Overview on biological hazards and detection methods

Dr. Anselm Lehmacher

Institut für Hygiene und Umwelt, Hamburg

Microbiological hazards by spices and dried herbs

- **Not only salmonellae!**
- **But also spore-formers:**
- ***Bacillus cereus* and bacilli of the *Bacillus subtilis* group**
- ***Clostridium perfringens* and *Clostridium botulinum***
- **One recall because of *Listeria monocytogenes* (Vij et al., 2006)**
- ***Staphylococcus aureus* detected occasionally with numbers below 10^3 cfu/g (Leitao et al., 1974; Donia, 2008)**

Prevalence and number of *Bacillus* in spices and dried herbs

Year	Country	No. of samples	Prevalence[a] of *Bacillus* (not *B. cereus*) (%)	Range of *Bacillus* (not *B. cereus*) numbers (cfu/g)	Prevalence[a] of *B. cereus* (%)	Range of *B. cereus* numbers (cfu/g)	Authors
1995	Netherlands	6	100	10^3 - 10^5	100	10^2 - 10^6	te Giffel et al. (1996)
1995	Italy	200	88	10^2 - 10^7	4	10^3 - 10^5	Giaccone et al. (1996)
1999	UK	425	63 (28 ≥ 10^4)	10^2 - 10^7	27 (2 ≥ 10^4)	10^2 - 10^6	Little et al. (2003)

[a]Lower limit of detection: 100 or 200 cfu/g (Little et al., 2003)

Giaccone et al. (1996), Little et al. (2003) Sagoo et al. (2009): The *Bacillus subtilis* group prevails among *Bacillus* spp., especially *B. subtilis, B. licheniformis*, and *B. pumilus*.

Bacillus subtilis group bacteria overgrowing *B. cereus* in spices and dried herbs

1:100 dilution of ginger on

MYP agar **BACARA™ agar**

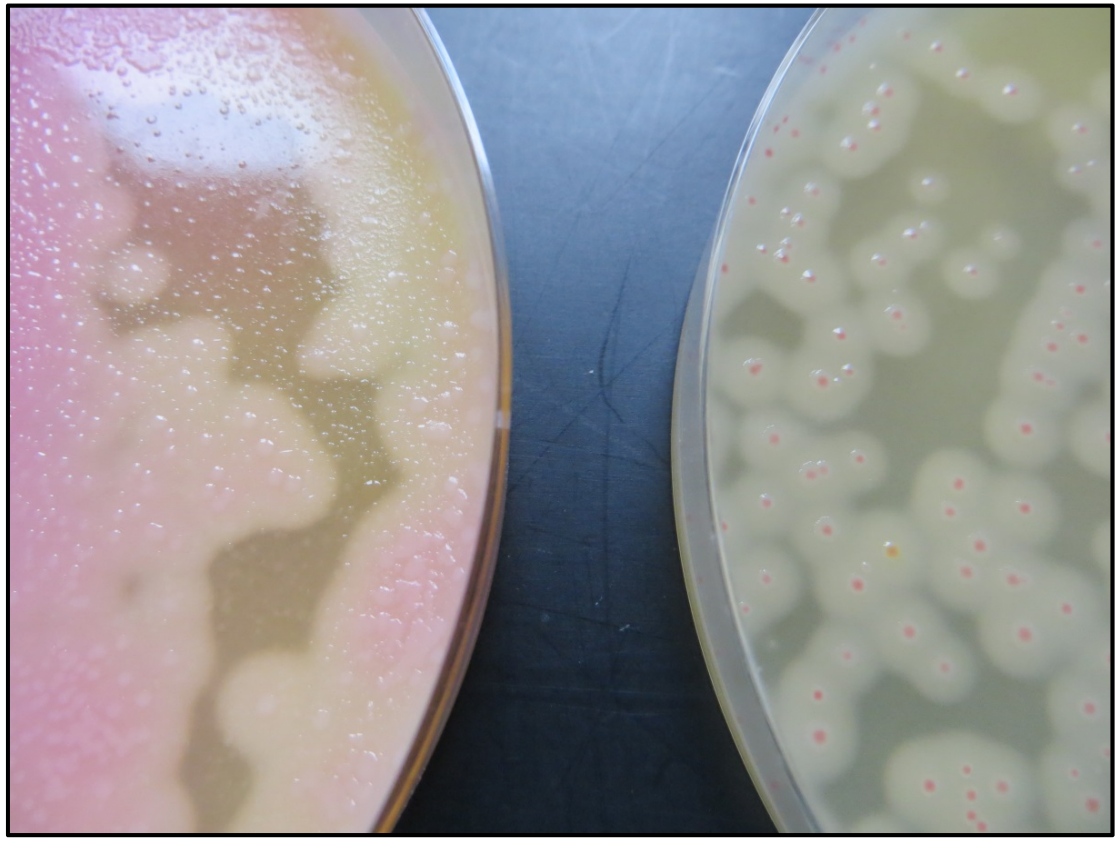


Photo: Lehmacher

Small outbreaks traced to spices contaminated with strains of the *Bacillus subtilis* group

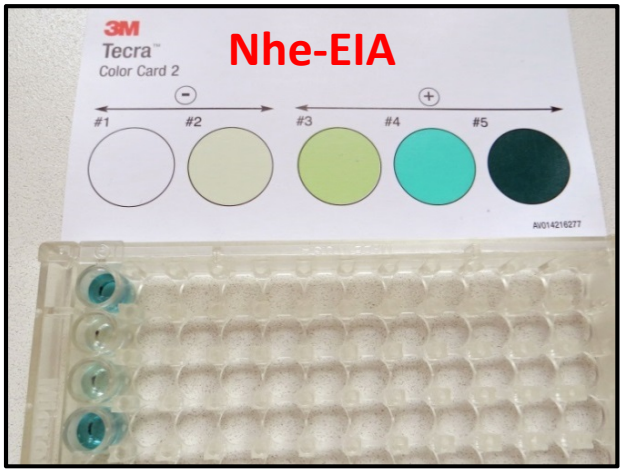
Year	Country	Source	*Bacillus* spp.	Cases	Reference
1995	England & Wales	Turmeric (kebab)	*B. subtilis* & *B. pumilus*	2	Little et al. (2003), Little (2012), HPA (2011)
1997	New Zealand	Pepper	*Bacillus subtilis*	2	Cameron (1998)

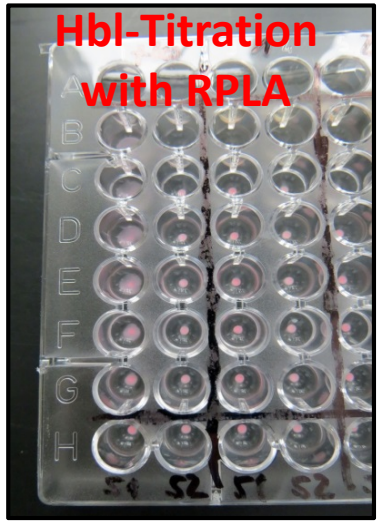
Number of *Bacillus cereus* in spices and dried herbs

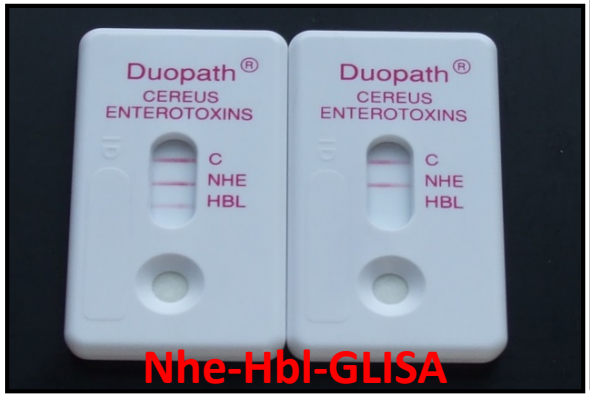
Year	Country	No. of samples	Number (% of samples)			Authors
			$< 10^2$ cfu/g	10^2 - $< 10^4$ cfu/g	$\geq 10^4$ cfu/g	
1973-74	Brasil	111	80	20	0	Leitao et al. (1974)
1975	USA	110	59	41	0	Powers et al. (1976)
1983	Netherlands	150	53	45	2	de Boer et al. (1985)
1986	Australia	523	50	43	7	Pafumi (1986)
1999	UK	425	80	18	2	Little et al. (2003)
2004	UK	2833	≤ 90	≥ 8	2	Sagoo et al. (2009)
2014	USA	247	96	4	0	Hariram & Labbé (2015)

Toxin production of *B. cereus* strains from spices and dried herbs

Commercially available Nhe- and Hbl-test kits:







Photos: Lehmacher

Enterotoxigenicity of isolates from spices:

- Powers et al. (1976): 89% of *B. cereus* isolates (n = 88) were enterotoxigenic (rabbits - vascular permeability assay).
- Hariram and Labbé (2015): 82% Nhe and 64% Hbl enterotoxin-producers (n = 77 *B. cereus* isolates)

Emetic toxigenicity of isolates from spices:

- Roberts et al. (1982): No serotypes of emetic *B. cereus* (n = 59 isolates).
- Hariram and Labbé (2015): No (n = 77 isolates) *ces* gene of cereulide found.
- Messelhäusser et al. (2014): Detection of emetic *B. cereus* in 11 of 135 dried mushroom samples.

Outbreaks traced to *B. cereus*-contaminated spices

Year	Country	Source	*B. cereus*/g	Cases	Reference
1975	Finland	Garlic salt & black pepper mix	1 x 10^2 (garlic salt), 4.5 x 10^3 (pepper)	18 (50% attack rate)	Raevuori et al. (1976)
2007	France	Spice blend used in couscous	?	146	van Doren et al. (2013)
2009	Belgium	Curry powder	?	7	EU (2010)
2009	Denmark	Paprika	?	48	Anonymous (no date)
2010/11	Denmark	Black pepper (1 x to season stew)	?	112 & 52 (2 outbreaks)	EFSA (2013), van Doren et al. (2013)
2011	Denmark	Cinnamon	5 x 10^3	30	EU (no date)
2011	Finland	Turmeric	?	23 (2 outbreaks)	EFSA (2013)
2011	USA	Cumin	1.6 x 10^4 (& *C. perfringens* & *S.* Caracas)	3	EFSA (2013)

Intoxication by *Bacillus cereus*

Soil – Dust:

- Crop plants, pasture

Enterotoxigenic *B. cereus*: Spices/herbs and dairy products

Emetic *B. cereus*: Rice and pasta

Inadequate preparation:

- **heating, warming, cooling, dwell time: growth: ≥ 10^5 cfu/g**

  Enterotoxigenic *B. cereus*: growth in food; production of heat-labile enterotoxins in the gut

  Emetic *B. cereus*: Cereulide (emetic toxin) production in food

Patient:

- **Enterotoxigenic *B. cereus*: Toxi-infection ► Diarrhoea**
- **Emetic *B. cereus*: Intoxication ► Emesis**

Number of *C. perfringens* in spices and dried herbs

Year	Country	No. of samples	Number (% of samples)			Authors
			$< 10^2$ cfu/g	10^2 - $< 10^3$ cfu/g	$\geq 10^3$ - 10^4 cfu/g	
1973-74	Brasil	111	98	2	0	Leitao et al. (1974)
1975	USA	114	89	10	2	Powers et al. (1975)
1983	Netherlands	150	41	57	2	de Boer et al. (1985)
1986	Australia	523	77	16	7 (0,4% > 10^4 cfu/g)	Pafumi (1986)
1998	Mexiko	300	96	4	0	Rodriguez-Romo et al. (1998)
2004	Argentina	115	97	3	0	Aguilera et al. (2005)
2004	UK	2833	96	3	0,4	Sagoo et al. (2009)

Prevalence and detection of *cpe*-positive *Clostridium perfringens* in spices and dried herbs

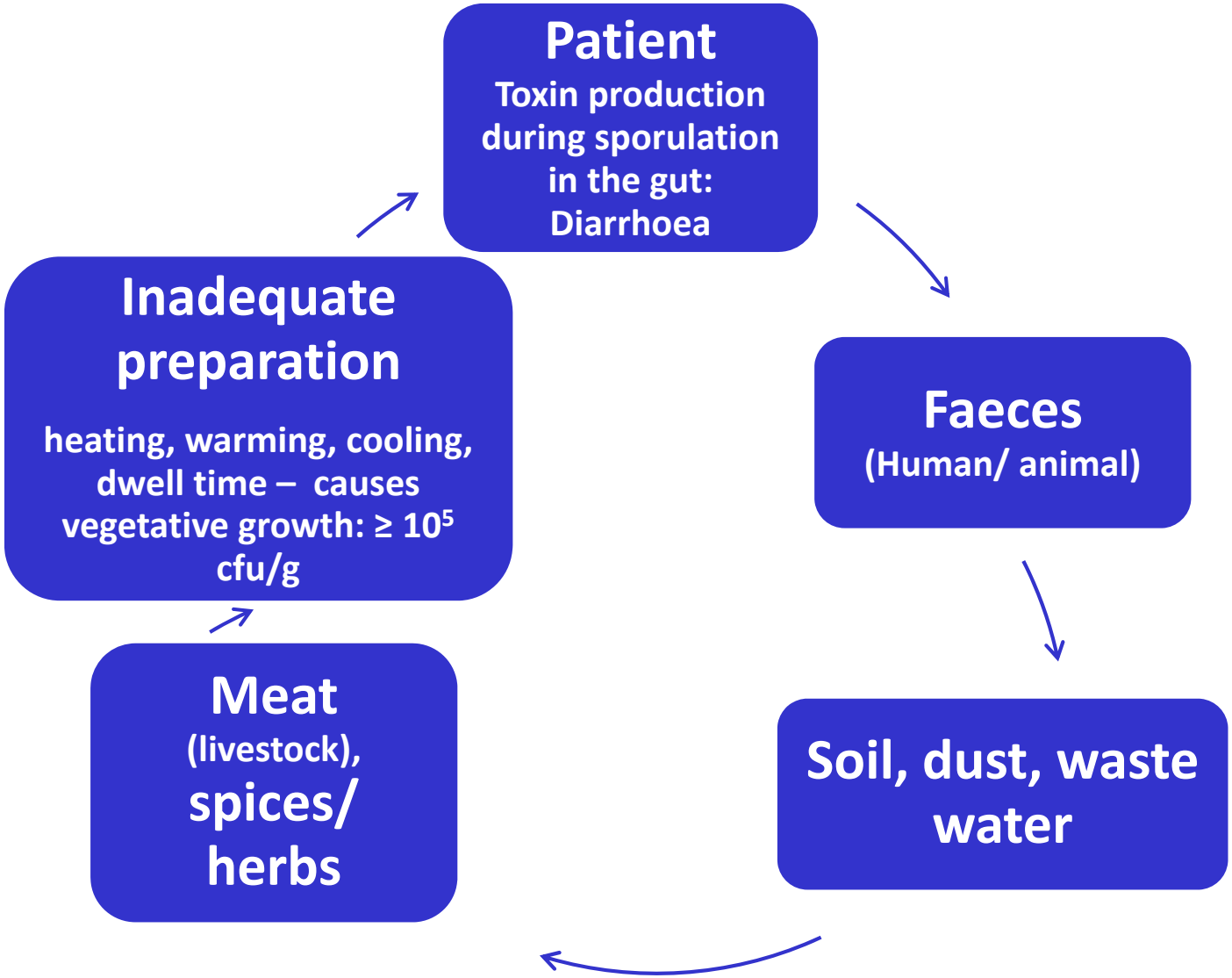
Year	Country	Detection method	Prevalence of *cpe*-positive strains	Number of *cpe*-positive strains (cfu/g)	Authors
1996	Mexiko	Isolation, confirmation, dot blot hybridization	4.3% of *Clostridium perfringens* isolates (n = 188)	n. d.	Rodriguez-Romo et al. (1998)
2004	Argentina	Isolation, confirmation, RPLA, PCR	3.5% of samples (n = 115)	≤ 1100	Aguilera et al. (2005)
2013	Germany	Selective agar, real-time PCR	1.5% of samples (n = 67)	20	Bauer & Lehmacher (2014)

n. d.: not determined

Outbreaks traced to *C. perfringens*-contaminated spices in 2011

Country	Source	*C. perfringens*/g	Cases	Reference
Denmark	Dried chilies	?	3	EU (no date)
Denmark	Red pepper	?	37	EU (no date)
Denmark	Black pepper	$3{,}3 \times 10^8$	19 (2 outbreaks)	EU (2012)
Denmark	BBQ spices	?	4	EU (no date)
Finland	Cumin	180 (& *B. cereus*, & *S.* Caracas)	3	EFSA (2013)

Transmission of enterotoxigenic *Clostridium perfringens*



Cases traced to *C. botulinum*-contaminated spices and herbal infusion

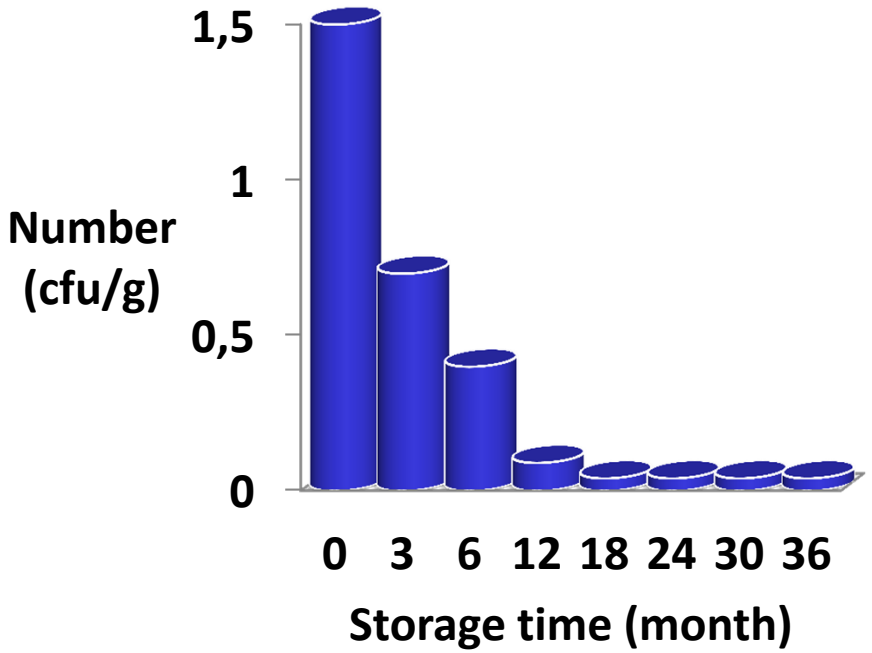
Year	Country	Source	*C. botulinum*/g	Cases	Reference
1984	Japan	Mustard with fried lotus rhizomes	? (Neurotoxin A)	36 (11 deaths)	Otofuji et al. (1987)
1985	Canada	Garlic in soybean oil (pH 4.5)	? (Neurotoxin B)	36	St. Louis et al. (1988)
1989	USA	Garlic in olive oil (pH 5.7)	> 10/g garlic (Neurotoxin A)	3 (42 to 45 yr old)	Morse et al. (1990)
2002	Denmark	Canned garlic in chilli oil	? (Neurotoxin B)	1 (38 yr old)	Lohse et al. (2003)
2009	Portugal	Chamomile tea/ honey (both positive)	? (Neurotoxin B)	1 (Infant botulism)	Saraiva et al. (2012)

Water for growth and toxin production?

Prevalence of *Salmonella* in spices and dried herbs

Year	Country	No. of samples	Stage of food chain	Prevalence (%)	Reference
1979-1980	UK	100	Retail	1	Roberts et al. (1982)
1986	Australia	97, 66, and 14, respect.	Shipment	8.2 (black peppercorn), 1.5 (white peppercorn), 7.1 (fenugreek seed)	Pafumi (1986)
1987	Spain	83 (pepper)	Retail	4.8	Salmeron et al. (1987)
1997-1998	Germany	975	Production	2.6	Schmoll (1999)
1994-2005	Germany	5754	Production	4.3	Hartwig et al. (2006)
2004	UK	2833	Retail	1.1	Sagoo et al. (2009)
2004-2006	Brazil	236	Retail	5.6	Moreira et al. (2009)
2007-2009	USA	2844	Shipment	6.6	van Doren et al. (2013)

Survival of *Salmonella* in spices and dried herbs



Salmonella survive in naturally contaminated food at least

- 3 years in spice mixture of potato chips (Lehmacher et al., 1995; figure on the left) and dried mushrooms
- 1 year in black pepper (ICMSF, 1996)
- 6 months in dried oregano, elderflowers, and a cake spice mixture (Schmoll, 1999)

In artificially contaminated black pepper at least 1 year (Keller et al., 2013)

Detection of „resting“ *Salmonella* in spices and dried herbs

- **Resuscitation with pyruvate and ferrioxamine E (Schmoll, 1999)**
- **Inhibition by sulfite recommended (Andrews et al., 1979) for the preenrichment of *Salmonella* from onions and garlic (Schmoll, 1999)**
- **Dilution of inhibitory essential oils of spices and herbs with buffered peptone water 1:20 to 1:1000 (e.g. pepper, paprika, ginger, majoram, basil, pimento, clove, cinnamon, oregano: Pafumi, 1986; DIN V 10224)**
- **Feeding of Fe^{3+}-supplemented mice with 10^6 cfu did not resuscitate viable-but-not-culturable-*Salmonella* (Claußen, 2000).**

Dried *Salmonella* stained with SYTOX-Green and CTC

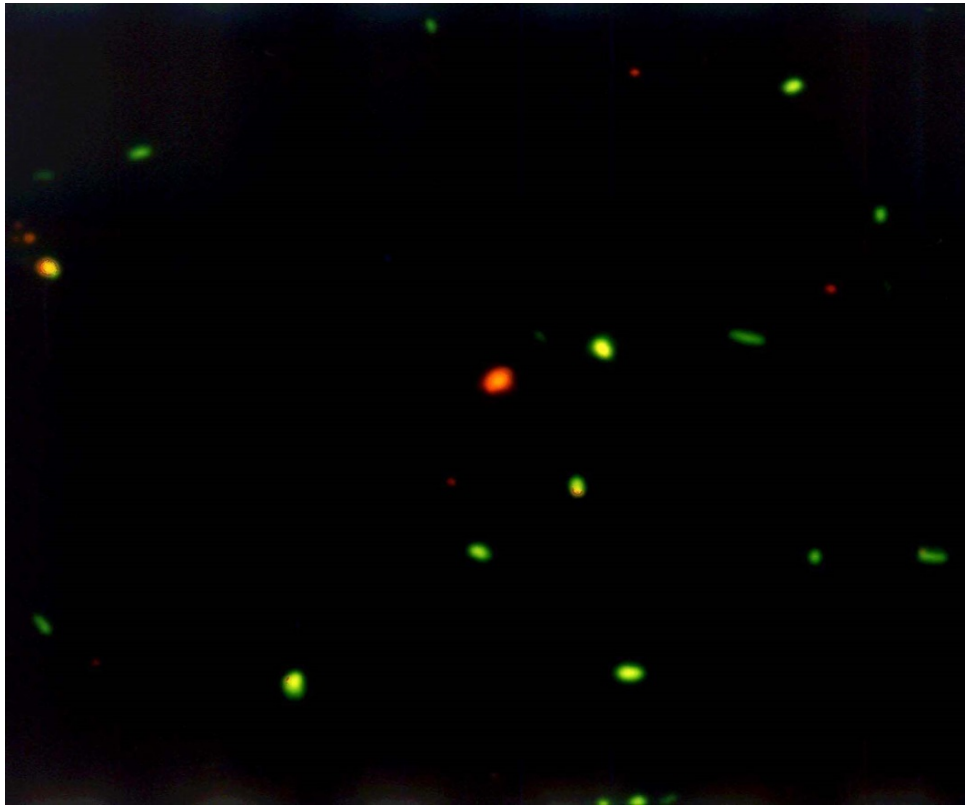
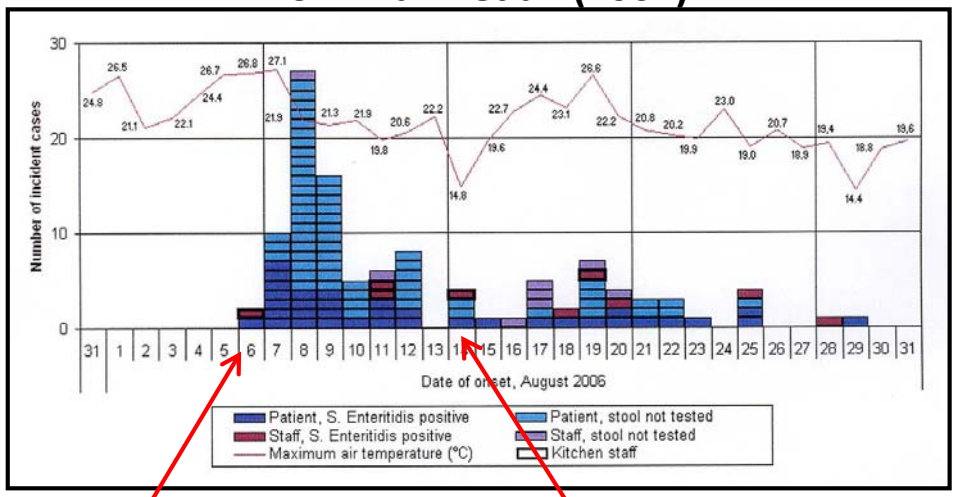


Photo: Claußen

Year	Country	Source	*Salmonella*/g	Serotype	Cases	Reference
1973	Canada	Black pepper	?	Weltevreden	17	Laidley et al. (1974), WHO (1974)
1981	Norway	Black pepper	0.1 – 2.4	Oranienburg	126	Gustavsen & Bren (1984)
1993	Germany	Paprika (potato chips)	0.04 – 0.45	Saintpaul, Rubislaw, Javiana	~ 1000	Lehmacher et al. (1995)
1996	England & Wales	Black pepper	?	Enteritidis PT4	8	Little et al. (2003), Little (2012), HPA (2011)
1999	USA	Coriander (salsa)	?	Thompson	76	
2002	England & Wales	Curry powder	?	Braenderup	20	Little (2012), HPA (2011)
2002	Germany	Anise seed	0.036	Agona	42	Koch et al. (2005). Rabsch et al. (2005)
2006	Germany	Vanilla (pastry)	?	Enteritidis PT21c	136	Frank et al. (2007)
2007	USA	Seasoning mix (snack)	?	Wandsworth & Typhimurium	87 & 18	Sotir et al. (2009)
2008	Serbia	Fennel seed	?	Senftenberg	14	Ilić et al. (2010)
2008	USA	White pepper	?	Rissen	87	USFDA (2009), et cetera
2009	USA	Pepper (salami)	?	Montevideo & Senftenberg	272 & 11	CDC (2010). Gieraltowski et al. (2012), DuVernoy (2012)

Infections of young and elderly persons by *Salmonella*-contaminated spices and dried herbs

Vanilla-and-cream pastry outbreak (2006)
From Frank et al. (2007):



Consumption of pastry with unheated filling

2nd epidemic of ill elderly persons

Baby tea outbreaks:

- **Anise seed tea: infants < 13 months (Koch et al., 2005)**
- **Fennel seed tea: 71% of ill persons < 1 year (Ilić et al., 2010)**

Paprika-powdered chips outbreak:

- **1 among 10,000 consumers became ill; majority of patients ≤ 14 years (Lehmacher et al., 1995)**

Seasoning outbreak

- **96% of ill persons < 6 years (Sotir et al., 2009)**

Vanilla-and-cream pastry outbreak (Frank et al., 2007)

- **Attack rate 0 – 23% among elderly persons from different wards**

Low numbers of *Salmonella* trigger a low infective dose and a low attack rate

- **Detected numbers of *Salmonella* in spices and dried herbs from outbreaks are low: 0.04 – 2.4 cfu *Salmonella*/g**
  (Gustavsen & Bren, 1984; Koch et al., 2005; Lehmacher et al., 1995)
- **In case the food product does not promote growth of *Salmonella*, low numbers of *Salmonella* only trigger a low infective dose:**
  Assumed consumption of 100 g contaminated potato chips resulted in a dose of 4 – 45 cfu *Salmonella* (Lehmacher et al., 1995).
- **An attack rate of 1 per 10000 exposed persons was calculated from roughly estimated 1000 cases and a total of 10 million contaminated packs of potato chips (Lehmacher et al., 1995).**

Transmission routes of *Salmonella*

„Classical route“

- Food rich in water and protein (Products made from meat, fish, milk, and egg)
- Often self-produced foods or communal catering
- Local distribution
- Vegetative *Salmonella*
- High infective dose (≥ 1 million cfu)
- High attack rate
- Outbreak limited in place and time

„Spiced route“

- Dried food (Spices, herbs, herbal infusions, oilseeds, nuts, and flavours)
- Industrially processed foods
- Large-scale distribution
- „Resting“ *Salmonella*, long-term survival in dried food; protection by essential oils? Protracted outbreak
- low infective dose (≤ 100 cfu)
- low attack rate
- Outbreak less limited in place and time

Thank you very much
for your kind attention!