Recovery, Characterization, and Control of *Salmonella* spp. in/on Chicken Liver and in Pâté Made with Chicken Liver

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Mitigating Risk from *Salmonella* in/on poultry liver & pâté...

1. **Presentation by Becker et al. @ 2017 FSIS:ARS Food Safety Summit:**

   - Risks not fully understood...eludes FSIS’s strategic priority to reduce pathogens in poultry...notable increase in outbreaks since 2012
   - Prevalence in/on liver not adequately elaborated
     - Data available for giblets (necks, heart, gizzards, and liver)
   - Undercooking by consumers (preferred) & restaurants (16 of 22 outbreaks = 73%) is a contributing factor, resulting in elevated risk

2. **Research questions and data voids being addressed by CRIS-019:**

   - What are the true prevalence and levels in/on raw livers?
   - What interventions reduce levels associated with liver or pâté?

*Salmonella* are frequently associated with recalls and human illness attributed to undercooked and/or improperly stored whole chicken liver or chicken liver pâté
Research on *Salmonella* in/on liver and pâté

ERRC CRIS-019 Team

**SURVEY & STORAGE**
(Aug 2017 - to present)

- **LIVER**
  - **RETAIL**
  - **ABATTOIR**

- **Prevalence, Levels, & Types**

**CHALLENGE STUDIES**
(Jun 2017 - May 2018)

- **LIVER**
- **PÂTÉ**

- **PHYSICAL & CHEMICAL INTERVENTIONS**
  - **HPP, Heat, & Antimicrobials**
Questions addressed

• How many chicken liver samples contain *Salmonella* spp.?
  • Are there any differences in prevalence among retail, farm, and abattoir samples?

• How many cells of *Salmonella* are present in a positive sample?
  • What are the types/relatedness of these isolates?

• Does *Salmonella* survive in liver during storage?
  • If present, what is the viability of *Salmonella* over time at refrigeration and freezing temperatures?
**“Chicken Liver Survey - *Salmonella*”**

<table>
<thead>
<tr>
<th>Key parameters</th>
<th>Matrix</th>
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<tbody>
<tr>
<td>Sources</td>
<td>Retail</td>
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<td>Farm</td>
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<td>Abattoir</td>
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<td>Types/species</td>
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<td>Duck</td>
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<td>Turkey</td>
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<td>Cattle</td>
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<td>Micro data</td>
<td>Prevalence, levels, &amp; types of <em>Salmonella</em> and native flora</td>
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<td>Challenge Studies/Risk</td>
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<td>Assessments</td>
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Microbiological Methods - Survey

- Presence/absence
  - (MLG 4.09 for *Salmonella*)

- Enumeration
  - by MPN and/or direct plating (CFU/g)

- Confirmation
  - Serological and biochemical tests

- Retention of isolates
  - (2-20 isolates per a sample)

- Molecular characterization
  - PFGE, serotype, WGS

- Environmental/Control Samples = 5 samples/week (selected randomly) = Enrichment/Screening

- Positive and Negative Controls = Enrichment/Screening (weekly)
Viability of *Salmonella* in Chicken Livers

- Storage at 4°C for 10 days or -20°C for 120 days resulted in reductions of 1.5 and 2.1 log CFU/g, respectively, for cells inoculated in or on livers.

- Pre-treatment of raw livers with buffered or table vinegar for up to 2 min (ca. 0.2 to 1.2 log CFU/g) had no additional effect on levels of *Salmonella* compared with non-treated livers (1.1 log CFU/g) after 7 days of storage at 4°C (ongoing studies).

FSIS Directive 7120.1 (01/19/18):
*Acetic acid, as an antimicrobial agent in chicken livers, For use as an antimicrobial immersion dip at a concentration of up to 5 percent and not to exceed two minutes*

- Cocktail of Rif® Salmonella @ 5.5 log CFU/g
Summary – Survey & Storage

• There was an appreciable difference in prevalence of *Salmonella* associated with specie [Duck > Chicken > Turkey > Cattle (more samples to be tested)].

• Differences in prevalence and levels in livers obtained at retail were observed among brands, states, season, and species of poultry.

• Refrigeration and freezing of raw chicken livers has an appreciable effect on viability of *Salmonella*.

• Data can be used to support risk assessments, inform policy decisions, and develop interventions to reduce the risk of salmonellosis associated with chicken liver, as well as other liver from poultry species and poultry liver products.

Greater number of samples should be tested
INTERVENTIONS

HEAT
- PÂTÉ
  - Water Bath
    - Temperature
  - Pan Fry
    - Time

PRESSURE
- LIVER
- PÂTÉ
  - Time and Pressure

CHEMICALS
- LIVER
- PÂTÉ
  - Dip
  - Formulation
    - Storage

INTEGRATED LETHALITY TOWARDS SALMONELLA
Chicken Liver Pâté – Thermal Inactivation

Pâté Formulation:
• 1 kg chicken liver
• 112 g butter
• 5 g salt
• 2.5 g pepper
• 100 g onion
• 2 hard-boiled eggs

1. Pâté made with raw liver - pâté cooked in a water bath held at 74.8°C
   ➢ Cook pâté at 60°, 63°, 65°, 68°, 71.1°, and 73.8°C (instantaneous internal)

2. Pâté made with cooked liver – liver cooked in a frying pan
   ➢ Single flip/mid point time
   ➢ Cook liver for 3, 4, 5, 6, 7, 8 min, prepare pâté, and then cool down at 4°C or 21°C for 2 h

3. Other
   ➢ Interactions of cooking, holding, storage, and formulation
Chicken Liver Pâté – Thermal Inactivation

Reductions of:

- ca. 0.3 to 6.5 log CFU/g = water bath
- ca. 1.0 to 4.7 log CFU/g = frying pan
  - Increase of 0.2 to 0.8 log CFU/g in *Salmonella* numbers were observed during cooling down at 4°C or 21°C for 2 h

ca. 5.0 log reduction ≥ 73.8°C or 7 min
High Pressure Inactivation of *Salmonella* in Chicken Liver and in Pâté

- **Inoculation level**
  - 7 log CFU/g

- **Chicken liver and pâté:**
  - 600 MPa (87,000 psi)
    - 0, instantaneous, 1, 2, & 3 min
  - 483 MPa (70,000 psi)
    - 0, instantaneous, 1, 3, & 5 min

3 trials, 3 replicates per sampling interval
High pressure inactivation of *Salmonella* in liver and pâté

- Inactivation of:
  - ca. 1.7 to 4.9 and 2.3 to 6.0 log CFU/g at 483 MPa and 600 MPa, respectively
  - Regardless of the pressure and time, greater reductions were achieved in livers than pâté
  - Longer times (≥ 1 min) had detrimental effect on color and texture of livers
  - No appreciable effects on quality were observed in pâté
Long Story Short ...

• In general, greater reductions were achieved at:
  • Higher temperature, higher pressure, and longer exposure times

• A 5-log reduction was achieved when pâté prepared and cooked to a target internal temperature of $\geq 73.8^\circ C$ in water bath or prepared by cooking livers in a frying pan for $>8$ min (spurious survivors)

• Factors impacting *Salmonella* inactivation in pâté:
  • Multiple flips of the livers during cooking
  • Variability in temperature at the cooking surface, appliances
  • Cooking methods and holding times (e.g., raw livers vs. cooked livers)
  • Differences in formulation (e.g., moisture, salt, and fat content)
  • Strain-to-strain variation, recovery method

• Pressurization can appreciably reduce the risk of salmonellosis associated with consumption of undercooked chicken liver and/or pâté

• Data may be useful for establishing cooking guidelines for pâté
Ongoing experiments...

• Expand the nature and number of samples to be analyzed from retail outlets, farms, and abattoirs – include other species of liver
  o Determine relatedness of isolates retained from positive samples – correlate with virulence potential, poultry species, store type, & viability under food relevant conditions
    • PFGE, antimicrobial susceptibility, WGS, serotyping, etc.
  o Correlate presence/levels and types of isolates recovered with states, brands, stores, geography, season, pre-packed vs/ store packed, species/source, dressing practices, etc.

• Investigate alternative preparation strategies for liver and/or pâté to reduce risk, yet maintain desired organoleptic and sensory properties
  o Conduct challenge studies using different formulations of pâté in combination with chemical and physical interventions
    • Clean label chemicals, HPP, heating parameters, cooking appliances, etc.

• Develop and implement tools and messages to better educate/train employees and consumers about proper methods for preparing and storing pâté
  o Cooking instructions not correct and/or not adequate – must achieve 165°F
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Thank you for your attention!