AVIAN FORUM ASIA

STRENGTHENING POULTRY HEALTH: UNDERSTANDING THE PILLARS FOR BETTER PERFORMANCE

TOKYO, JAPAN

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WIL J. M. LANDMAN
Escherichia coli peritonitis syndrome in laying chickens in the Netherlands: clinical and pathological features, incidence and economic impact, and molecular typing of Avian Pathogenic E. coli involved

W.J.M. Landman¹, G.J. Buter¹, R. Dijkman¹ & J.H.H. van Eck²

¹GD - Animal Health Service, Deventer, the Netherlands
²Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands
Diagnosis

• Clinical signs & postmortem examination
• Bacteriological analysis required
  – Blood
  – Organs
  – Bone marrow
• Serotyping
• Molecular techniques
  – Virotyping? (Multiplex PCR)
  – Molecular epidemiology (PFGE)
Bacteriology

• Scheep blood agar 37 °C overnight

• Kovac’s indole test
  – Red +
  – Unchanged –

• β-glucuronidase test (β-GUA)
  – Physiological salt solution 0.25 ml + β-GUA
    • Waterbath 37 °C 4 h
  – Yellow +
  – White –
V-factors

• Molecules expressed & secreted by pathogens enabling
  – Colonization (adhesins)
  – Immunoevasion
  – Immunosuppression
  – Entry & exit of cells
  – Obtain nutrition from host
Classical typing vs V-factors

• Sero- & biochemical typing
  – APEC & commensal undistinguishable

• V-factors are no unequivocal markers for pathogenicity
  – Combination V-factors are indicative
Colibacillosis

• Localized or generalized infection
• Avian Pathogenic *Escherichia coli* (APEC)
• Secondary?
  – Age?

• Mamals ⇄ Enteric
Localized forms

- Coliform omphalitis/yolk sac infection
- Coliform cellulitis
- Swollen head syndrome
- Diarrheal disease
- Acute vaginitis
- **Salpingitis/peritonitis/salpingoperitonitis (SPS)**
  - Egg peritonitis
Intravaginally inoculated
Generalized forms

• Coligranuloma
• Colisepticemia
  – Respiratory-origin colisepticemia (polyserositis)
  – Acute septicemia laying hens (*E. coli* peritonitis syndrome)
• Colisepticemia sequelae
  – Meningitis/encephalitis
  – Panophthalmitis
  – Osteomyelitis/spondylitis/arthritis/polyarthritis/synovitis
Colibacillosis broilers

- Fibrinous polyserositis
  - Generalized
Colibacillosis laying hens

• Salpingitis/peritonitis/salpingoperitonitis (SPS)
  – Egg peritonitis
  – Localized

• EPS (*E. coli* peritonitis syndrome)
  – Generalized
Clinical & pathological features
Coliform SPS (egg peritonitis)

- Incidence low
- Chronic
- Part ‘normal’ mortality
- Oviduct distended, thin-walled & with exudate
  - Spread *E. coli* into abdomen induces concurrent peritonitis (salpingoperitonitis)
• >mid-1990s incidence & severity *E. coli* peritonitis flocks laying hens increased
  – USA & many European countries
• Start egg production onwards
• Mortality increased (up to ≥10%)
• Severe septicaemia & polyserositis lesions
  – Peritonitis! (low incidence salpingitis)
• Flock & house associations
Peracute EPS
Acute EPS
Chronic EPS
Control no EPS
Pathogenesis EPS obscure

- Publications scarce
  - Gross & Siegel, 1959
  - Zanella et al., 2000
  - Raviv et al., 2007
Induction of EPS

• 5 experiments (23- & 33-week-old)
  – Intravenous
  – Intraperitoneal
  – Intratracheal
  – Aerosol
  – Intravaginal
  – Oral
• Brown layers n = 7 – 14 per route
• Pm, bacteriology & PFGE (2-4 wks p.i.)
Inocula

- Volume inocula 1 ml per hen
- Aerosol exposure 100 ml 1:2 in diluted BPW per isolator
  - Exposure time was 30 min
- 25 ml bacteriological sterile egg yolk was injected intraperitoneally
## Induction of EPS

<table>
<thead>
<tr>
<th>Inoculation route</th>
<th>E. coli dose (10^x cfu/hen)</th>
<th>% EPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>7.6-8.9</td>
<td>84^A</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>8.7-8.9</td>
<td>76^A,B</td>
</tr>
<tr>
<td>Oral</td>
<td>8.7-8.9</td>
<td>13^C</td>
</tr>
<tr>
<td>Intravaginal</td>
<td>8.7-8.9</td>
<td>49^B</td>
</tr>
<tr>
<td>Intratracheal</td>
<td>8.7-9.1</td>
<td>80^A</td>
</tr>
<tr>
<td>Aerosol 1x</td>
<td>5.1-5.7</td>
<td>57^B</td>
</tr>
<tr>
<td>Aerosol 4x</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Intraperitoneal (placebo)</td>
<td>BPW</td>
<td>0^C</td>
</tr>
<tr>
<td>Intratracheal (placebo)</td>
<td>BPW</td>
<td>0^C</td>
</tr>
</tbody>
</table>

^A-C Figures having identical superscript do not differ significantly (P ≥0.01)
Intratracheally inoculated
Aerosol exposed
Intravaginally inoculated
Bacteriology

- Dead birds with EPS 89% (159/179)
  - 12 h to 5 days after *E. coli* inoculation
  - *E. coli* isolation positive
    - Septicaemia (155/159 = 97%)
    - Septicaemia & peritonitis (126/159 = 79%)
- Surviving hens with EPS 11% (20/179)
  - *E. coli* isolation negative
PFGE

- Reisolates clonal
  - (n = 5/group)
- 3 controls
  - Parent strain
  - Other cases peritonitis
Incidence & economic impact
Incidence & calculation economic impact EPS

**Six reference flocks**
- Loss eggs (no. phh)
- Loss slaughter weight (kg phh)
- Costs destruction hens (€ phh)
- Reduction feed consumption (kg phh)

**Different layer- & meat-type flocks**
- Average losses four layer flocks (phh)
- Average losses two meat-type flocks (phh)
- Losses multiplied with prices per poultry category (= loss € phh)

**Dutch poultry farming**
- Incidence data EPS of GD (farm & house level)
- Data medication
- Number hens in houses with EPS x loss € phh each category + costs medication
# Mortality in flocks with EPS

Flock D1 was medicated & flock D2 was vaccinated

<table>
<thead>
<tr>
<th>Farm</th>
<th>Flock</th>
<th>Breed</th>
<th>Housing</th>
<th>Flock size</th>
<th>Period of increased mortality</th>
<th>Mortality % (norm)</th>
<th>Mortality % EPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1&lt;sup&gt;4&lt;/sup&gt;</td>
<td>Brown layers</td>
<td>Cage</td>
<td>51932</td>
<td>41-57</td>
<td>4.3 (1.6)</td>
<td>2.7</td>
</tr>
<tr>
<td>B</td>
<td>1&lt;sup&gt;5&lt;/sup&gt;</td>
<td>White layers</td>
<td>Floor</td>
<td>26646</td>
<td>80-106&lt;sup&gt;6&lt;/sup&gt;</td>
<td>14.1 (3.8)&lt;sup&gt;7&lt;/sup&gt;</td>
<td>10.3</td>
</tr>
<tr>
<td>C&lt;sup&gt;8&lt;/sup&gt;</td>
<td>1</td>
<td>Brown layers</td>
<td>Free range</td>
<td>15826</td>
<td>40-73&lt;sup&gt;6&lt;/sup&gt;</td>
<td>18.0 (5.8)</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Brown layers</td>
<td>Free range</td>
<td>15547</td>
<td>36-77&lt;sup&gt;6&lt;/sup&gt;</td>
<td>19.8 (3.4)</td>
<td>16.4</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>Broiler breeders</td>
<td>Floor</td>
<td>13890</td>
<td>24-57&lt;sup&gt;6&lt;/sup&gt;</td>
<td>22.5 (6.8)</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td>2&lt;sup&gt;9&lt;/sup&gt;</td>
<td>Broiler breeders</td>
<td>Floor</td>
<td>13680</td>
<td>25-59&lt;sup&gt;6&lt;/sup&gt;</td>
<td>13.9 (7.0)</td>
<td>6.9</td>
</tr>
</tbody>
</table>
## Economic impact in six flocks

<table>
<thead>
<tr>
<th>Farm-flock</th>
<th>Breed</th>
<th>Number of eggs lost (value = I)(^a)</th>
<th>Slaughter weight loss of flock in kg (value = II)(^a)</th>
<th>Reduction of feed consumption in kg (value = III)(^a)</th>
<th>Costs of destruction dead hens (IV)(^a)</th>
<th>Total loss (I + II - III + IV = V)(^a)</th>
<th>Loss per flock € (flock size x V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1(^b)</td>
<td>Brown layers</td>
<td>2.68 (0.14)</td>
<td>0.053 (0.02)</td>
<td>0.326 (0.09)</td>
<td>0.00</td>
<td>0.07</td>
<td>3635</td>
</tr>
<tr>
<td>B-1(^c)</td>
<td>White layers</td>
<td>8.89 (0.54)</td>
<td>0.178(^h) (0.07)</td>
<td>1.062(^h) (0.29)</td>
<td>0.01</td>
<td>0.33</td>
<td>8793</td>
</tr>
<tr>
<td>C-1</td>
<td>Brown layers</td>
<td>10.38 (1.40)</td>
<td>0.242 (0.11)</td>
<td>1.609 (0.70)</td>
<td>0.01</td>
<td>0.82</td>
<td>12997</td>
</tr>
<tr>
<td>C-2</td>
<td>Brown layers</td>
<td>16.71 (2.26)</td>
<td>0.341 (0.15)</td>
<td>2.370 (1.03)</td>
<td>0.02</td>
<td>1.40</td>
<td>21766</td>
</tr>
<tr>
<td>D-1</td>
<td>Broiler breeders</td>
<td>15.19 (2.96)(^k)</td>
<td>0.627 (0.45)</td>
<td>3.374 (0.95)</td>
<td>0.03</td>
<td>2.49</td>
<td>34586</td>
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<tr>
<td>D-2</td>
<td>Broiler breeders</td>
<td>6.20 (1.21)</td>
<td>0.279 (0.20)</td>
<td>1.317 (0.37)</td>
<td>0.01</td>
<td>1.05</td>
<td>14364</td>
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</table>
The economic impact EPS in € on layer & meat chickens phh in the Netherlands in 2013

<table>
<thead>
<tr>
<th>Poultry sector &amp; categories</th>
<th>Number eggs lost (value = I)</th>
<th>Slaughter weight loss of flock in kg (value = II)</th>
<th>Reduction feed consumption in kg (value = III)</th>
<th>Costs destruction dead hens (value = IV)</th>
<th>Total loss (I + II - III + IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Layer-sector</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand parent</td>
<td>10 (10.00)</td>
<td>0.2 (0.09)</td>
<td>1.3 (0.35)</td>
<td>0.01</td>
<td>9.75</td>
</tr>
<tr>
<td>Parent</td>
<td>10 (1.50)</td>
<td>0.2 (0.09)</td>
<td>1.3 (0.35)</td>
<td>0.01</td>
<td>1.25</td>
</tr>
<tr>
<td>Layer cage</td>
<td>10 (0.53)</td>
<td>0.2 (0.09)</td>
<td>1.3 (0.35)</td>
<td>0.01</td>
<td>0.28</td>
</tr>
<tr>
<td>Layer barn</td>
<td>10 (0.61)</td>
<td>0.2 (0.09)</td>
<td>1.3 (0.35)</td>
<td>0.01</td>
<td>0.36</td>
</tr>
<tr>
<td>Layer free range</td>
<td>10 (0.71)</td>
<td>0.2 (0.09)</td>
<td>1.3 (0.35)</td>
<td>0.01</td>
<td>0.46</td>
</tr>
<tr>
<td>Layer organic</td>
<td>10 (1.35)</td>
<td>0.2 (0.09)</td>
<td>1.3 (0.57)</td>
<td>0.01</td>
<td>0.88</td>
</tr>
<tr>
<td>Layer vaccine</td>
<td>10 (0.81)</td>
<td>0.2 (0.09)</td>
<td>1.3 (0.35)</td>
<td>0.01</td>
<td>0.56</td>
</tr>
<tr>
<td><strong>Meat-sector</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand parent</td>
<td>11 (11.00)</td>
<td>0.5 (0.36)</td>
<td>2.3 (0.65)</td>
<td>0.02</td>
<td>10.73</td>
</tr>
<tr>
<td>Parent</td>
<td>11 (2.14)</td>
<td>0.5 (0.36)</td>
<td>2.3 (0.65)</td>
<td>0.02</td>
<td>1.87</td>
</tr>
</tbody>
</table>
Costs medication €

- Nine different antibiotics

<table>
<thead>
<tr>
<th>Layer-sector</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand parent</td>
<td>450</td>
</tr>
<tr>
<td>Parent</td>
<td>784</td>
</tr>
<tr>
<td>Layer cage</td>
<td>0</td>
</tr>
<tr>
<td>Layer barn</td>
<td>26496</td>
</tr>
<tr>
<td>Layer free range</td>
<td>4752</td>
</tr>
<tr>
<td>Layer organic</td>
<td>1344</td>
</tr>
<tr>
<td>Layer vaccine</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>33826</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Meat-sector</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand parent</td>
<td>14509</td>
</tr>
<tr>
<td>Parent</td>
<td>63644</td>
</tr>
<tr>
<td>Total</td>
<td>78153</td>
</tr>
</tbody>
</table>
## Economic impact in Duch poultry farming

<table>
<thead>
<tr>
<th>Poultry sector</th>
<th># farms</th>
<th>Average # hens per farm category x1000</th>
<th># farms with EPS (%)</th>
<th># hens in houses with EPS x1000 (%)</th>
<th>Loss € x1000 due to loss eggs, loss slaughter weight &amp; destruction costs compensated for reduced feed intake</th>
<th>Costs medication € x1000</th>
<th>Total losses € x1000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer-type</td>
<td>1198</td>
<td>36580</td>
<td>89 (7)</td>
<td>1813 (5)</td>
<td>375</td>
<td>33</td>
<td>408</td>
</tr>
<tr>
<td>Meat-type</td>
<td>190</td>
<td>4570</td>
<td>66 (35)</td>
<td>1144 (25)</td>
<td>3185</td>
<td>79</td>
<td>3264</td>
</tr>
<tr>
<td>Total</td>
<td>1388</td>
<td>41150</td>
<td>155 (11.2)</td>
<td>2957 (7.2)</td>
<td>3560</td>
<td>112</td>
<td>3672</td>
</tr>
</tbody>
</table>
Incidence layer-type

\[
\frac{89}{1198} = 7\% \text{ farms}
\]
Incidence meat-type

$$\frac{66}{190} = 35\% \text{ farms}$$
3.7 million € per year
Molecular typing
Study design

- 6 flocks with EPS
  - 4 unrelated farms A, B, C, D
  - 2 successive flocks farm C, D
- 12-25 birds per farm
- Postmortem examination
- Isolation *E. coli* bone marrow
- Genotyping colonies using PFGE & MLST
## Mortality in flocks with EPS

Flock D1 was medicated & flock D2 was vaccinated

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<tr>
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<th>Breed</th>
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<th>Period of increased mortality²</th>
<th>Mortality % (norm)³</th>
<th>Mortality % EPS</th>
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<tbody>
<tr>
<td>A</td>
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<td>Floor</td>
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<td>80-106⁶</td>
<td>14.1 (3.8)⁷</td>
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</tr>
</tbody>
</table>
Macroscopic lesions dead hens from six EPS flocks

• Age at sampling 28-82 weeks
• \( n = 102 \) (12-25/flock)
• EPS 96%
Bacteriology dead hens from six EPS flocks

- $n = 102$ (12-25/flock)
- *E. coli* bone marrow 88%
Effect of EPS on cumulative mortality % and effect antibiotic treatment

- Cumulative mortality % hens
- Cumulative mortality % norm
- Cumulative mortality % roosters
PFGE

- 5 colonies/bird (9 to 14 hens per flock = 320 colonies) within bird clonality
- 1 colony/bird (9 to 16 hens per flock = 67 colonies) within flock & between flock clonality
- Threshold clonality >95% similarity between colonies
PFGE farm A

- 9 Birds
- 3 genotypes
- 1 genotype per bird
PFGE farm C
Colonies flock 2 = colonies flock 1 (genotype 1 & 2)
PFGE farm D
Colonies flock 2 ≠ colonies flock 1
PFGE all farms

- 1 genotype per bird
- 1-5 genotypes per farm
- 1-2 genotypes dominate each outbreak
- 15 genotypes found
MLST

• 24 colonies
  • 9 PFGE types 2 colonies
  • 6 PFGE types = 1 colony (found in one hen)
• 39 colonies other EPS cases
  • 2001-2012
## Housekeeping genes MLST

**Target genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>adk</td>
<td>adenylate kinase</td>
</tr>
<tr>
<td>fumC</td>
<td>fumarate hydratase</td>
</tr>
<tr>
<td>gyrB</td>
<td>DNA gyrase</td>
</tr>
<tr>
<td>icd</td>
<td>isocitrate/isopropylmalate dehydrogenase</td>
</tr>
<tr>
<td>mdh</td>
<td>malate dehydrogenase</td>
</tr>
<tr>
<td>purA</td>
<td>adenylosuccinate dehydrogenase</td>
</tr>
<tr>
<td>recA</td>
<td>recombinase A</td>
</tr>
</tbody>
</table>

University of Warwick website
(http://mlst.warwick.ac.uk/mlst/dbs/Ecoli/documents/primersColi_html)
E. coli colonies this study (n = 24)

E. coli colonies 2001-2012 (n = 39)
Conclusions

• EPS caused by various *E. coli* genotypes
• In single birds one genotype
• *E. coli* genotypes from EPS may persist at a farm
• Consequences for composition autovaccines?
• PFGE types & MLSTs not unambiguously related to APEC from EPS
Final remarks

• EPS major health problem
  – Clinical & economic impact
  – Primary pathogenic *E. coli*

• Antibiotics inefficient/residues/resistance

• Autovaccines
  – Based on genotypes outbreak?

• Molecular markers APEC from EPS not identified yet
Thank you for your attention!