

**Gallibacterium anatis infection in chickens**

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**Introduction to G. anatis**

- Gram negative bacteria within the family Pasteurellaceae  
  Pohl 1981
- Previously known as: *Actinobacillus salpingitidis*, avian *Pasteurella haemolytica* or *Pasteurella anatis*
- Two biovars reported: haemolytic and non-haemolytic

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**Prevalence of G. anatis**

- Globally reported

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**Field reports of G. anatis**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type</th>
<th>Remarks/co-infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kjos-Hanssen (1950)</td>
<td>Hens</td>
<td>Referred as „cloacal bacteria”</td>
</tr>
<tr>
<td>Harbourne (1962)</td>
<td>Pullets</td>
<td>Coccioidosis, pullet disease</td>
</tr>
<tr>
<td>Harry (1962)</td>
<td>Layers</td>
<td>Healthy chickens/E.coli</td>
</tr>
<tr>
<td>Greenham &amp; Hill (1962)</td>
<td>Hen</td>
<td>Fibrile liver, gut lesions/E. coli</td>
</tr>
<tr>
<td>Hacking &amp; Pettit (1974)</td>
<td>Pullets/Layers</td>
<td>Peritonitis, salpingitis, low egg production</td>
</tr>
<tr>
<td>Biggaard (1977)</td>
<td>≥ 4 weeks</td>
<td>Healthy chickens/IB</td>
</tr>
<tr>
<td>Mustin et al. (1980)</td>
<td>6%-12 weeks</td>
<td>Normal tracheal flora</td>
</tr>
<tr>
<td>Jones &amp; Owen (1981)</td>
<td>Layer parents</td>
<td>Peritonitis, salpingitis, low egg production, internal laying/E. coli</td>
</tr>
<tr>
<td>Addis &amp; Mohan (1985)</td>
<td>Domestic flock</td>
<td>Nodular hepatic necrosis</td>
</tr>
<tr>
<td>Shaw et al. (1990)</td>
<td>Pullets/Layers</td>
<td>Mortality, respiratory problem/ILT virus</td>
</tr>
<tr>
<td>Mifsu et al. (1991)</td>
<td>Layers</td>
<td>Less mortality but low egg production</td>
</tr>
<tr>
<td>Suzuki et al. (1996)</td>
<td>Pullets/Layers</td>
<td>MOKA, paragallinarum/CAV</td>
</tr>
</tbody>
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**Field reports of G. anatis**

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<thead>
<tr>
<th>Reference</th>
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<th>Remarks/co-infection</th>
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<tbody>
<tr>
<td>Bojesen et al. (2003)</td>
<td>Layer/Parents</td>
<td>Healthy chickens</td>
</tr>
<tr>
<td>Neubauer et al. (2009)</td>
<td>Layers</td>
<td>Peritonitis, salpingitis, low egg production</td>
</tr>
<tr>
<td>Hwangfu et al. (2012)</td>
<td>24 days</td>
<td>Detection of G. anatis at the earliest age</td>
</tr>
<tr>
<td>Jones et al. (2013)</td>
<td>Broiler Parents/Broiler</td>
<td>Increasing cases of G. anatis in diagnostics</td>
</tr>
</tbody>
</table>

In reproductive tract:
- 6/31 flocks: G. anatis pure culture
- 10/31 flocks: E. coli pure culture
- 14/31: G. anatis + E. coli

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**Field reports of G. anatis**

Mississippi (USA): investigation of broiler and broiler breeder samples

Jones et al. (2013), *Poultry Science*, 92, 3166-71

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AFU Video lecture

3/7/2017
Experimental studies

<table>
<thead>
<tr>
<th>reference</th>
<th>age</th>
<th>route</th>
<th>remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harbourne (1962)</td>
<td>15 weeks</td>
<td>SC</td>
<td>1/14 birds died at 15 dpi, all birds positive in tracheas at 31 dpi</td>
</tr>
<tr>
<td>Bisgaard (1977)</td>
<td>day-old</td>
<td>IP</td>
<td>no mortalities</td>
</tr>
<tr>
<td>Mushin et al. (1980)</td>
<td>8 weeks</td>
<td>IM</td>
<td>IT, 1/3 birds died</td>
</tr>
<tr>
<td>Bjoensen et al. (2004)</td>
<td>18 weeks</td>
<td>IV and IP</td>
<td>observed for 24 hours</td>
</tr>
<tr>
<td>Zapeta et al. (2010)</td>
<td>12 weeks</td>
<td>IN</td>
<td>observed for 15 dpi</td>
</tr>
</tbody>
</table>

SC: subcutaneous, IP: intraperitoneal, IM: intramuscular, IT: intratracheal, IV: intravenous, IN: intranasal

Koch's postulates

Koch's postulates are used to prove the cause of an infectious disease:

1. The same microorganisms are present in all the cases of the disease.
2. The microorganisms are isolated from the tissues of dead animals and grown in a pure culture.
3. The disease is reproduced when pure culture of the microorganism is inoculated into healthy animals.
4. The same microorganisms are re-isolated from tissues of the diseased animals.

G. anatis infection in SPF layers

<table>
<thead>
<tr>
<th>group</th>
<th>infection</th>
<th>killing (dpi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>infected (n=37)</td>
<td>yes</td>
<td>3 3 3 3 23*</td>
</tr>
<tr>
<td>control (n=37)</td>
<td>no</td>
<td>3 3 3 3 25</td>
</tr>
</tbody>
</table>

* 2 animals died before scheduled killing

- age of infection: 30 weeks
- route: intranasal

Result: Clinical findings

- control birds: no clinical signs
- infected birds:
  - whitish diarrhoea
  - mortality

<table>
<thead>
<tr>
<th>dpi</th>
<th>no. of birds</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-14</td>
<td>2</td>
</tr>
<tr>
<td>15-20</td>
<td>5</td>
</tr>
<tr>
<td>21-24</td>
<td>2</td>
</tr>
</tbody>
</table>

Result: Egg production

- lowest egg production: 47.2 % at 3 wpi (vs 86.7 % in control)
- total egg loss in infected group = 836-553 = 283 (in 5 weeks)
- average weight of eggs did not differ

Distribution of G. anatis at different time points:
- choana, trachea, lungs, liver, heart, spleen, ovary, oviduct, testis, duodenum, cloaca

Paudel et al. (2013), Avian Pathology, 42, 527-535

Veterinärmedizinische Universität Wien (Vetmeduni Vienna)
Result: Macroscopic lesions
days post infection

<table>
<thead>
<tr>
<th>dpi</th>
<th>3</th>
<th>7</th>
<th>10</th>
<th>17</th>
<th>28</th>
<th>30</th>
<th>38</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>folliculitis (1)</td>
<td>pericarditis (2)</td>
<td>widespread</td>
<td>folliculitis</td>
<td>pericarditis, eggs and caseous material in abdomen (1)</td>
<td>haemorrhagic follicle (1), yolk in abdomen (1)</td>
<td>haemorrhagic follicle (1), yolk in abdomen (2)</td>
</tr>
</tbody>
</table>

Result: Histopathology

lesions in ovary

<table>
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<tr>
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<th>no. of birds</th>
</tr>
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<tbody>
<tr>
<td>7</td>
<td>3/3</td>
</tr>
<tr>
<td>10</td>
<td>2/3</td>
</tr>
<tr>
<td>28</td>
<td>1/2</td>
</tr>
<tr>
<td>38</td>
<td>2/2</td>
</tr>
</tbody>
</table>

histological evidence of folliculitis

Result: Bacterial distribution

dpi choana trachea lung liver spleen heart ovary oviduct duodenum cloaca

3 7 10 17 28 30 38

38 dpi re-isolation from the egg yolk at 10 dpi

Major outcomes of the trial

- G. anatis invades systemic organs in layers
- pathological lesions and bacterial reisolation from ovaries coincide with the drop in egg production
- bacterial invasion into the egg yolk was demonstrated

Paudel et al. (2014), Avian Pathology, 43, 443-449

G. anatis infection in adult males

- to investigate the impact of G. anatis infection in SPF roosters
Assessment of semen quality

- mass motility
- density
- viability & motility characteristics
- viability

Experimental design

- Group infection
- Killing (dpi)
  - Infected: n=12
    - dpi: 3, 7, 10, 28, 38
    - n=4
  - Control: n=8
    - dpi: 2, 2, 2, 2, 4

* Used for semen sampling
- Training for semen collection started at 32 weeks
- Age of infection: 35 weeks
- Route: Intranasal

Result: Histopathology

- Lesions in epididymis
  - Dpi: 7, 10, 28
  - No. of birds: 2/3, 1/3, 2/3

Histological evidence of epididymitis

Result: Semen quality

- Density (billion/ml)
  - Control: 60%, 80%, 100%
  - Infected: 60%, 80%, 100%

- Progressive motility
  - Control: 40%
  - Infected: 40%

- Viability
  - Control: 90%
  - Infected: 90%

- Pathomorphology
  - Control: 80%
  - Infected: 80%

Result: Bacterial distribution

- Dpi: 3, 7, 10, 28, 38

- G. anatis
  - 18 dpi: 4/4
  - 22 dpi: 3/4
  - 25 dpi: 1/4
  - 29 dpi: 1/4
Major outcomes of the trial

- G. anatis colonize gonads in mature males
- Infection with G. anatis leads to epididymitis
- The semen quality was lowered in experimentally infected birds
- G. anatis was found in semen from infected birds

Paudel et al. (2014), Avian Pathology, 43, 529-534

Gallibacterium anatis fulfills Koch’s postulates to be regarded as a primary pathogen in layers and cockerels

G. anatis infection in immunosuppressed chickens

G. anatis infection in immunosuppressed chickens

Bacterial load in spleen

Coinfection of chickens with G. anatis and Avibacterium paragallinarum
Clinical signs of infectious coryza

4 birds died at 5 dpi from non-vaccinated and co-infected groups.

elucidation of the severity of infectious coryza

severe clinical signs of infectious coryza

patho-morphology in sinus

histopathology in sinus

immunohistochemistry

Take home message

- G. anatis is a primary pathogen in adult chickens
- in layers, it causes folliculitis, haemorrhagic follicles and drop in egg production
- in cockerels, epididymitis and decreased semen quality were observed
- multiplication of G. anatis in systemic organs is increased in immunosuppressed chickens
- co-infection of G. anatis with Av. paragallinarum causes mortality and increased clinical signs of infectious coryza

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