Emergence and predominance of a hypervirulent, tetracycline-resistant *Campylobacter jejuni* clone as a major cause of sheep abortion in the United States

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Presentation Outline

• Overview on *Campylobacter*
• Recent emergence of a hypervirulent clone in the U.S.
  – Identification of clone SA in sheep
  – Tetracycline resistance in *C. jejuni*
  – Tetracycline concentrations in tissues
  – Conclusions
Campylobacter--characteristics

- Gram-negative; spiral to comma/coccoid shape
- Single or bipolar flagella --- motile
- Obligate microaerophilic
- Thermophilic
- A small (~1.7 Mbp), but plastic genome
- Broad host range---commensal/pathogenic
- Multiple species
**Campylobacter** spp. important for animal and public health

- **C. fetus:**
  - Abortion in cattle and sheep
  - Rare cause of bacteremia and abortion in humans

- **C. jejuni** (and **C. coli**)
  - Abortion in sheep, cattle, goat and rarely in humans
  - Major cause of foodborne enteritis in humans
  - Generally considered asymptomatic in poultry (and livestock)

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*Campylobacter* species tree

Lefebure and Stanhope, Genome Res. 2009.
Campylobacter as a zoonotic pathogen

• A leading cause of foodborne bacterial gastroenteritis in humans worldwide
  – 845,000 annual cases in the U.S. with over 8,000 hospitalization (recent CDC estimate)
• Commonly present in food producing animals
  – Poultry, ruminants, and swine
  – Poultry meat is the main source; followed by raw milk
• Usually self-limiting infections, but antibiotic treatment is indicated in certain cases
Emergence of a highly pathogenic and tetracycline-resistant *C. jejuni* clone in ruminants in the U.S.
Campylobacter Abortion in Sheep

• Leading cause of ovine abortion worldwide
• Commonly found in the intestine and bile as commensal
• Abortion rates can exceed 50% in some cases
• Major economic loss
• Multiple species and strains of *Campylobacter* involved (*C. fetus* mostly and *C. jejuni*)
Campylobacter species shift in sheep abortion in the U.S.

• Gradual shift toward *C. jejuni* (displacement of *C. fetus*)
• Occurred during 1980s and 1990s
• *C. jejuni* became the predominant species
• Different *C. jejuni* strains were involved (genetically diverse)
  
  Kirkbride, 1993; Delong et al., 1996; Sahin et. al. 2008.
Identification of a Highly Virulent *C. jejuni* Clone

- Collection of all *Campylobacter* isolates from sheep abortions since 2003 from VDLs in IA, ID, SD, ND, OR, NV, CA.
- High genetic similarity (clonal) of the clinical isolates from different farms and lambing seasons (PFGE and MLST genotyping)
- Not expected as *C. jejuni* strains from abortion had been genetically diverse
- The abortion clone named *clone SA* (Sheep Abortion)
PFGE of *C. jejuni* isolates from sheep abortions in the U.S.

MLST confirmed the clonality as all isolates were of the same sequence type, ST-8.

Predominance of clone SA in recent sheep abortions in the U.S.

All clone SA isolates belong to ST-8 type

1990-2000
21 total isolates

66.6% ST-8

2003-2015
145 total isolates

91% ST-8

9% Other
Presence of *C. jejuni* clone SA in healthy sheep (slaughterhouse survey)

N= 48 total isolates
C. jejuni from sheep abortion in the U.K. are genetically diverse

- From 2002-2008 (n=42 isolates)
- No ST-8 in the U.K. collection
- Confirmed by PFGE
C. jejuni Clone SA is also present in other ruminant abortions

11 of 27 (40%) abortion isolates from IA, CA, and ND
## Clone SA is associated with human disease in the U.S.

<table>
<thead>
<tr>
<th>Case</th>
<th>No. of isolates</th>
<th>State</th>
<th>Year</th>
<th>Isolation source</th>
<th>Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outbreak-1</td>
<td>1</td>
<td>VT</td>
<td>2003</td>
<td>Unknown</td>
<td>Raw milk</td>
</tr>
<tr>
<td>Outbreak-2</td>
<td>4</td>
<td>SC</td>
<td>2007</td>
<td>Stool</td>
<td>Raw milk</td>
</tr>
<tr>
<td>Outbreak-3</td>
<td>16*</td>
<td>PA</td>
<td>2008</td>
<td>Unknown</td>
<td>Raw milk</td>
</tr>
<tr>
<td>Outbreak-4</td>
<td>4</td>
<td>RI</td>
<td>2008</td>
<td>Unknown</td>
<td>Chicken</td>
</tr>
<tr>
<td>Outbreak-5</td>
<td>32</td>
<td>WI</td>
<td>2009</td>
<td>Stool</td>
<td>Raw milk</td>
</tr>
<tr>
<td>Outbreak-6</td>
<td>2</td>
<td>MA</td>
<td>2010</td>
<td>Stool/Blood</td>
<td>Raw milk</td>
</tr>
<tr>
<td>Outbreak-7</td>
<td>7</td>
<td>MI</td>
<td>2010</td>
<td>Stool</td>
<td>Raw milk</td>
</tr>
<tr>
<td>Outbreak-8</td>
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<td>MT</td>
<td>2010</td>
<td>Unknown</td>
<td>Well water</td>
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<tr>
<td>Outbreak-9</td>
<td>2</td>
<td>VT</td>
<td>2010</td>
<td>Stool</td>
<td>Raw milk</td>
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<tr>
<td>Sporadic†</td>
<td>56</td>
<td>Multiple</td>
<td>2004-10</td>
<td>Stool</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

- CDC PulseNet *Campylobacter* database was used (since 2003)
- 123 (9.03%) human *C. jejuni* isolates were of clone SA through 2010
- Raw milk consumption was the main exposure source

Hypothesis

Clone SA is highly pathogenic for sheep and may have unique virulence characteristics
Clone SA is abortifacient in pregnant guinea pigs

Survival Rate

Days Post Inoculation

IP inoculation

Oral inoculation

Genomic approaches to understanding pathogenesis of clone SA

Wu et al. PNAS: 113 (38), 2016
Point mutations in the major outer membrane protein drive hypervirulence of a rapidly expanding clone of *Campylobacter jejuni*

Zuowei Wu, Balamurugan Periaswamy, Orhan Sahin, Michael Yaeger, Paul Plummer, Weiwei Zhai, Zhangqi Shen, Lei Dai, Swaine L. Chen, and Qijing Zhang

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Infections due to clonal expansion of highly virulent bacterial strains are clear and present threats to human and animal health. Association of genetic changes with disease is now a routine, but identification of causative mutations that enable disease remains difficult. *Campylobacter jejuni* is an important zoonotic pathogen transmitted to humans mainly via the foodborne route. *C. jejuni* typically colonizes the gut, but a hypervirulent and rapidly expanding clone of *C. jejuni* recently emerged, which is able to translocate across the intestinal tract, causing systemic infection and abortion in pregnant animals. The genetic basis responsible for this hypervirulence is unknown. Here, we developed a strategy, termed “directed states alone, *Campylobacter* accounts for more than 800,000 cases of foodborne illnesses each year (10). As a zoonotic pathogen, *C. jejuni* is widely distributed in the gut microbiota of wild and domesticated animal species, such as cattle, sheep, and poultry (11, 12). Transmission of *C. jejuni* to humans is mainly via contaminated meat, milk, and water. Although *C. jejuni* is primarily a gut colonizer, some hypervirulent strains may be able to translocate across intestinal epithelium, producing bacteremia and systemic infections (13). In addition to causing foodborne illnesses, *C. jejuni* is also a primary etiological agent for ruminant abortion (14). Recently, we reported the emergence of an antibiotic-
Resistance to tetracyclines (TCs) among *C. jejuni* clone SA isolates
**C. jejuni** clone SA isolates from sheep abortion are universally resistant to TCs

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC$_{90}$</th>
<th>Resistance breakpoint</th>
<th>No. (%) resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftiofur</td>
<td>&gt;64</td>
<td>≥8</td>
<td>74 (100)</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>&lt;0.13</td>
<td>≥4</td>
<td>0</td>
</tr>
<tr>
<td>Florfenicol</td>
<td>2</td>
<td>≥32</td>
<td>0</td>
</tr>
<tr>
<td><strong>Oxytetracycline</strong></td>
<td><strong>64</strong></td>
<td><strong>≥16</strong></td>
<td><strong>74 (100)</strong></td>
</tr>
<tr>
<td>Penicillin</td>
<td>16</td>
<td>≥16</td>
<td>40 (54)</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>2</td>
<td>≥32</td>
<td>0</td>
</tr>
<tr>
<td>Tulatromycin</td>
<td>0.5</td>
<td>≥32</td>
<td>0</td>
</tr>
<tr>
<td>Tylosin</td>
<td>8</td>
<td>≥32</td>
<td>2 (2.7)</td>
</tr>
</tbody>
</table>

### Antibiotic Resistance of C. jejuni clone SA isolates from healthy sheep

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (μg/ml)</th>
<th>90%</th>
<th>Resistance breakpt.</th>
<th>No. (%) of resistant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Azithromycin</td>
<td>0.03–0.12</td>
<td>0.12</td>
<td>≥8</td>
<td>0 (0)</td>
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<tr>
<td>Ciprofloxacin</td>
<td>0.06–16</td>
<td>0.12</td>
<td>≥4</td>
<td>2 (4.1)</td>
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<tr>
<td>Clindamycin</td>
<td>0.06–1</td>
<td>0.25</td>
<td>≥8</td>
<td>0 (0)</td>
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<tr>
<td>Erythromycin</td>
<td>0.25–2</td>
<td>1</td>
<td>≥32</td>
<td>0 (0)</td>
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<tr>
<td>Florfenicol</td>
<td>0.5–4</td>
<td>1</td>
<td>≥16</td>
<td>0 (0)</td>
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<tr>
<td>Gentamycin</td>
<td>0.25–2</td>
<td>1</td>
<td>≥8</td>
<td>0 (0)</td>
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<tr>
<td>Nalidixic acid</td>
<td>&lt;4 → &gt;64</td>
<td>8</td>
<td>≥32</td>
<td>3 (6.2)</td>
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<tr>
<td>Telithromycin</td>
<td>0.25–2</td>
<td>1</td>
<td>≥16</td>
<td>0 (0)</td>
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<tr>
<td><strong>Tetracycline</strong></td>
<td>0.25→64</td>
<td>&gt;64</td>
<td>≥16</td>
<td><strong>40 (83.3)</strong></td>
</tr>
</tbody>
</table>

TC resistance among the U.S. and U.K. *C. jejuni* sheep abortion isolates

<table>
<thead>
<tr>
<th></th>
<th>UK Isolates (41)</th>
<th>US Isolates (54)</th>
<th>Early-US Isolates (21)</th>
<th>Late-US Isolates (33)</th>
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</thead>
<tbody>
<tr>
<td><strong>% Resistance</strong></td>
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<tr>
<td>Azithromycin</td>
<td>2.44</td>
<td>1.85</td>
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<tr>
<td>Ciprofloxacin</td>
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<td>1.85</td>
<td>0</td>
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<td>Clindamycin</td>
<td>9.76</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Erythromycin</td>
<td>0</td>
<td>1.85</td>
<td>4.76</td>
<td>0</td>
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<tr>
<td>Florfenicol</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Gentamycin</td>
<td>0</td>
<td>1.85</td>
<td>0</td>
<td>3.03</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>17.1</td>
<td>3.7</td>
<td>4.76</td>
<td>3.03</td>
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<td>Telithromycin</td>
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<td>1.85</td>
<td>4.76</td>
<td>0</td>
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<tr>
<td>Tetracycline</td>
<td>4.88</td>
<td>68.5</td>
<td>19.05</td>
<td>100</td>
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</table>

Tetracycline resistance in *C. jejuni* clone SA isolates is mediated by *tet*(O) gene.

*tet*(O)-specific PCR
**tetO is located in the chromosome in recent clone SA isolates**

<table>
<thead>
<tr>
<th>tetO / loct.</th>
<th>#isolate</th>
<th>Country</th>
<th>Year</th>
<th>ST</th>
<th>CC</th>
<th>Remarks</th>
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<tbody>
<tr>
<td>Yes / Chrom.</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>85</td>
<td>U.S.</td>
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<td>21</td>
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<td>45</td>
<td>45</td>
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<tr>
<td></td>
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<td>U.S.</td>
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<td>239</td>
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<tr>
<td></td>
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<td>982</td>
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<td></td>
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<td>4843</td>
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<td>Yes / pTet</td>
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<td>38</td>
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<td></td>
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<td></td>
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<td>U.S.</td>
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<td>42</td>
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<td>U.S.</td>
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<td>5189</td>
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</table>
Tetracycline Resistance Implications

• The drug is the only class of antibiotics approved in the U.S. for prevention and control of sheep abortion and is commonly used for these purposes

• The high resistance suggests that the treatment is no longer effective

• Use of tetracycline might have served as a selection force for the emergence of *C. jejuni* clone SA
Are tetracyclines effective for *C. jejuni* abortion storms?

- **Typical recommendation**: In-feed tetracycline (TC) or chlortetracycline (CTC) during the last trimester.
  - FDA-approved dose of CTC: 80 mg/head/day. Nonprescription (OTC).
  - Extra-label dose of CTC: 500 mg/head/day (unapproved in the US, but used commonly!). FDA CPG 615.115 sort of “allows” it (*Extra-Label Use of Medicated Feeds for Minor Species*).
  - Do these doses result in effective concentration of the drug at target tissues (placenta)?
Non-pregnant ewes (6 per group) were given either dose orally for 8 days; plasma concentration of CTC were determined before, during and 36 h after the last dose.

- Mean cont. of CTC were 20 ng/ml (80 mg dose), and 101 ng/mL (500 mg dose).
- >90% of *C. jejuni* clone SA have MIC of 64,000 ng/mL (~600 to 3000 fold difference).

What about the concentrations in fetal tissues?

- CTC was given orally to pregnant ewes (n=5) at 500 mg/head/day for 8 days during the last trimester.
- On day 7, placenta and amniotic fluid were harvested from the fetus via implanted venous lines (sampled for 36 h after the last dose).
- At necropsy, amniotic fluid, placenta, fetal kidneys, liver and stomach contents were tested for CTC concentrations.
- In two fetuses only: Fetal liver: 4.6-125 ng/mL; fetal kidney: 8-17 ng/mL.
- All the other tissues were below the limit of detection (1 ng/mL).

Washburn, Plummer et al. manuscript is in prep.
Conclusion

• High level of TC resistance among *C. jejuni* sheep abortion isolates.

• Lack of efficacy of TCs for sheep abortion (anecdotal reports).

• Far less concentrations of TCs are achieved in plasma/fetal tissues than the MIC for *C. jejuni*.

• These indicate that feed grade TCs used may not provide the therapeutic drug concentration in target tissues for effective treatment of *C. jejuni* associated abortion storms in sheep.

• Common use of TCs (sub-therapeutic level in body) may have provided selective advantage for the emergence, spread and persistence of clone SA in the sheep abortions in the U.S.
Conclusions-implications

- New U.S. FDA Guidance (#209, 213); and new VFD release will take effect in Jan 1, 2017:
  - Judicious use of medically important antibiotics (e.g., TCs) in food animals
  - Remove the growth promoting indications from the label
  - Veterinary oversight for addition of these drugs to feed/water for any reason
  - Removal of all these drugs out of OTC status, including TCs.
  - These will change the practice substantially:
    - Sheep producers can no longer buy oral OTC TCs.
    - CPG 615.115 is expiring soon and there is no news on the new CPG for use of TCs in small ruminants. I.e.:
    - Prescription of extra-label use of TCs beyond approved dose (80 mg) will not be allowed.
Alternatives to TCs

• **Tulatromycin (Draxxin)**
  – Safe to use in sheep at the approved cattle dose
  – Two doses, 14-days apart, given SQ: Appears effective.
  – During an abortion storm.

• **Florfenicol, tilmicosin, tylosin** have low MIC values; *C. jejuni* sheep abortion isolates are highly sensitive *in vitro*. No real data available!

• **Others**: E.g., vaccines?
Acknowledgements

Dr. Qijing Zhang  Dr. Paul Plummer  Dr. Michael Yaeger  Dr. Zuowei Wu

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