemiological significances.
  - VNUA, NIAS

• Learning for best practice, bespoke training, dissemination, stakeholder/policy/community engagement
  - CIRAD and partners

P1: People, Poultry, Production

P2: Host-pathogen Dynamics

P3: Flexible Fund

P4: Translation to Impact

• Characterise the evolution and transmission dynamics of health risks spreading through the chicken networks
  - NIAS, NIVR, NIHE

• Staff exchange, fellowships, pump-priming, interventions
  - NIVR, NIHE
Mapping chicken production and distribution networks

**Coloured chicken broiler, white chicken broiler, layer**

A risk assessment based on the likelihood of introduction, transmission, and evolution of zoonotic health risks (e.g., AIV, foodborne pathogens) was carried out by local and international researchers.

Coloured chicken and white chicken broiler networks were selected for further studies.

Sociological and ethnographic studies were implemented to investigate behaviours and practices of actors in the chicken networks that can increase the health risks.
P2: HOST-PATHOGEN DYNAMICS

- **Link tracing study**: identifying chicken movement along the chicken broiler networks to define the sampling frame.

  - To ensure sampled chickens belonging to the study networks

- **Biological sampling (first round)**: March to December 2021 (1700 chickens in 102 sampling sites)

<table>
<thead>
<tr>
<th>Province</th>
<th>Market</th>
<th>Slaughtering facilities</th>
<th>Farm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bac Giang</td>
<td>7</td>
<td>8</td>
<td>14</td>
<td>29</td>
</tr>
<tr>
<td>Hanoi</td>
<td>11</td>
<td>8</td>
<td>23</td>
<td>42</td>
</tr>
<tr>
<td>Hai Duong</td>
<td>5</td>
<td>5</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Quang Ninh</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>27</td>
<td>25</td>
<td>50</td>
<td>102</td>
</tr>
</tbody>
</table>
SAMPLE COLLECTION

• Random selection of sampling sites (farms, markets, slaughter facilities) from the list provided by local veterinarians

• Randomly select clinically healthy chicken at the end of production cycle

• Sampled both colour and white chickens on the same sampling site if any
**SAMPLE TYPES**

**Whole blood**
- Serum
- FTA card

**Breast tissue**

**Wing feather**

**Caecal pounches**
- Caecal content
- Caecal swab

**Intestine tissue**

**Environment sample (boot swab)**

**Oropharyngeal swab, Cloacal swab**

** stata**
- RNAprotect Bacteria reagent
- Preston broth
- BHI broth
ANTIMICROBIAL RESIDUES IN CHICKEN MEAT

- 110 chicken meat samples tested for 70 different antimicrobials (1 bird from each sampling site)
  - 41% from farms, 59% from endpoints (markets, slaughtering facilities)
  - 87% from coloured chicken broilers, 13% from white chicken broilers

- 91 of the 110 (82%) had at least 1 residue detected at any level

- Proportion of chicken broiler with residues ≥1 Maximum Residue Level (MRL): 12% (95% confident interval: 7-19%)
  - 1 in category B (Norfloxacin)
  - 3 in category C (Tilmicosin, Florfenicol, Azithromycin)
  - 5 in category D (3 sulphonamides and 2 tetracyclines)

- Bird type and sampling site type were not found to be associated with the prevalence
ANTIMICROBIAL RESIDUES IN CHICKEN FEATHERS

• 111 feather samples tested:
  • Broiler farm (n=49)
  • Slaughterhouses (n=33)
  • Market (n=29)

• Highest level of sulfonamides across all sampling sites

SLF: Sulfonamides
TET: Tetracyclines
QN: Quinolones
BL: Beta-Lactams
ANTIMICROBIAL RESIDUES IN CHICKEN FEATHER (CONT.)

• 51 feather samples tested:
  • Broiler farm (n=29)
  • Slaughterhouse (n=15)
  • Market (n=7)

• SPC (Spectinomycin) KAN (Kanamycin) NEO (neomycin) STP (streptomycin)

• Greater level of residues in the start point (farms) than endpoints (market, slaughterhouse).

• No STP detected across all samples
TESTING FOR ANTIMICROBIAL RESISTANCE (AMR) – CAECAL SWAB

• 92 isolates completed with whole genome sequencing, AMR genes were found as follows:
  • 90: Tetracycline (TetO 66; TetL 24)
  • 85 :Fluoroquinolone ciprofloxacin; 81/85 strains have Cipro resistance mutation
  • 83: Beta-lactam (blaOXA)
  • Aminoglycoside resistant gens, including APH 3-IIIa: 55; APH2-Iff: 32; aad6: 7; and AAC6: 29
  • 10: Streptothricin (SAT)
  • 36: Chloramphenicol (cat)
  • Multi-drug resistant gens: cmeA 80/92, cmeB 71/92, cmeC 92/92; cmeR 34, e.g resistant to cephalosporin, macrolide-antibiotic, fusidance antibiotic, and fluoroquinolone antibiotic.

• High level of antimicrobial resistance in all isolates
TESTING FOR ANTIMICROBIAL RESISTANCE (AMR) – CAECAL CONTENT

- 114 caecal content samples were extracted and sequenced to identify bacteria genes.
- Targeted PCR and sequencing of fragments of genes associated with resistance to antimicrobials (>300 AMR genes) to identify the presence or absence of AMR genes.
- Subsequent analysis to pool genes that resist antimicrobials and class of antimicrobials.
## Antimicrobial Resistance Results

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Class</th>
<th>Ameg</th>
<th>Proportion of samples contained AMR genes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMIKACIN</td>
<td>AMINOGLYCOSIDE</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>APRAMYCIN</td>
<td>AMINOGLYCOSIDE</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>TOBRAMYCIN</td>
<td>AMINOGLYCOSIDE</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>GENTAMICIN</td>
<td>AMINOGLYCOSIDE</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>HYGROMYCIN</td>
<td>AMINOGLYCOSIDE</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>KANAMYCIN</td>
<td>AMINOGLYCOSIDE</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>SPECTINOMYCIN</td>
<td>AMINOGLYCOSIDE</td>
<td>D</td>
<td>100</td>
</tr>
<tr>
<td>STREPTOMYCIN</td>
<td>AMINOGLYCOSIDE</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>CARBAPENEM</td>
<td>BETA-LACTAM</td>
<td>A</td>
<td>5.3</td>
</tr>
<tr>
<td>CEPHALOSPORIN</td>
<td>BETA-LACTAM</td>
<td>unk</td>
<td>100</td>
</tr>
<tr>
<td>METHICILLIN</td>
<td>BETA-LACTAM</td>
<td>unk</td>
<td>100</td>
</tr>
<tr>
<td>CHLORAMPHENICOL</td>
<td>PHENICOL</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>ERYTHROMYCIN</td>
<td>MACROLIDE</td>
<td>C</td>
<td>100</td>
</tr>
<tr>
<td>STREPTOTHRICIN</td>
<td>STREPTOTHRICIN</td>
<td>unk</td>
<td>100</td>
</tr>
<tr>
<td>FLORFENICOL</td>
<td>PHENICOL</td>
<td>C</td>
<td>99</td>
</tr>
<tr>
<td>TRIMETHOPRIM</td>
<td>TRIMETHOPRIM</td>
<td>D</td>
<td>99</td>
</tr>
<tr>
<td>COLISTIN</td>
<td>COLISTIN</td>
<td>B</td>
<td>18</td>
</tr>
<tr>
<td>VANCOMYCIN</td>
<td>GLYCOPEPTIDE</td>
<td>A</td>
<td>8</td>
</tr>
</tbody>
</table>
AMR BETWEEN SAMPLING SITES

- Class-level resistance
  - No evidence for difference between farms, markets, and slaughtering facilities
  - Resistance to **glycopeptides** in white chicken broilers is much higher than coloured chicken broilers (p=0.003)

- Drug-level resistance
  - No evidence for difference between farms, markets, and slaughtering facilities
  - Resistance to **colistin (AMEG class B)** and **vancomycin (glycopeptide, AMEG class A)** in white chicken are higher than coloured chicken broilers. (p=0.003)
## Antimicrobial Resistance vs. Residues in Chicken Meat (Any Level)

<table>
<thead>
<tr>
<th>Class</th>
<th>AMR+ residue+</th>
<th>AMR- residue-</th>
<th>AMR+ residue-</th>
<th>AMR- residue+</th>
</tr>
</thead>
<tbody>
<tr>
<td>BETA.LACTAM</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>79 (100%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>MACROLIDE</td>
<td>18 (23%)</td>
<td>0 (0%)</td>
<td>61 (77%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>PHENICOL</td>
<td>2 (3%)</td>
<td>0 (0%)</td>
<td>77 (97%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>QUINOLONE</td>
<td>11 (14%)</td>
<td>17 (22%)</td>
<td>49 (62%)</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>SULFONAMIDE</td>
<td>21 (27%)</td>
<td>18 (23%)</td>
<td>29 (37%)</td>
<td>11 (14%)</td>
</tr>
<tr>
<td>TETRACYCLINE</td>
<td>46 (58%)</td>
<td>0 (0%)</td>
<td>33 (42%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
## Antimicrobial Resistance vs. Residues in Chicken Feathers

<table>
<thead>
<tr>
<th>Class</th>
<th>AMR+ residue+</th>
<th>AMR- residue-</th>
<th>AMR+ residue-</th>
<th>AMR- residue+</th>
</tr>
</thead>
<tbody>
<tr>
<td>BETA.LACTAM</td>
<td>13 (13%)</td>
<td>0 (0%)</td>
<td>84 (87%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>QUINOLONE</td>
<td>42 (43%)</td>
<td>15 (15%)</td>
<td>28 (29%)</td>
<td>12 (12%)</td>
</tr>
<tr>
<td>SULFONAMIDE</td>
<td>38 (39%)</td>
<td>8 (8%)</td>
<td>17 (18%)</td>
<td>34 (35%)</td>
</tr>
<tr>
<td>TETRACYCLINE</td>
<td>42 (43%)</td>
<td>0 (0%)</td>
<td>55 (57%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>
CONCLUSIONS

• Proportion of antimicrobial residues ≥ MRL in chicken meat ranges from 7% to 19% with 9 compounds detected

• Microbiomes in chicken broilers resist most antimicrobial classes

• There is a difference in antimicrobial class-level and drug-level resistance between white chicken broilers and coloured chicken broilers

• Ongoing analysis in comparing resistances to residues in chicken meat and feathers
RECOMMENDATIONS

• Encourage poultry farmers to use antimicrobial alternatives and strengthen biosecurity measures.

• Recommend farmers choose antibiotics with low rates of resistance to treat diseases.

• Enhance the management and control of the purchase, sale, and use of antibiotics in poultry production (including withdrawal period)
THANK YOU VERY MUCH FOR YOUR ATTENTION