The epidemiology of hepatitis E virus and the relationship between infection in pigs and human in a community of agricultural-food system in Thailand

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Outlines

1. Introduction HEV
2. Methodology
3. Results
4. Discussions
Geographic Distribution of Hepatitis E

Outbreaks or confirmed infection in > 25% of sporadic non-ABC hepatitis

Credit: CDC
Table 1: Types of acute viral hepatitis in patients in 2008, Thailand

<table>
<thead>
<tr>
<th>Type</th>
<th>No. of cases</th>
<th>Proportion (%)</th>
<th>Incidence per 100,000 pop.</th>
<th>Case fatality rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>515</td>
<td>4.91</td>
<td>0.81</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>5304</td>
<td>50.53</td>
<td>8.39</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>1357</td>
<td>12.93</td>
<td>2.15</td>
<td>0.07</td>
</tr>
<tr>
<td>D</td>
<td>113</td>
<td>1.08</td>
<td>0.18</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>30</td>
<td>0.29</td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>Unspecified</td>
<td>3177</td>
<td>30.27</td>
<td>5.03</td>
<td>0.16</td>
</tr>
</tbody>
</table>

True?

What?

- A case is compatible with the clinical description and/or has a general laboratory of increasing ALT >100 U/L, but no laboratory confirmed for etiological diagnosis

- A limitation of a few available laboratories to identify HEV
Genotypes 3 & 4 infect both humans and pigs, whereas genotype 1 & 2 is restricted to humans.

HEV genotype 3 RNA detected in:
1. Domestic pig 25-30%
2. Wild boar 5-10%
3. Deer
4. Mungo
5. Mussel
6. Birds
Survival and persistence of the pathogens HEV

1. Ingestion of HEV
2. Colonization of intestinal epithelial cells
3. Entry to bloodstream with macrophages
4. Liver

- Feces
- Water
- Plants
Conceptual framework of zoonotic HEV infection in the community of agricultural system
Specific aim

Specific Aim 1: To assess the point prevalence and incidence of HEV infection in pigs and human and to hypothesize the risk factors for this infection

STUDY 1.1: A cross sectional study of HEV infection in pigs distributed in different farm sizes

STUDY 1.2: A cross sectional study of HEV infection in human distributed in different occupations

Specific Aim 2: To identify the risk factors of HEV infection in human and determine the phylogenetic relationship between concurrent isolates from pigs and humans in the same community of agricultural-food system

STUDY 2: A prospective cohort study in an area of integrated pig and human agricultural-food system
Sub-study 1.1 A cross sectional study of HEV infection in pigs distributed in different farm sizes

1. Pig farms in Nan province
2. Stratified by farm sizes (large, medium, small)
3. Proportional to size cluster sampling the farms
4. Sampled farms
5. Simple random sampling pigs from each farm

Study Population
Sub study 1.1: Data measurements (Exposure)

Primary exposure is “Farm sizes” in 3 categorical variables (large > 300 pigs, medium ≥30X≤300, small < 30)

→ Assessing information from the registration of pig farms from Nan Provincial Livestock Health Office

Potential confounders (By asking information from pig owners)

→ Sex
→ Number of stable units
→ Stables hygiene level
→ Source of water
→ Type of production
→ Type of feeding system
→ Evidence of rodent infestation
→ Sources of purchased pigs
Sub study 1.2 A cross sectional study of HEV infection in human distributed in different occupation

Study Population

Exposed group
- Pig farmers who work with pigs

Unexposed group
- No history of working in pig farms or relate to pigs
- Sampled from population lists of all residents

Direct contact to human
- Pig farmers

HEV seroprevalence

The association between pig farmers and HEV infection

• Pig farmers who are exposed

• No history of working in pig farms or relate to pigs

• Sampled from population lists of all residents
Primary exposure is pig exposure (Assessed by the questionnaire)
- Ask questions at the enrollment: “Have you worked in pig production?” If he says “never”, it will be classified as unexposed
- If say “yes” or “ever”, then job characteristics (years in pig production, days per week working with pigs, handling waste from pig farms, use of personal protective equipment, farm size, and type of pig productions)

Potential confounders (Assessed by the questionnaire)
→ Water - food exposure: how often of eating raw or undercooked pork, drinking untreated water and cooking preferences
→ Socio demographic characteristics → Travel
→ Recreational activity
→ Illness
→ Smoking habits
→ Educational level
→ Alcohol consumption
→ Contact with animals
→ BMI
→ History of chronic viral hepatitis
Sub study 1.2

Pig owners: 94 farms (875 pigs)
- Made an appointment
- Informed consent

Interview

Nasal swab: Flu test

Blood sample: Brucellosis test

Blood sample: JE test

Blood sample: HEV-Ig test

Fecal sample: RT-PCR HEV test

Fecal sample: Parasites

Blood sample: Flu test
Sub study 1.1 Data measurements (Outcome)

1. Sampled:
   - Pig farmers (171)
   - General pop. (342)

2. Make appointment:
   - To be collected blood
   - To be interviewed

3. Informed consent

4. Study team: collect blood and interview
   - ELISA Ig test
     - If have acute sign, then ALT test and acute hepatitis A, B, C test
     - ELISA IgM test
       - If +, then RT-PCR
         - If +ALT, then RT-PCR test
         - If +, then sequencing
       - + result or - result
Results

Figure 1. Six districts and average seroprevalence under different farm sizes

Figure 2. Six districts and average seroprevalence in exposed and unexposed to pig farms
Results in pigs

- Overall prevalence of IgG anti-HEV was 9.9% (87/879)
- 29 herds from 94 tested were positive for HEV antibody (30.9% herd prevalence).
- The prevalence:
  - Pua = 11.4% (26/229)
  - Tha Wang Pa = 8.3% (15/180)
  - Ban Luang = 7.5% (3/40)
  - Mueang Nan = 18.6% (13/70)
  - Wiang Sa = 6.3% (17/270)
  - Phu Phiang = 14.4% (13/90)
Results in pigs

Positive for HEV RNA
- Overall was 25 (2.9%) from 875 stool samples
- 2 paired samples showed Genotype 3
- 11 (20.0%) from 55 stool samples of 2-month-old pig
- 7 (5.1%) from 137 stool samples of 3-month-old pig
- 6 (3.3%) from 184 stool samples of 3-month-old pig
- All pigs of ≥ 5-month-old were negative

Districts
- Pua 4.8% (11/229)
- Tha Wang Pa 0% (0/180)
- Ban Luang 2.5% (1/40)
- Mueang Nan 1.4% (1/70)
- Wiang Sa 4.4% (12/270)
- Phu Phiang 0% (0/86)

Farm size
- 12 (3.8%) of 313 from the large farms
- 7 (2.6%) of 269 from the medium farms
- 6 (2.0%) of 293 from the small farms
Multivariate logistic regression analysis of factors associated with HEV infection in pigs under the **medium farm size versus the large farm size** in Nan, Thailand 2010-2011

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted odds ratio (95%CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd size with 30 – 300 pigs</td>
<td>4.946 (1.786-13.696)</td>
<td>0.002</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 3 - ≤ 6</td>
<td>1.465 (0.517 – 4.156)</td>
<td>0.473</td>
</tr>
<tr>
<td>&gt; 6</td>
<td>1.766 (0.822-3.795)</td>
<td>0.145</td>
</tr>
<tr>
<td>Industrial and agricultural by-products (Yes vs. No)</td>
<td>0.156 (0.062-0.390)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Water treated before use in a farm (Yes vs. No)</td>
<td>2.916 (0.762-11.163)</td>
<td>0.118</td>
</tr>
<tr>
<td>Veterinarian provided to pigs in farm (Yes vs. No)</td>
<td>0.120 (0.042-0.341)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Presence of other pig farm(s) (Yes vs. No)</td>
<td>0.256 (0.099-0.663)</td>
<td>0.005</td>
</tr>
<tr>
<td>A rodent control program in farm (Yes vs. No)</td>
<td>0.969 (0.410-2.288)</td>
<td>0.942</td>
</tr>
</tbody>
</table>

** Adjusted for age, feeding practice, water treatment, veterinarian provided to pigs in farm, presence of other pig farm(s) and a rodent control program in place
Multivariate logistic regression analysis of factors associated with HEV infection in pigs under the small farm size versus the large farm size in Nan, Thailand 2010-2011

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted odds ratio (95%CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd size less 30 pigs</td>
<td>0.914 (0.291-2.870)</td>
<td>0.878</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt; 3 - ≤ 6</td>
<td>0.850 (0.233-3.098)</td>
<td>0.806</td>
</tr>
<tr>
<td>&gt; 6</td>
<td>2.332 (0.929-5.855)</td>
<td>0.072</td>
</tr>
<tr>
<td>Industrial and agricultural by-products (Yes vs. No)</td>
<td>0.545 (0.187-1.586)</td>
<td>0.265</td>
</tr>
<tr>
<td>Water treated before use in a farm (Yes vs. No)</td>
<td>2.695 (0.653 – 11.119)</td>
<td>0.171</td>
</tr>
<tr>
<td>Veterinarian provided to pigs in farm (Yes vs. No)</td>
<td>0.195 (0.042-0.912)</td>
<td>0.038</td>
</tr>
<tr>
<td>Presence of other pig farm (s) (Yes vs. No)</td>
<td>0.740 (0.284-1.926)</td>
<td>0.537</td>
</tr>
<tr>
<td>A rodent control program in farm (Yes vs. No)</td>
<td>0.345 (0.071-1.684)</td>
<td>0.942</td>
</tr>
</tbody>
</table>

** Adjusted for age, feeding practice, water treatment, clean floor with disinfectant, management of manure, veterinarian provided to pigs in farm, presence of other pig farm(s), presence of wild birds or feces of birds inside the pig house/pen and rodent control program in place **
Results in human

- **Overall prevalence was 23.0% (118/513)**
- **Districts**
  - Pua = 17.9% (32/179)
  - Tha Wang Pa = 33.5% (61/182)
  - Ban Luang = 9.4% (5/53)
  - Mueang Nan = 24.1% (13/54)
  - Wiang Sa = 23.1% (6/26)
  - Phu Phiang = 5.3% (1/19)
- **Tested positive for IgG anti-HEV**
  - 39 (22.8%) of the 171 serum samples of the exposed group
  - 79 (23.1%) of the 342 serum samples of the unexposed to pig groups
Multivariate logistic regression analysis of healthy people distributed in exposed and unexposed to pig groups factors associated with positive HEV infection in a community of agricultural-food system in Nan, Thailand 2011

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted odds ratio (95%CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed vs. unexposed to pig groups</td>
<td>0.808 (0.490 – 1.330)</td>
<td>0.402</td>
</tr>
<tr>
<td>Age ≥ 65 vs. 15 – 24 years old</td>
<td>3.395 (0.838 – 13.757)</td>
<td>0.087</td>
</tr>
<tr>
<td>Wash hands with water and soap vs. water only</td>
<td>0.520 (0.305 – 0.884)</td>
<td>0.016</td>
</tr>
<tr>
<td>Household flooded in the rainy season vs. not flooded</td>
<td>1.640 (1.004 – 2.679)</td>
<td>0.048</td>
</tr>
<tr>
<td>Consuming internal pig organs &gt; 2 times per week vs. never</td>
<td>3.225 (1.154 – 9.008)</td>
<td>0.026</td>
</tr>
</tbody>
</table>

*adjusted for age, hand washing, flooding, consuming internal pig organs and drinking alcoholic*
Discussion

Overall positive for HEV RNA: 2.9% (25/875) pig stool samples, Genotype 3

Overall prevalence in pigs: 23.0% (118/513) pig blood samples

Exposed vs. unexposed groups:
Adjusted OR = 0.808 (0.490 – 1.330)

Consuming internal pig organs > 2 times per week vs. never
Adjusted OR = 3.225 (1.154 – 9.008)

It is likely that a route of HEV transmission in Nan was an indirect contact as Food-borne zoonosis

It seems this food borne transmission play more important role than the direct contact with the pigs
Conclusion

Thank you

No pork meat disease can be a wonderful “Larb”!!!