# Meat as a source of food-borne agents

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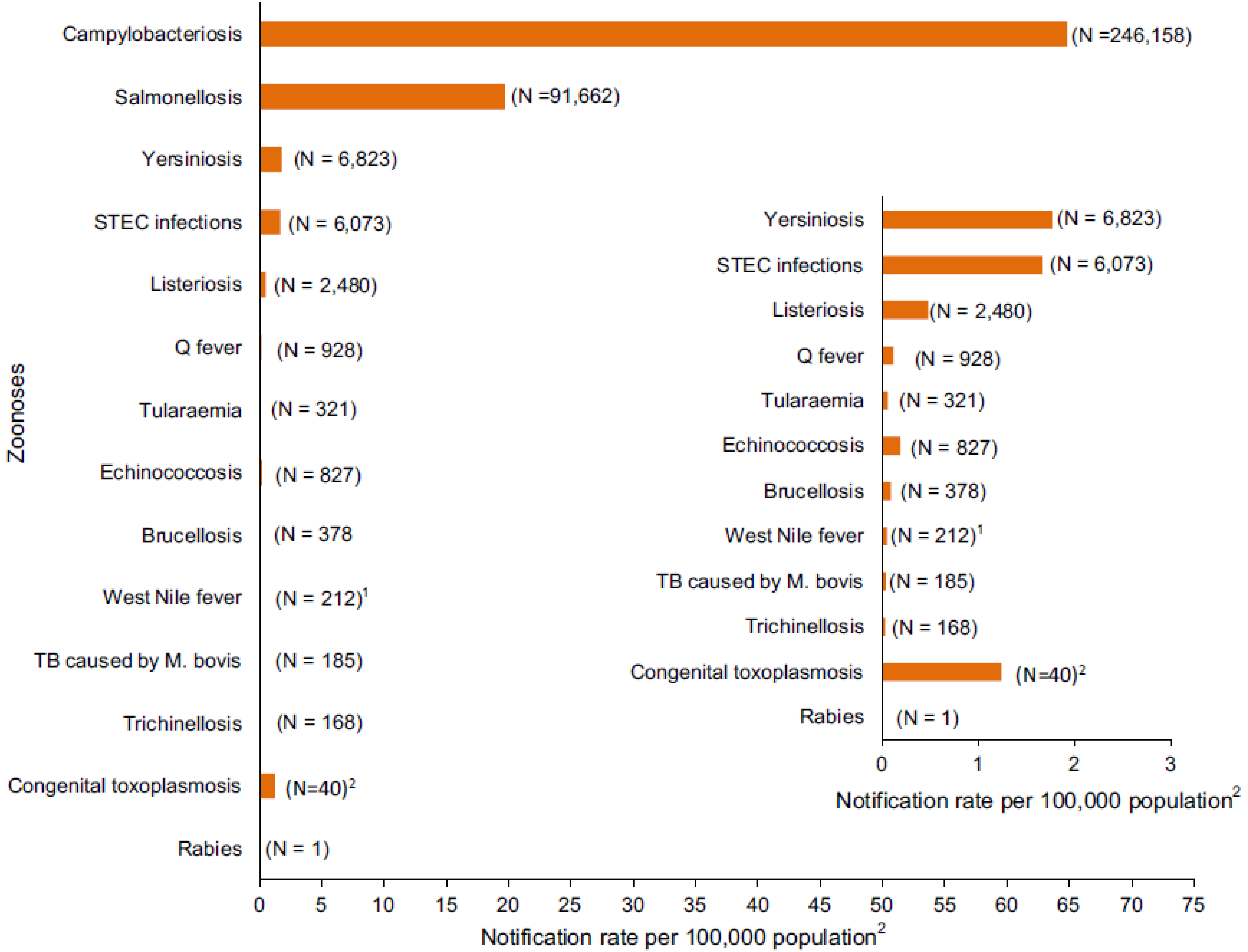
# EFSA report
(2018)



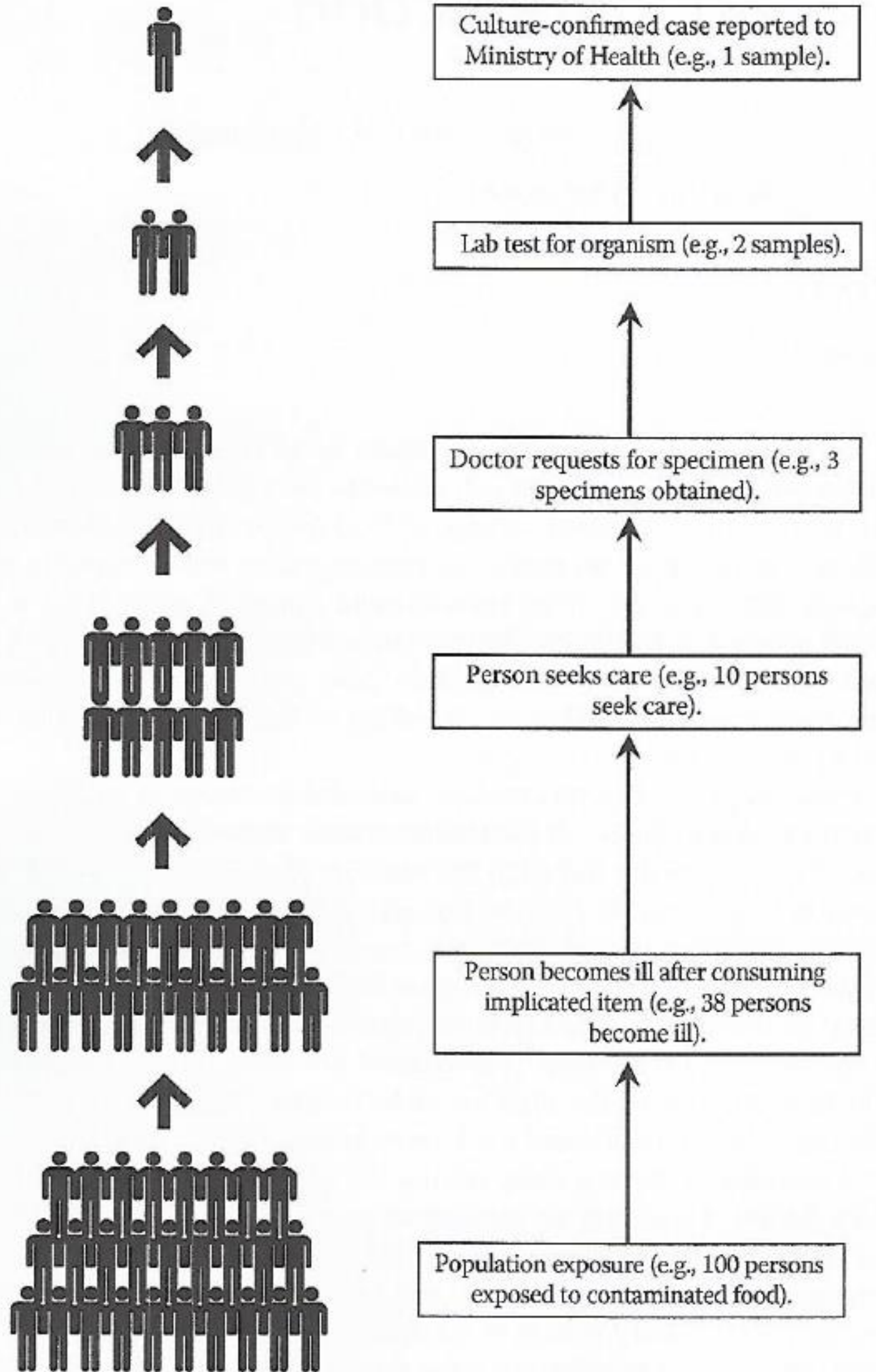
# Foodborne Disease Outbreaks

- two or more confirmed case reports
- linked to the same incident
- involving a pathogen after the ingestion of a common food/ingredient
- **by healthy individuals**

(Manning et al., 2016)

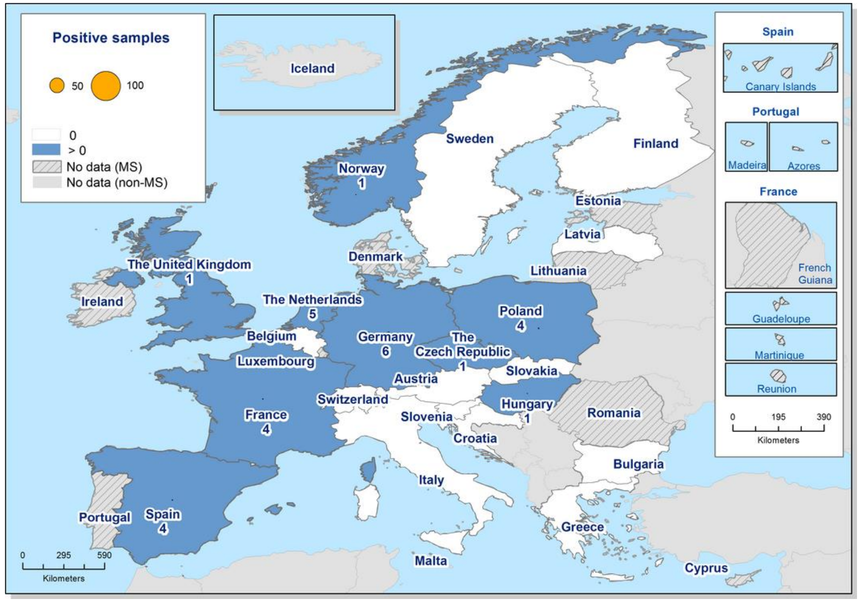
# Surveillance pyramid

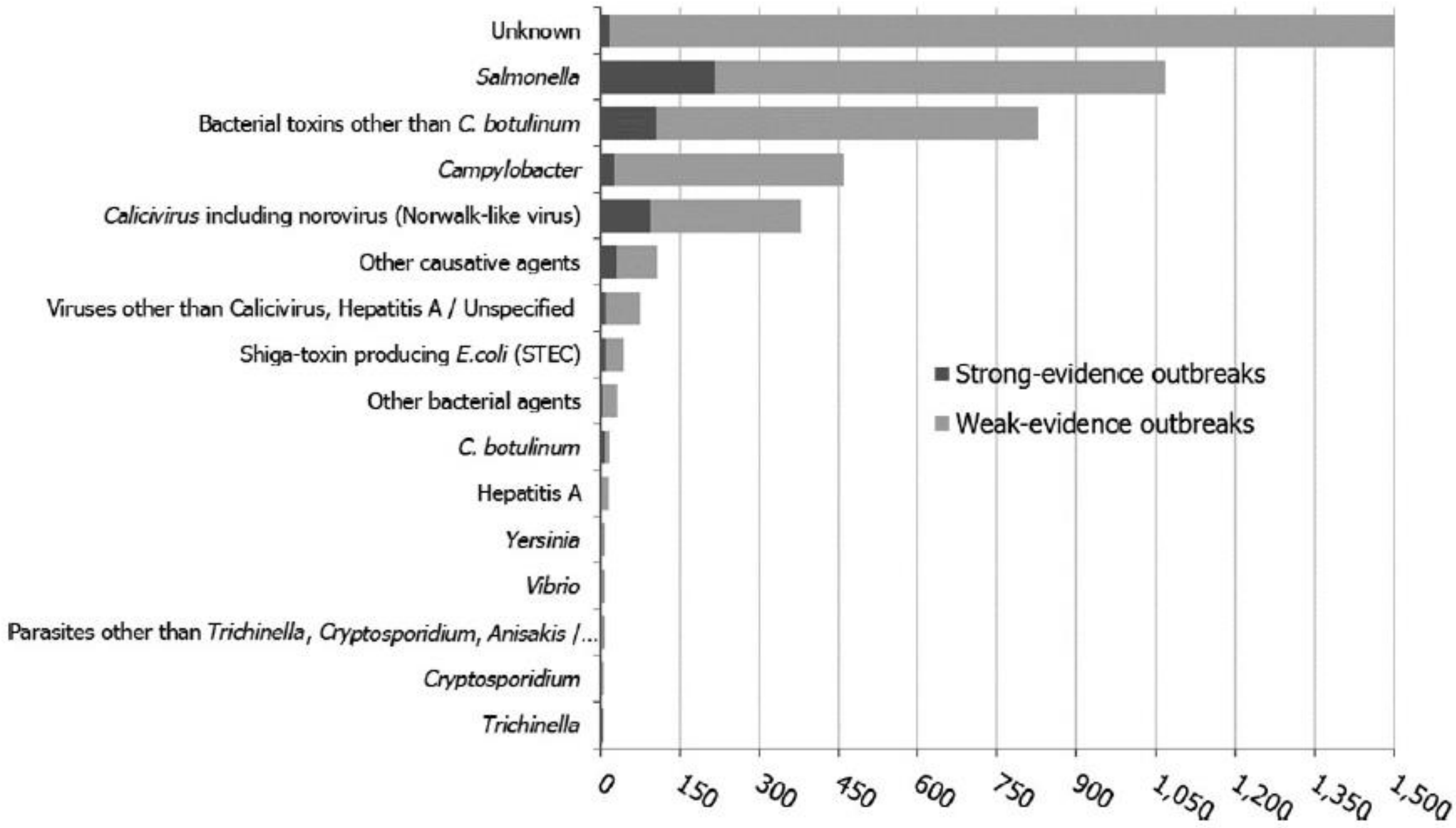
(Manning et al., 2016)



# EFSA report (2016)

- **4 786 outbreaks**
  - bacterial agents: 33.9%
    - *Salmonella*: 22.3% (65. 8% from bacterial agents)
  - bacterial toxins: 17.7%
  - viruses: 9.8%
  - parasites: 0.4%
  - others: 2.2%




Unknown
Salmonella
Bacterial toxins other than C. botulinum
Campylobacter
Calicivirus including norovirus (Norwalk-like virus)
Other causative agents
Viruses other than Calicivirus, Hepatitis A / Unspecified
Shiga-toxin producing E.coli (STEC)
Other bacterial agents
C. botulinum
Hepatitis A
Yersinia
Vibrio
Parasites other than Trichinella, Cryptosporidium, Anisakis /...
Cryptosporidium
Trichinella
Strong-evidence outbreaks
Weak-evidence outbreaks
0
150
300
450
600
750
900
1,050
1,200
1,350
1,500

# EFSA report – strong evidence outbreaks

- the classification of outbreaks as either strong or weak evidence is based on **an assessment of all available evidence**:

    - epidemiological evidence
    - microbiological evidence

# EFSA report – strong evidence outbreaks

- **Epidemiological evidence**:
  - ❑ descriptive epidemiological evidence
  - ❑ analytical epidemiological evidence

- Microbiological evidence

# Descriptive epidemiology

- a story is incomplete if it does not describe the **what, who, where, when, and why/how of a situation**,

- **Epidemiologists strive for similar comprehensiveness** in characterizing an epidemiologic event (**the five W´s**).

# Descriptive epidemiology

- Epidemiologists strive for similar comprehensiveness in characterizing an epidemiologic event (**the five W´s**):
  - What = health issue of concern
  - Who = person
  - Where = place
  - When = time
  - Why/how = causes, risk factors, modes of transmission

# Descriptive epidemiology

- Descriptive epidemiology covers
  - **time**,
    - The occurrence of disease changes over time.
    - Some of these changes occur regularly, while others are unpredictable.
    - For diseases that occur seasonally, health officials can anticipate their occurrence and implement control and prevention measures,
  - **place**,
    - Analyzing data by place can identify communities at increased risk of disease
  - **person**

# Descriptive epidemiology

- descriptive epidemiology can **identify patterns** among cases and in populations **by time, place and person**.

- From these observations, **epidemiologists develop hypotheses:**
  - ❑ **about the causes** of these patterns and
  - ❑ **about the factors that increase risk of disease**.

# Analytic epidemiology

- epidemiologists can use descriptive epidemiology to generate hypotheses, but only rarely **to test those hypotheses**.

- For that, epidemiologists must turn to analytic epidemiology.

- The key feature of analytic epidemiology is **a comparison (control) group**.

# Microbiological evidence

- Detection in food vehicle (or its component) and Detection of indistinguishable causative agent in humans
- Detection in food chain or its environment and Detection of indistinguishable causative agent in humans
- Detection in food vehicle (or its component) and Symptoms and onset of illness pathognomonic of the causative agent found in food vehicle or in food chain or its environment
- Detection in food chain or its environment and Symptoms and onset of illness pathognomonic of the causative agent found in food vehicle or in food chain or its environment

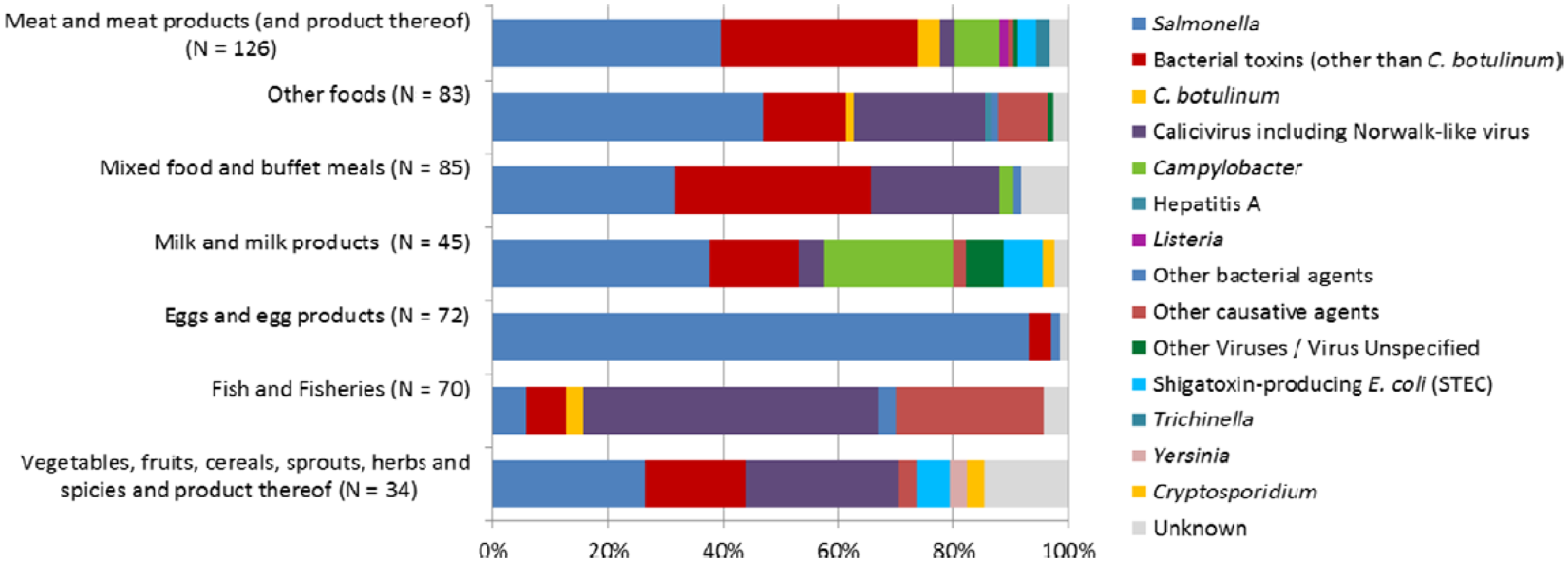
# EFSA report – strong evidence outbreaks

- according to the reporting specifications, an outbreak is defined as either:
    - a household outbreak, in which only members of a single household are affected, or as
    - **a general outbreak**, in which members of more than one household are affected

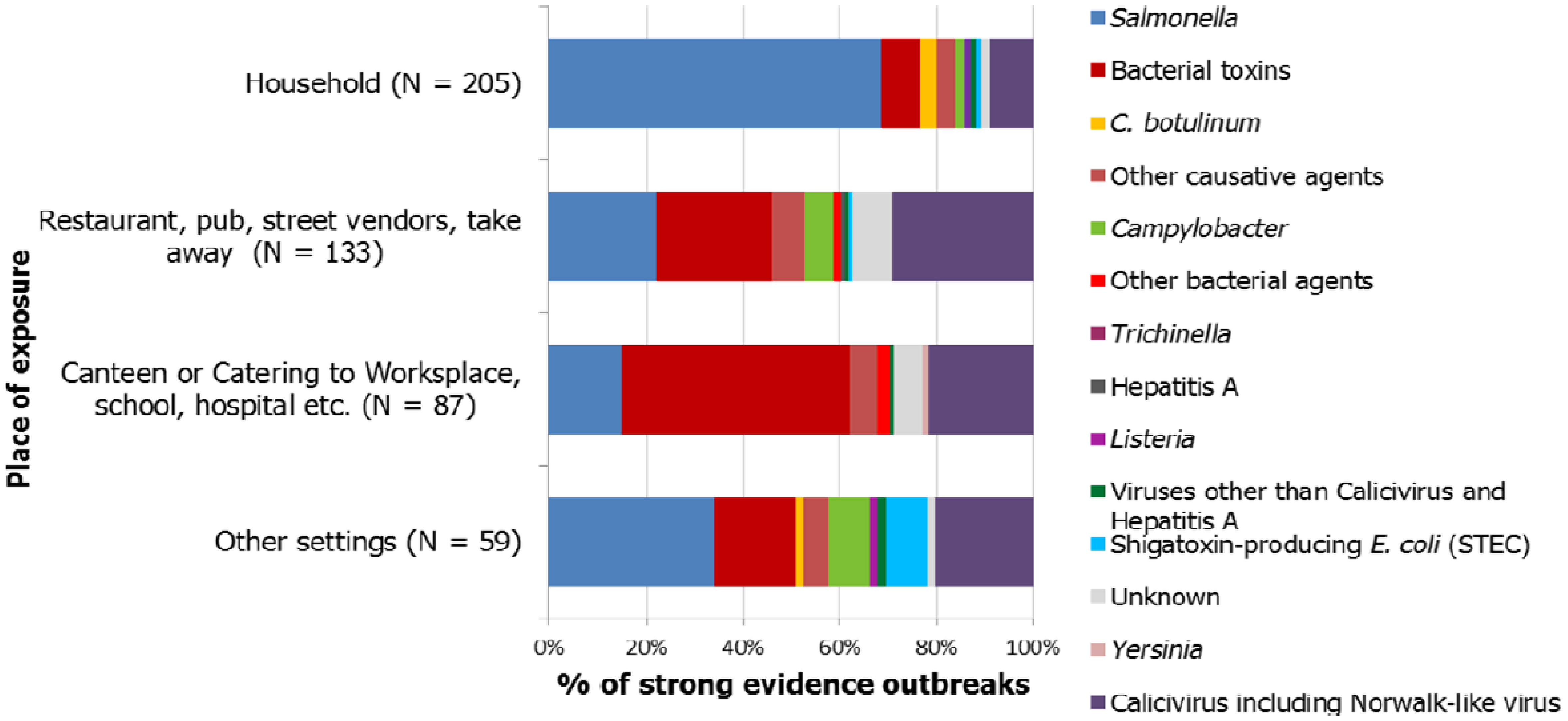
# EFSA report (2016)

- „**strong-evidence**“ outbreaks (n = 521; i. e. 10.9% of the total outbreaks)
  - **food of animal origin** (n = 313)
    - eggs: 23.0 %
    - fish & fisheries: 22.4%
    - meat & meat products: 21.7%
    - poultry meat: 18.5%
    - milk & milk products: 14.4%

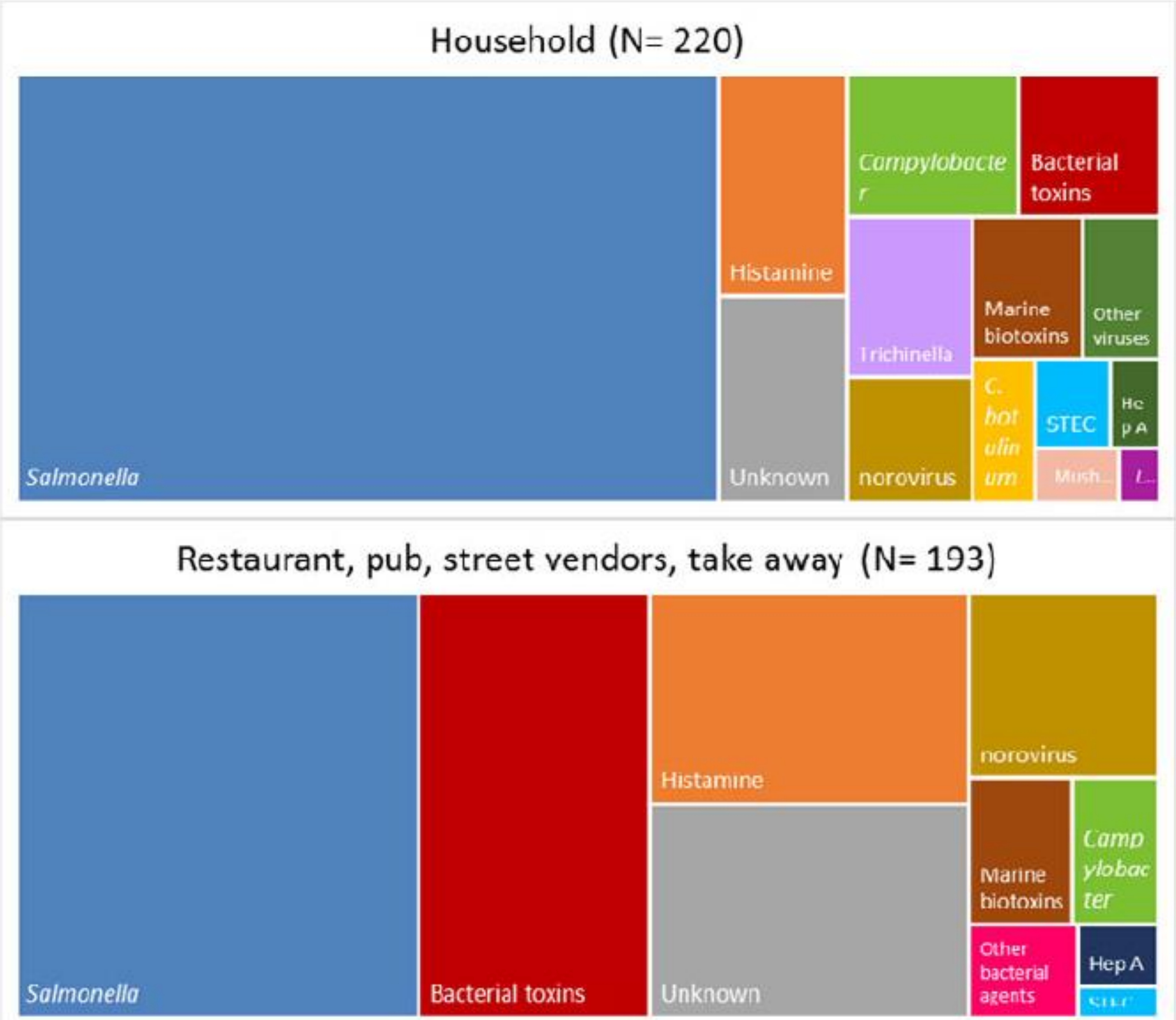
# EFSA report (2016)

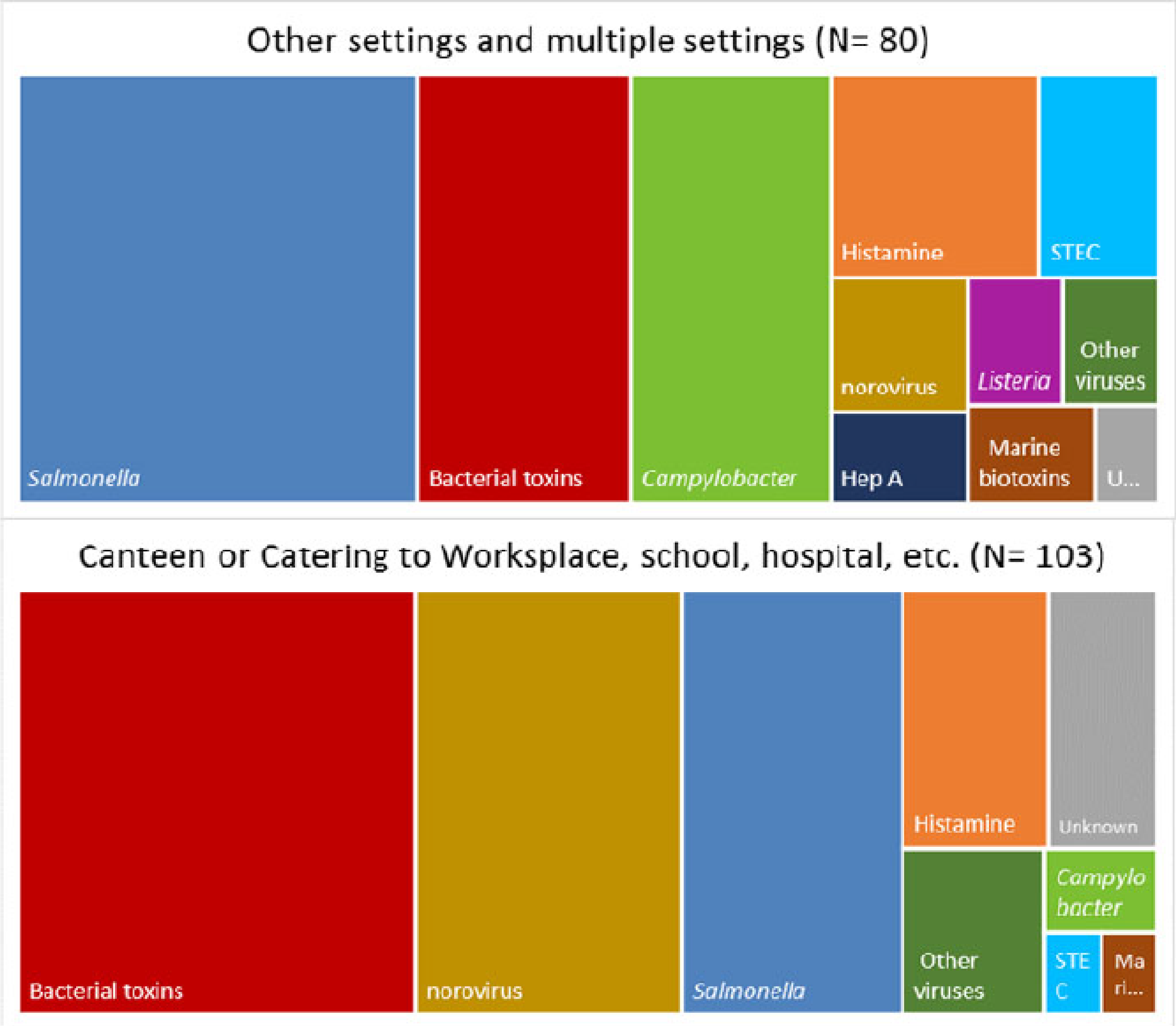


# EFSA report (2016)



EFSA, 2018


Household (N= 220)
Salmonella
Histamine
Unknown
Campylobacter
Bacterial toxins
Trichinella
Marine biotoxins
Other viruses
norovirus
C. botulinum
STEC
Hep A
Restaurant, pub, street vendors, take away (N= 193)
Salmonella
Bacterial toxins
Histamine
Unknown
norovirus
Marine biotoxins
Campylobacter
Other bacterial agents
Hep A

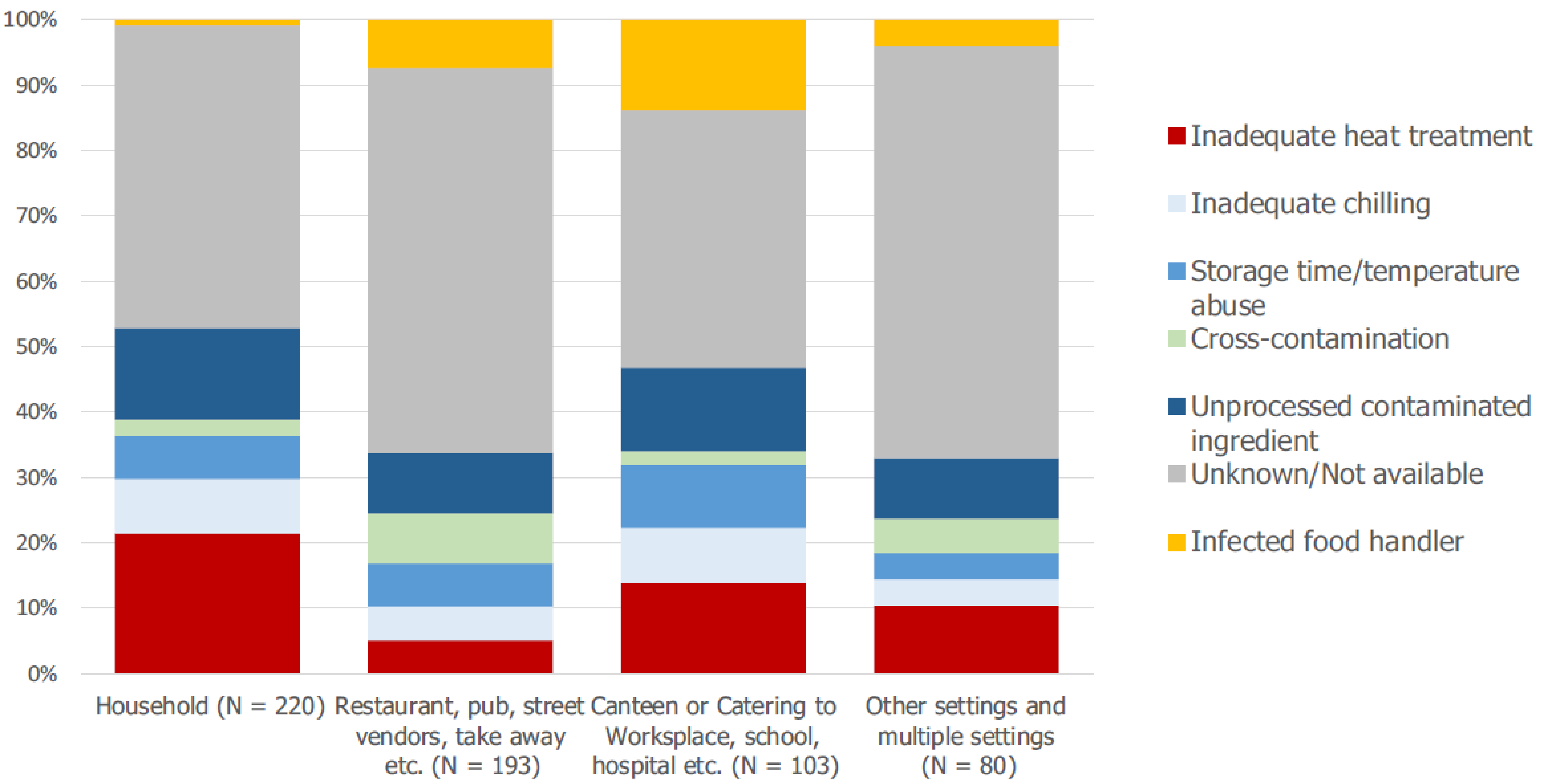


Other settings and multiple settings (N= 80)
Salmonella
Bacterial toxins
Campylobacter
Histamine
STEC
norovirus
Listeria
Other viruses
Hep A
Marine biotoxins
U...
Canteen or Catering to Worksplace, school, hospital, etc. (N= 103)
Bacterial toxins
norovirus
Salmonella
Histamine
Unknown
Other viruses
Campylo bacter
STE C
Ma ri...

# Outbreak contributing factors

- inadequate heat treatment
- an infected food handler
- inadequate chilling
- inaccurate cooling (storage time/temperature abuse)
- cross-contamination
- unprocessed contaminated ingredient
- a combination of different contributory factors

# EFSA (2018)



100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Household (N = 220)
Restaurant, pub, street vendors, take away etc. (N = 193)
Canteen or Catering to Workspace, school, hospital etc. (N = 103)
Other settings and multiple settings (N = 80)
Inadequate heat treatment
Inadequate chilling
Storage time/temperature abuse
Cross-contamination
Unprocessed contaminated ingredient
Unknown/Not available
Infected food handler

# EU: meat as a vehiculum of food-borne agents

(EFSA, 2018)

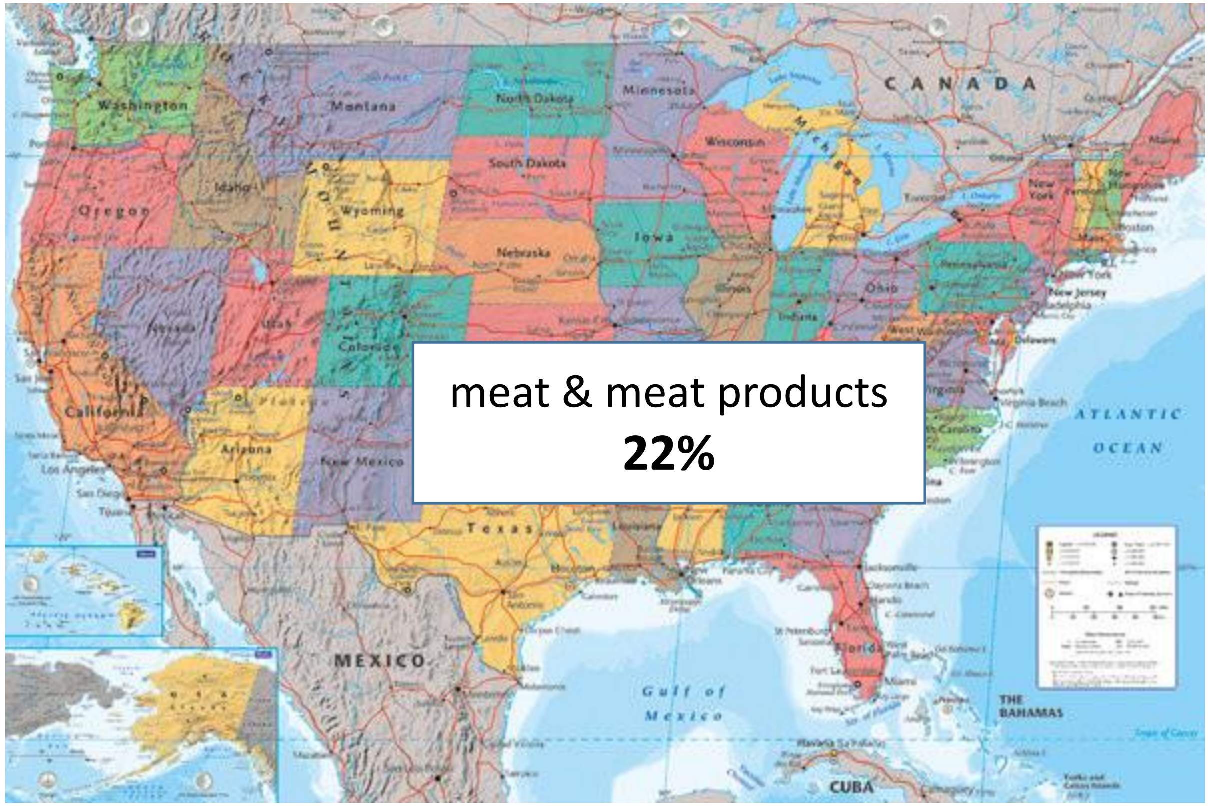
| | | Number of outbreaks* | % of total outbreaks | Number of cases | % of total cases |
|---|---|---|---|---|---|
| **total** | | **639** | **100.0** | **11,844** | **100.0** |
| **meat & meat products** | | **121** | **18.9** | **2,888** | **24.4** |
| * (*strong evidence*) | | | | | |

# EU: meat as a vehiculum of food-borne agents

(EFSA, 2018)

| | | Number of outbreaks* | % of total outbreaks | Number of cases | % of total cases |
|---|---|---|---|---|---|
| **total** | | **639** | **100.0** | **11,844** | **100.0** |
| **Meat & meat products** | **subtotal** | **121** | **18.9** | **2,888** | **24.4** |
| | Poultry meat | 30 | 4.7 | 613 | 5.2 |
| | unspecified | 39 | 6.1 | 681 | 5.7 |
| | pork | 27 | 4.2 | 821 | 6.9 |
| | beef | 13 | 2.0 | 350 | 3.0 |
| | sheep meat | 2 | 0.3 | 110 | 0.9 |
| | other/mixed | 10 | 1.6 | 313 | 2.6 |

# USA: EU: meat as a vehiculum of food-borne agents (Gorton, Stasiewicz, 2017)



# EU: meat as a vehiculum of food-borne agents

(EFSA, 2017)

| | | Number of outbreaks | Number of cases |
|---|---|---|---|
| ***Campylobacter*** | Poultry meat | **9** | **3,231** |
| **Bacterial toxins other than *C. botulinum*** | poultry meat | **25** | **813** |
| ***Salmonella*** | poultry meat | **23** | **328** |
| ***Salmonella*** | meat products | **17** | **307** |
| ***Listeria*** | meat products | **1** | **11** |

# What is the origin of the causative agents of food-borne diseases?

# *Campylobacter* spp.

***Campylobacter*** → poultry meat

- **2017** (EFSA 2018):
  - 37,4 % positive foundings – chicken meat
  - 31,5 % - turkey meat
- *Campylobacter jejuni* nebo *C. coli* (EFSA, 2018).

# *Salmonella* spp.

- ***Salmonella***: less common
  - 2,15 % pig carcasses,
  - 1,58 % pork cuts,
  - 0,17 % beef cuts,
  - 4,85 % chicken meat,
  - 4,18 % turkey meat.

# Shiga-toxigenic *Escherichia coli* (STEC)

- **STEC**:
  - 1,0 % beef cuts
  - 5,3 % sheep meat cuts
  - 3,0 % pork cuts

# The origin of causative agents in meat?

- **Direct occurrence in tissues**
  - rare (exception – parasites)

- **Cross-contamination**
  - **transmission from the carrier (skin, gut)**:
    - hide removal
    - evisceration
  - **from the environment**
    - tools, equipment, work surfaces
    - staff

# Microbiological quality of carcass

The microbiological quality of meat depends on:

- the physiological status of the animal at slaghter
- the spread of contamination during slaughter/processing
- the temperature and other conditions of storage/distribution

# Bacteria on the surface or in the depth of meat?

- bacteria can penetrate **from the surface** of meat where they have been cross-contaminated **to deeper parts**

- bacteria enter the meat **between the muscle fibers** after the post mortem stiffness - *rigor mortis*

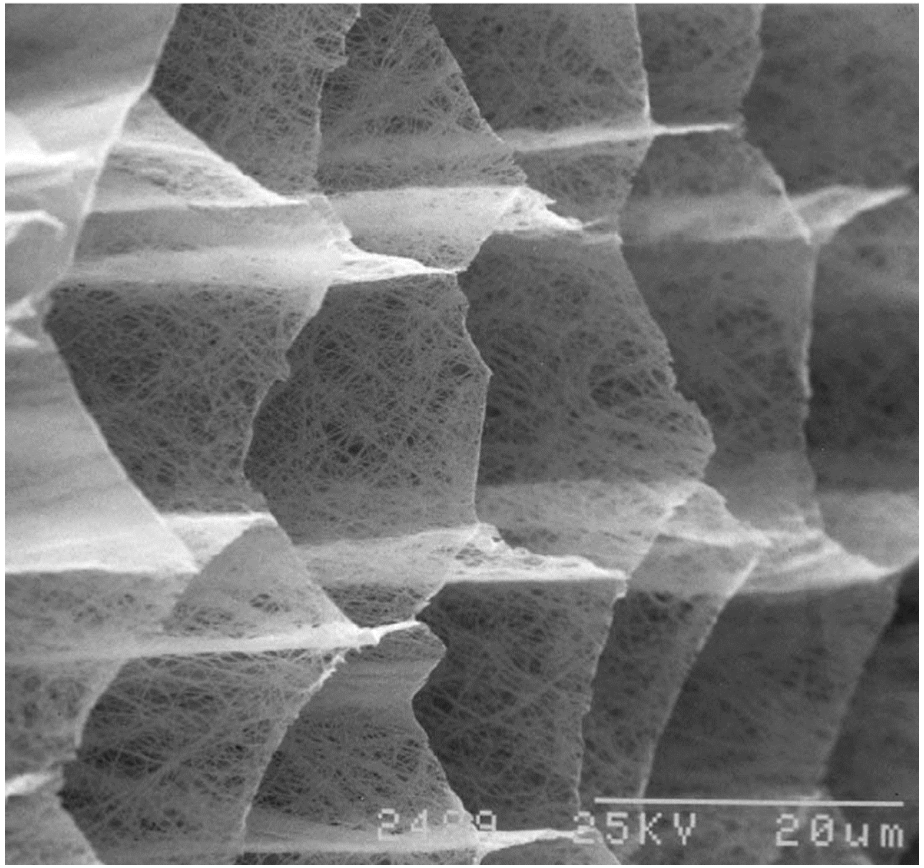
# Bacteria on the surface or in the depth of meat?

- bacteria enter the meat **between the muscle fibers** after the post mortem stiffness - *rigor mortis*

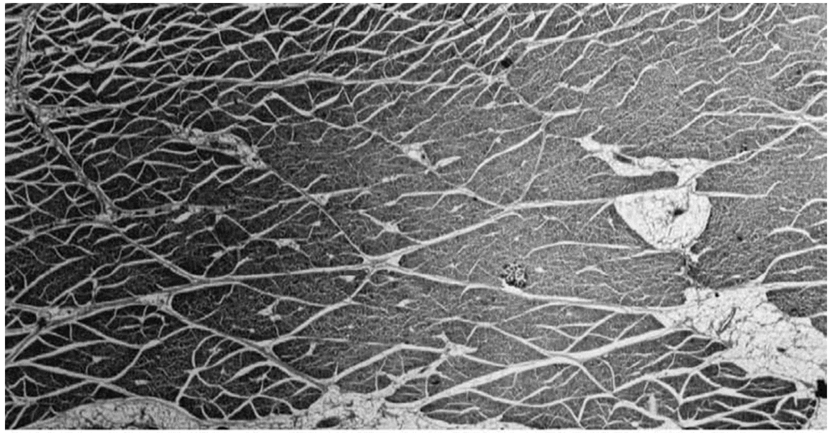
- **Before the onset of *rigor mortis* bacteria are not able to overcome muscle structures - endomysium is closely associated with muscle fibers**

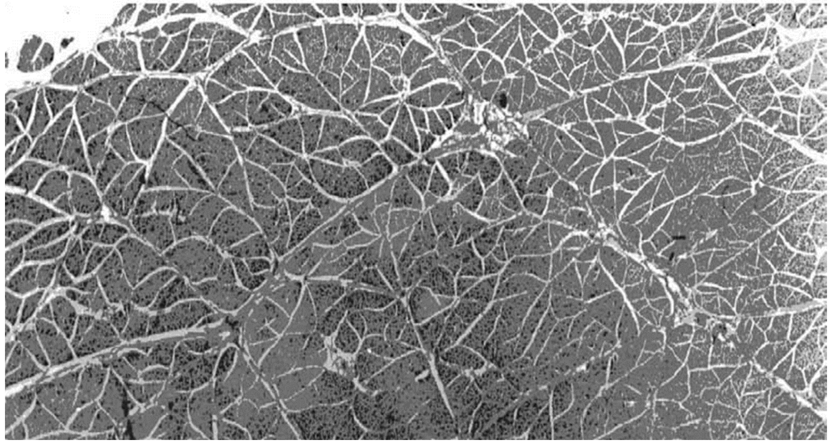
# Intramuscular connective tissue

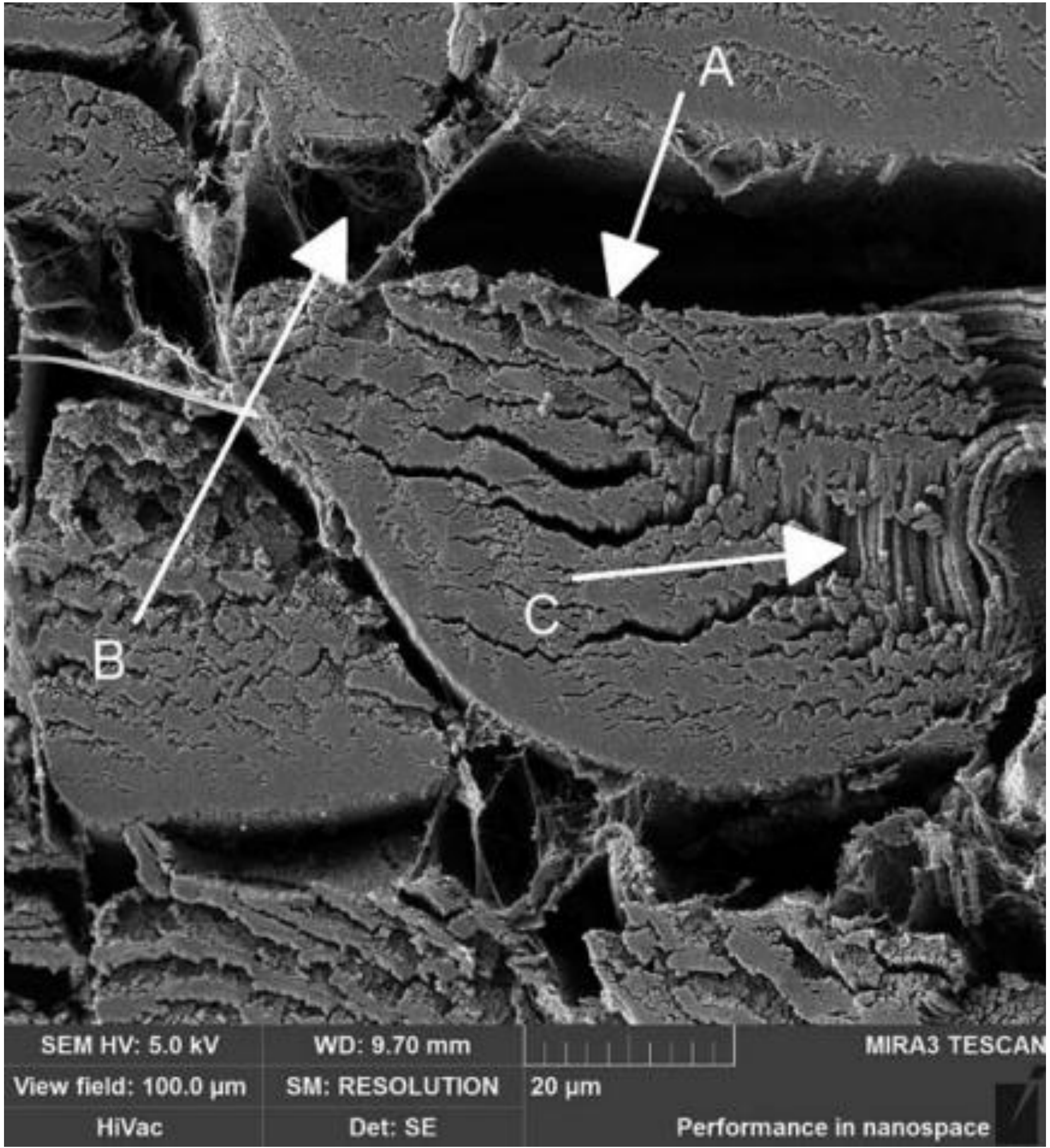
**Endomysium**




**Perimysium**







A
B
C
SEM HV: 5.0 kV
WD: 9.70 mm
MIRA3 TESCAN
View field: 100.0 μm
SM: RESOLUTION
20 μm
HiVac
Det: SE
Performance in nanospace

# Bacteria on the surface or in the depth of meat?

- contraction of muscle fibers on the advent of rigor mortis creates **gaps between contractile elements and the surrounding endomysium**
- **The resulting spaces offer a way for bacterial invasion from the meat surface into its deeper layers.**
- **This process does not require the proteolytic activity of the ingressing bacteria.**

# Survival of salmonella during meat cooking

- Heat treatment of meat - the most effective method of eliminating vegetative bacteria causing food-borne diseases

- The combination of temperature and time of **70 ° C for 2 min** guarantees **a reduction of more than 6 log orders** (99.9999%) of vegetative forms of bacteria.

# Equivalent heat treatment to reduce *Listeria monocytogenes* by 6 log (Stringer, Metris, 2018)

| Temperature (°C) | time (min) |
|---|---|
| 60 | 43,5 |
| 65 | 9,3 |
| 70 | 2,0 |
| 75 | 0,4 |
| 80 | 0,09 |
| 85 | 0,02 |

# Survival of salmonella during meat cooking

- for **chicken breast fillets**:
  - $D_{55}$ (temp. 55 °C) 24,0 min,
  - $D_{60}$ 3,83 min,
  - $D_{70}$ 0,10 min

- for **duck breast meat**:
  - $D_{55}$ 28,6 min,
  - $D_{60}$ 6,79 min,
  - $D_{70}$ 0,11 min

# Survival of salmonella during meat cooking

- If the contamination of poultry meat with salmonella occurs, then naturally, **the probable level of contamination is 10 cfu/g**, resp. even less.

- If the number of cfu/g is higher (100-1000/g), this means **bacterial cell proliferation due to inappropriate storage at higher temperatures.**

# Survival of salmonella during meat cooking

- If the number of cfu/g is higher (100-1000/g), this means bacterial cell proliferation due to inappropriate storage at higher temperatures.

- Analysis of food-borne outbreaks revealed that the number of salmonella bacterial cells received by patients was of $10^6$/person **(= infectious dose)**

# Temperature resistance of selected vegetative bacteria

| Bacterium | D-value (min) at: | | | | z-value (°C) |
|---|---|---|---|---|---|
| | 70 °C | 65 °C | 60 °C | 55 °C | |
| *Escherichia coli* | | | | 4 | |
| *Salmonella* Senftenberg | | | 6,1 | | 6,8 |
| *Salmonella* Typhimurium | | 0,056 | | | |
| *Staphylococcus aureus* | | | 7,8 | | 4,5 |

# How does high temperature affect bacteria?

- **D-value**:
  - time (in minutes) needed for decimal reduction (by 1 log = 90%) at given temperature

- **z-value**:
  - the change in temperature (in ° C) required to reduce the D-value decimally

# How does high temperature affect bacteria?

(chicken breast fillets, Karyotis et al., 2017)

| **Temp. (°C)** | **D-value (min)** | | | |
|---|---|---|---|---|
| | *Listeria monocytogenes* | | *Salmonella* spp. | |
| | control | marinated | control | marinated |
| **55** | 54,8 | 45,1 | 47,7 | 34,1 |
| **57,5** | 14,9 | 11,6 | 12,0 | 10,4 |
| **60** | 10,4 | 7,3 | 7,5 | 5,9 |

# Growth ability and resistance of bacterial species/genera in food

- **Vegetative bacteria without the ability to grow in cold temperatures**:
    - *E. coli*
    - *Salmonella* spp.
    - *Staphylococcus aureus*
    - *Campylobacter* spp.

# Growth ability and resistance of bacterial species/genera in food

- Vegetative bacteria without the ability to grow in cold temperatures
- **Vegetative bacteria with the ability to grow in cold temperatures**:
  - *Listeria monocytogenes*
  - *Yersinia enterocolitica*
  - *Aeromonas*

# Growth ability and resistance of bacterial species/genera in food

- Vegetative bacteria without the ability to grow in cold temperatures
- Vegetative bacteria with the ability to grow in cold temperatures

- **Psychrotrophic sporogenic bacteria (with the ability to grow at cold temperatures):**
  - nonproteolytic *Clostridium botulinum* (group II),
  - psychrotrophic *Bacillus cereus*

# Growth ability and resistance of bacterial species/genera in food

- Vegetative bacteria without the ability to grow in cold temperatures
- Vegetative bacteria with the ability to grow in cold temperatures
- Psychrotrophic sporogenic bacteria (with the ability to grow at cold temperatures)
- **Mesophilic sporogenic bacteria without growth at cold temperatures**

# Growth ability and resistance of bacterial species/genera in food

- Psychrotrophic sporogenic bacteria (with the ability to grow at cold temperatures)
- **Mesophilic sporogenic bacteria without growth at cold temperatures:**
    - proteolytic *C. botulinum, C. perfringens,* mesophilic *B. cereus*

# Adequate food handling during preparation

- 40-60% of foodborne diseases → inadequate food handling during preparation

- **cross-contamination or undercooking**?

# Adequate food handling during preparation

- **poultry meat** as a source of cross-contamination to:
  - other food products
  - hands of cook
  - surfaces during preparation
    - wooden/plastic cutting boards
    - knives

- **bacterial cells require only a few minutes to attach to a surface**
- they are often difficult to remove after attachment

# Adequate food handling during preparation

- most of the transfer rates for *Salmonella* spp. & *Campylobacter* spp.: ~ 0 to 3% (*Sarjit, Dykes,* 2017)

- after the surfaces were **rinsed with water/rinsed with water & wiped with a kitchen towel** → reduction by 0.3-4.1 log

# Case study: *E. coli* O157:H7 outbreak associated with restaurant hamburgers

- **Shiga toxin-producing *Escherichia coli*** (STEC)
  - serotype O157:H7: a significant risk to public health
  - emerged in the 1980s
  - ruminants (cattle, sheep) as zoonotic reservoir
  - **infectious dose is low (10-100 organisms)**
  - outbreaks linked to the consumption:
    - contaminated undercooked beef
    - contaminated raw vegetables & salad leaves

# Case study: *E. coli* O157:H7 outbreak associated with restaurant hamburgers

- October 2013, Pennsylvania, USA:
  - **14 confirmed (9 employees) & 10 probable (3 employees) cases**
  - the median age 25 years (range, 3 to 72)
  - symptoms: diarrhea (92 %), vomiting (37 %), abdominal cramps (87 %); 8 cases hospitalized
  - **100 % confirmed cases ate restaurant (A) beef burgers**
  - **71 % confirmed cases ate burger rare to medium**

# Case study: *E. coli* O157:H7 outbreak associated with restaurant hamburgers

- **deficiencies at restaurant A**:
  - improper temperature holding
  - unsafe cooling
  - possibility for cross-contamination

- 78% samples of ground beef were positive for *E. coli* O157:H7

# Case study: *E. coli* O157:H7 outbreak associated with restaurant hamburgers

- **contaminated boxed beef introduced pathogen into the ground product**

- hamburgers were undercooked

# Case study: *E. coli* O157:H7 outbreak associated with restaurant hamburgers

- **general rules:**
  - there is **no guarantee that beef will be free** from *E. coli* O157:H7
  - CDC recommendation: **to cook beef to an internal temperature of 160 °F (71 °C)**
  - *E. coli* O157:H7 is killed at temperature of 155 °F (68,3 °C)
    - **rare:** 52-55 °C/4-5 min
    - **medium rare:** 55-60 °C/6-7 min
    - **medium:** 60-65 °C/8-9 min
    - **well done:** 70-100 °C/12 min

# Temperature for food storage

- USA: „**a rule 40-140**“
  - store food at temperatura < 40 °F (4,4 °C)
  - store food at temperature > 140 °F (60 °C)