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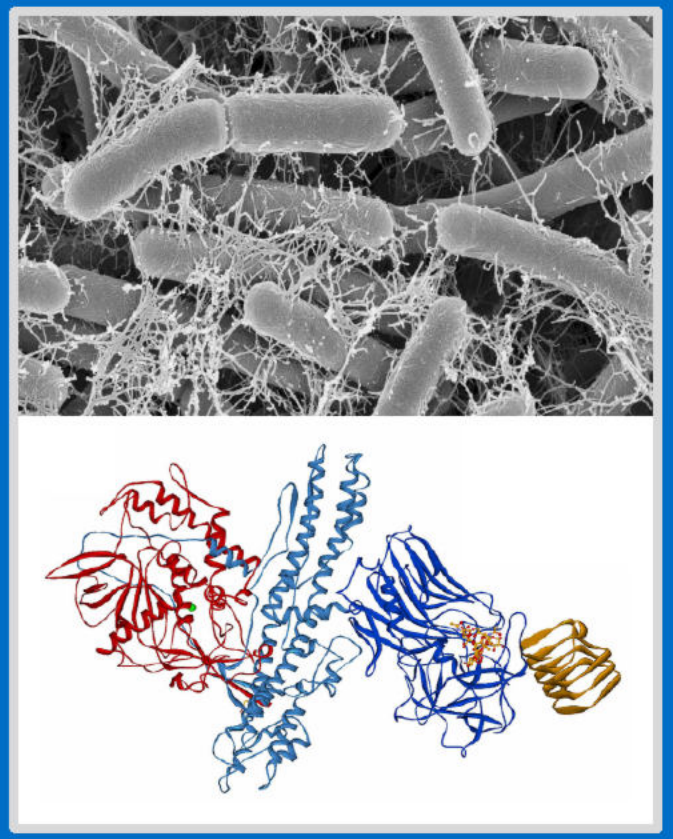

An outbreak of foodborne botulism caused by commercially canned mushrooms from Russia

Martin B. Dorner
Konsiliarlabor für Neurotoxin-produzierene Clostridien (Botulismus, Tetanus)
DVG-KL für Clostridium botulinum/Botulinum Toxin in Lebensmitteln
Robert Koch Institute, Berlin, DE

19. Fachsymposium Lebensmittelmikrobiologie, 1.-3. Apr 2025, Berlin

Botulism: Disease caused by botulinum neurotoxins effecting humans and animals (mammals, birds, fish)

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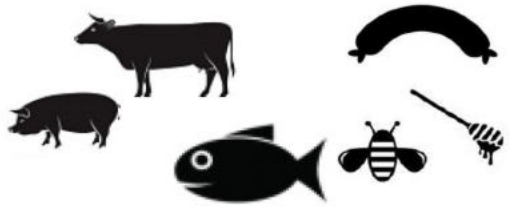
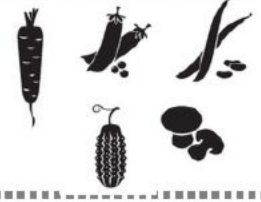
Classical (naturally occurring) forms of botulism and tetanus

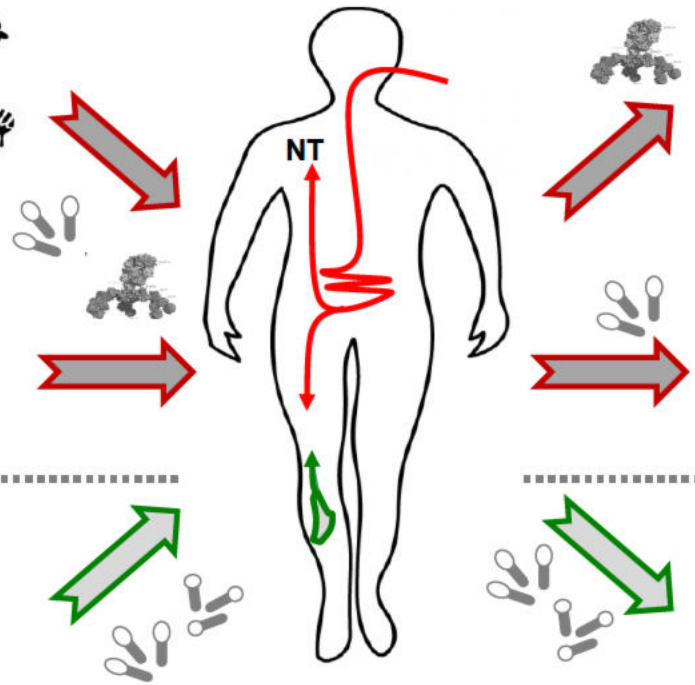
Zoonotic source



Non-zoonotic source



Environmental source



LD_{50} (BoNT)
1 ng/kg

Foodborne botulism

Intoxication

Onset:
12–48 hrs

Infant botulism

Toxico-infection

Onset:
3–30 days

Wound botulism **Tetanus**

Toxico-infection

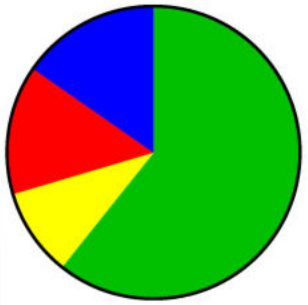
Onset:
5–15 days

Medical intervention

Iatrogenic botulism

Intoxication

Onset:
few days



60.71% Lebensmittel
9.69% Säugling
14.29% Wund
15.31% iatrogener

Total=196

8,5 Fälle p.a.

Botulinum neurotoxins, a diverse group of 8 serotypes and > 40 subtypes

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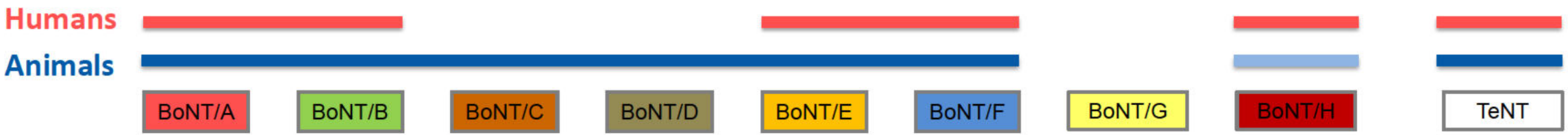
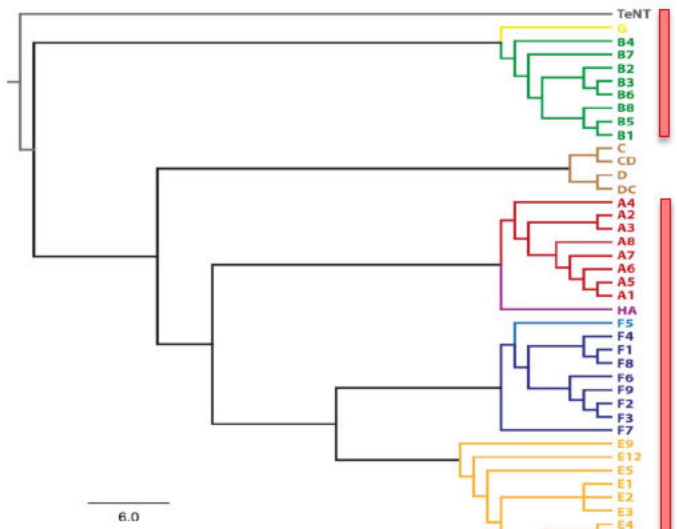


	A	B	C	D	E	F	G	H	TeNT
A		37.8	30.5	31.7	37.4	38.4	37.7	49.9	32.0
B			32.1	33.6	35.9	37.5	57.2	39.7	39.4
C				52.2	30.3	30.8	32.9	30.8	30.6
D					31.4	32.2	34.2	32.5	31.3
E						62.4	36.1	47.3	34.0
F							37.4	51.8	34.6
G								39.4	38.9
H									37.1

30 to 62% identity at amino acid levels



- **> 40 subtypes and mosaic forms**
- **Up to 36% differences at aa level**

NT-producing *Clostridium* spp.	Types
Clostridium botulinum **Group I**	**A, B, F, H**
Clostridium sporogenes	**B**
Clostridium botulinum **Group II**	**B, E, F**
Clostridium butyricum	**E**
Clostridium baratii	**F**
Clostridium botulinum **Group III (*C. novyi sensu lato*)**	**C, D, CD, DC**
Clostridium botulinum **Group IV (*C. argentinensis*)**	**G**
Clostridium tetani	**TeNT**

Botulinum neurotoxins, a diverse group of 8 serotypes and > 40 subtypes

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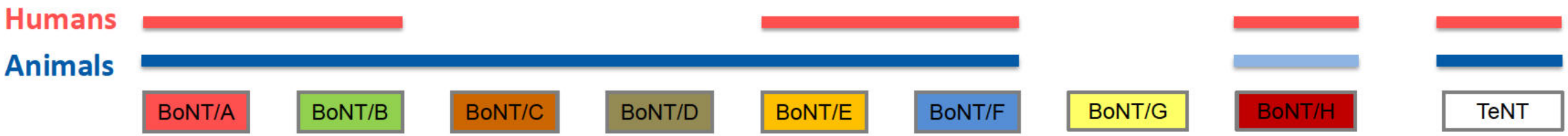

	A	B	C	D	E	F	G	H	TeNT
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D									
E						62.4	36.1	47.3	34.0
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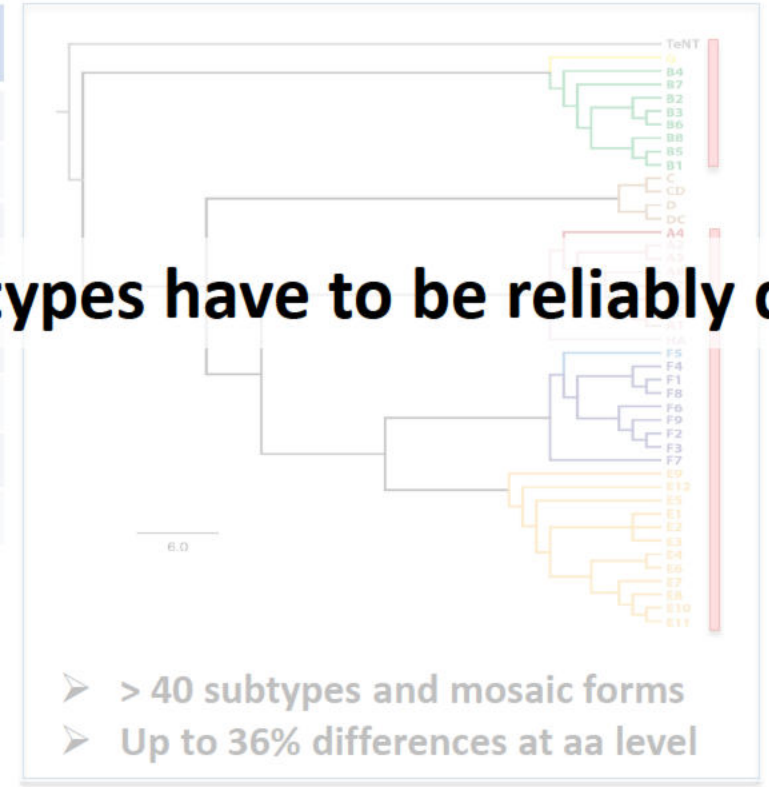
G								39.4	38.9
H									37.1

30 to 62% identity at amino acid levels

All sero- und subtypes have to be reliably detected



- > 40 subtypes and mosaic forms
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Clostridium botulinum Group I	A, B, F, H
Clostridium sporogenes	B
Clostridium botulinum Group II	B, E, F
	E
Clostridium baratii	F
Clostridium botulinum Group III (*C. novyi sensu lato*)	C, D, CD, DC
Clostridium botulinum Group IV (*C. argentinensis*)	G
Clostridium tetani	TeNT

Methods used for botulinum detection

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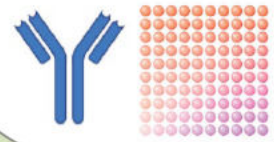
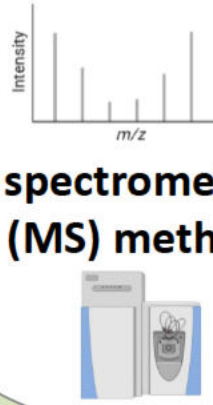
Protein-based detection

functional methods

immunological methods

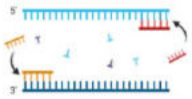


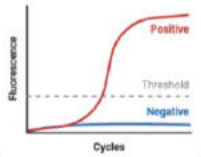
spectrometric (MS) methods



Anaerobic microbiology and DNA-based detection

DNA-based detection

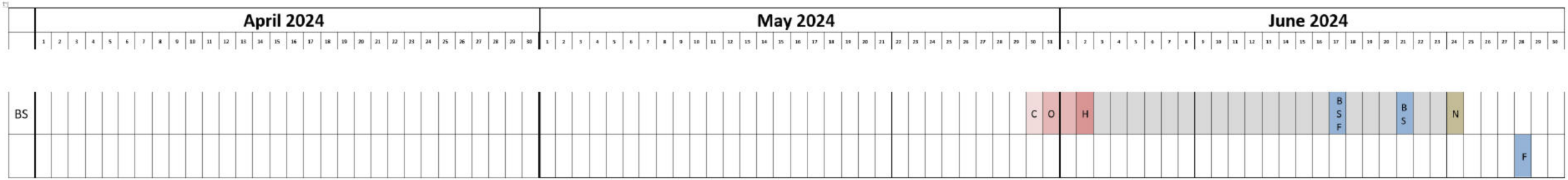




Challenges:

- Extreme low concentrations in clinical samples (fM, low pg/mL)
- Variety of molecules!
- Different matrices (clinical, food, household items, feed, animals, environmental)

Description of cases in two unrelated households in Lower Saxony



C: consumed; O: disease onset; H: hospitalized; N: notification of laboratory confirmation; D: discharged; B: blood/serum, S: stool, F: fish sample arrived; hospitalized

Case "BS"

- Patient with Eastern European background
- hospitalized 2 June (suspected GBS)
- suspected botulism 16 June
- Blood + stool + food (mushrooms) received 17 June
- Food (mushrooms) positive for BoNT/A: 18 June
  however: initial stool + serum: negative
- New blood + stool received 21 June
  >> stool positive for *bont/A* (24 May)

Description of cases in two unrelated households in Lower Saxony

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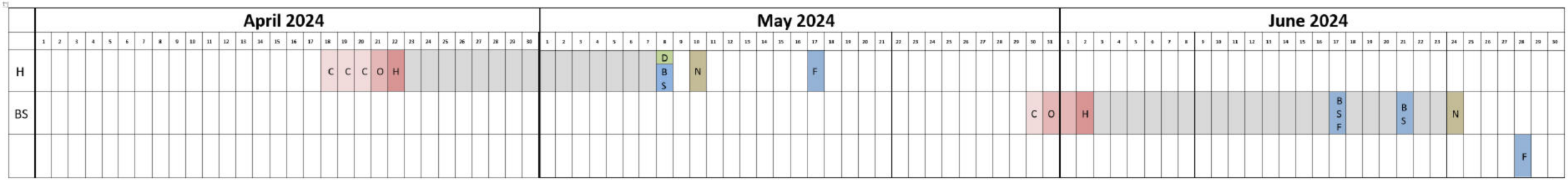

C: consumed; O: disease onset; H: hospitalized; N: notification of laboratory confirmation; D: discharged; B: blood/serum, S: stool, F: fish sample arrived; hospitalized

Case "H"

- Patient with Eastern European background
- hospitalized 23 April (suspected Myasthenia gravis)
- Suspected botulism: 7 May
- Blood + stool received 8 May
- Stool positive for *bont/A* (10 May)
- Food items collected from household 17 May
  - Tomatoes: *bont/A* positive, but BoNT/A negative
    >> not the source of the botulism
  - Patient had consumed the same mushrooms!

Case "BS"

- Patient with Eastern European background
- hospitalized 2 June (suspected GBS)
- suspected botulism 16 June
- Blood + stool + food (mushrooms) received 17 June
- Food (mushrooms) positive for BoNT/A: 18 June
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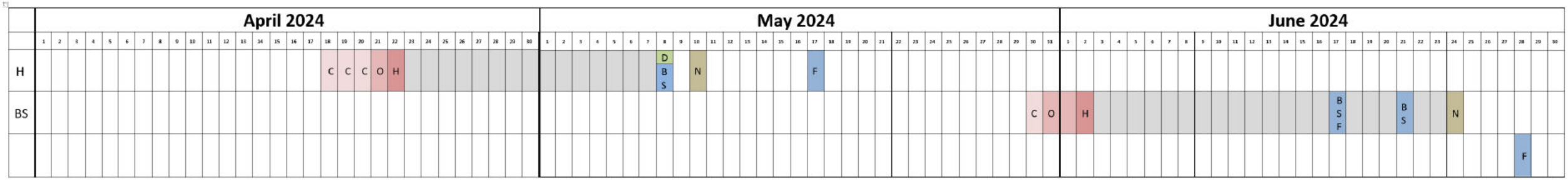

C: consumed; O: disease onset; H: hospitalized; N: notification of laboratory confirmation; D: discharged; B: blood/serum, S: stool, F: fish sample arrived; hospitalized

Case "H"	MBA	Endopep-SIA	ELISA	Cultivation + PCR	
Serum (d2)	BoNT/A pos	BoNT/A pos	BoNT/A pos		
Feces				*bont/A* pos	
Food, different items - Tomatoes in brine - Mushrooms not suppl.			negative	*bont/A* pos	**Likely not the source (no toxin)**
Case "BS"					
Serum (≈14 d)	negative	BoNT/A +/-	negative		
Feces				*bont/A* pos	
Food - mushrooms (unopened jar)		BoNT/A pos	BoNT/A pos	*bont/A* pos	**Source identified!**

Food analysis

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Case “BS”: unopened glass jar from household was positive for *bont/A* and BoNT/A

>> Official food recall and collection of additional glasses of mushrooms from supermarkets

>> Different pickled mushrooms from a Russian producer were imported by a German distributor specialized in Eastern European foods

Same producer

12/15 positive **4/4 negative** **1/1 negative** **4/4 negative** **2/2 negative**

12/12 from distributor
3/3 from local supermarket

https://www.lebensmittelwarnung.de/___lebensmittelwarnung.de/Meldungen/2024/06_Juni/240626_17_BW_Eingelegte_Pilze/240626_17_BW_Eingelegte_Pilze.html

Food analysis

ROBERT KOCH INSTITUT

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>> Different pickled mushrooms from a Russian producer were imported by a German distributor specialized in Eastern European foods

Same producer

12/15 positive

4/4 negative

1/1 negative

4/4 negative

2/2 negative

Case “H” confirmed to have consumed same mushrooms as case “BS” (no left-overs, glass jar washed, see photo)

Additional information

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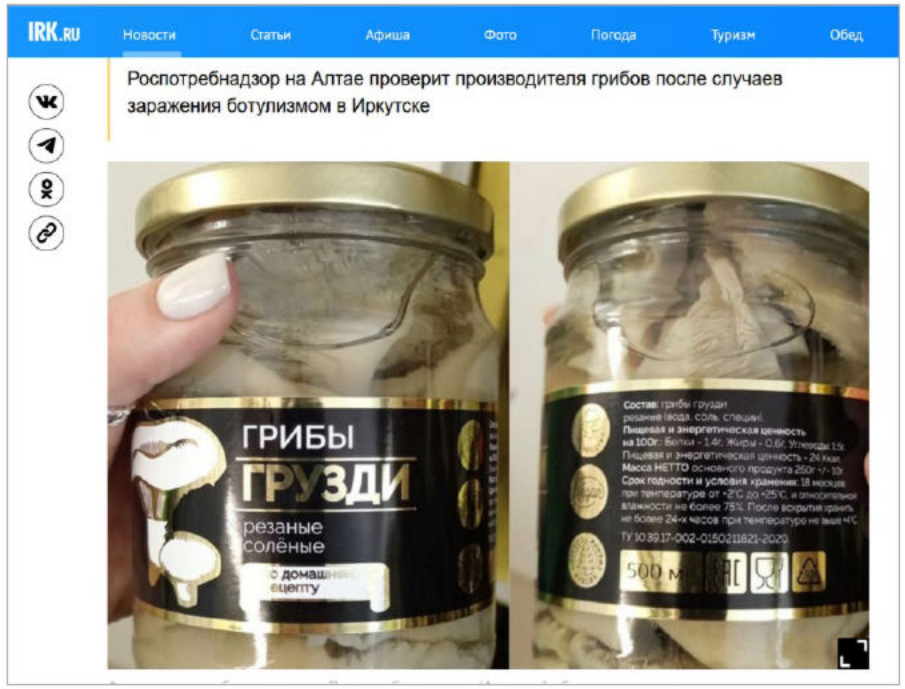
Recall of contaminated mushrooms “milk-white brittlegill” (Грзуди), *Russula delica* produced by a company from Altai Krai

25 March 2024: product warning in Russia due to two botulism cases in Irkutsk involving the same producer from Altai Krai



Russian news

http://www.irk.ru/news/20240325/disease/

Additional information

ROBERT KOCH INSTITUT

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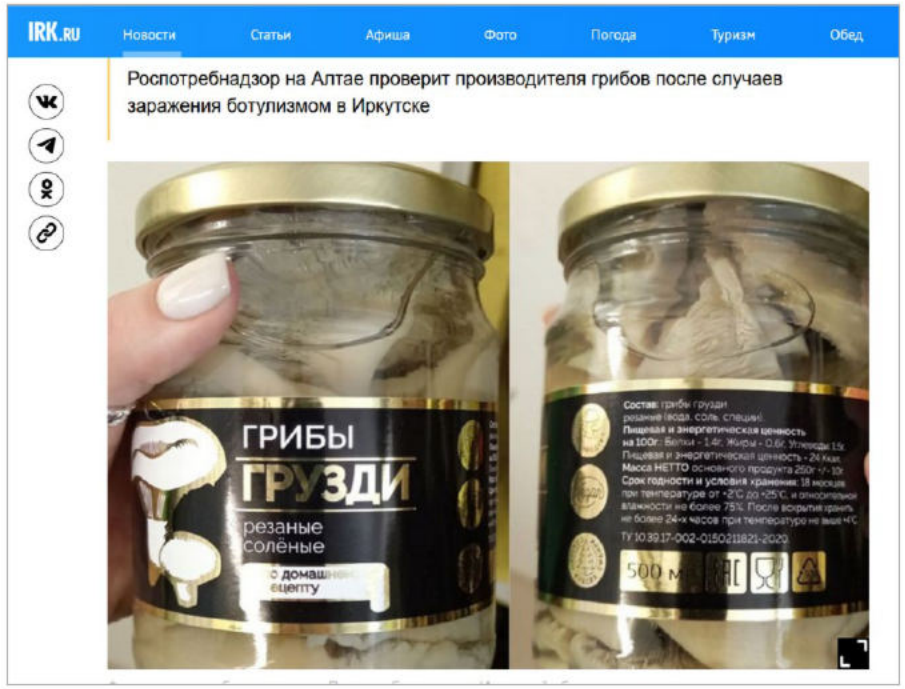


Russian news

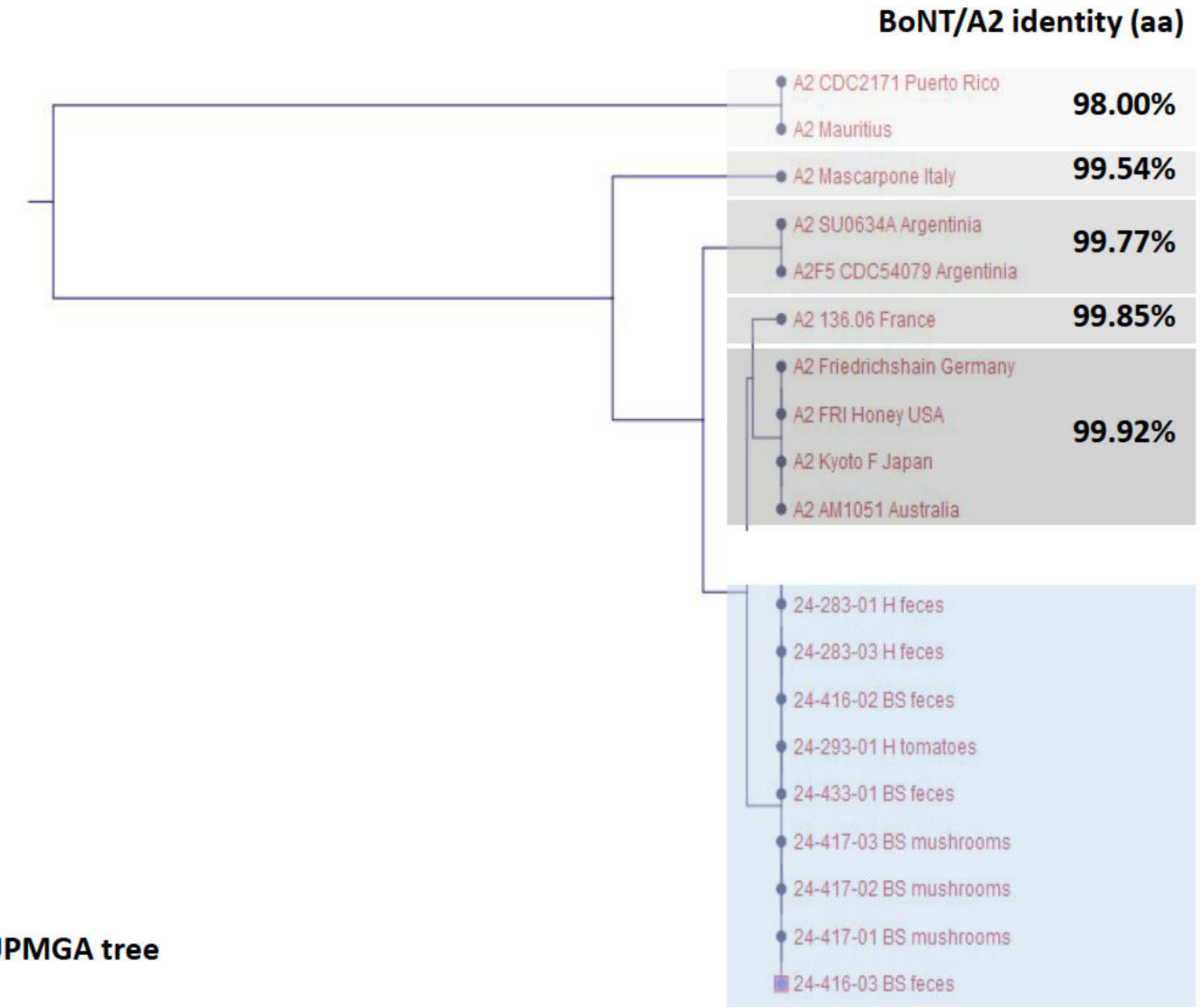
http://www.irk.ru/news/20240325/disease/

Germany

- **Very similar glass jars**
- **Same producer**
- **Different label**

- **Criminal investigation was filed against the German distributor for “negligence and serious personal injuries”**

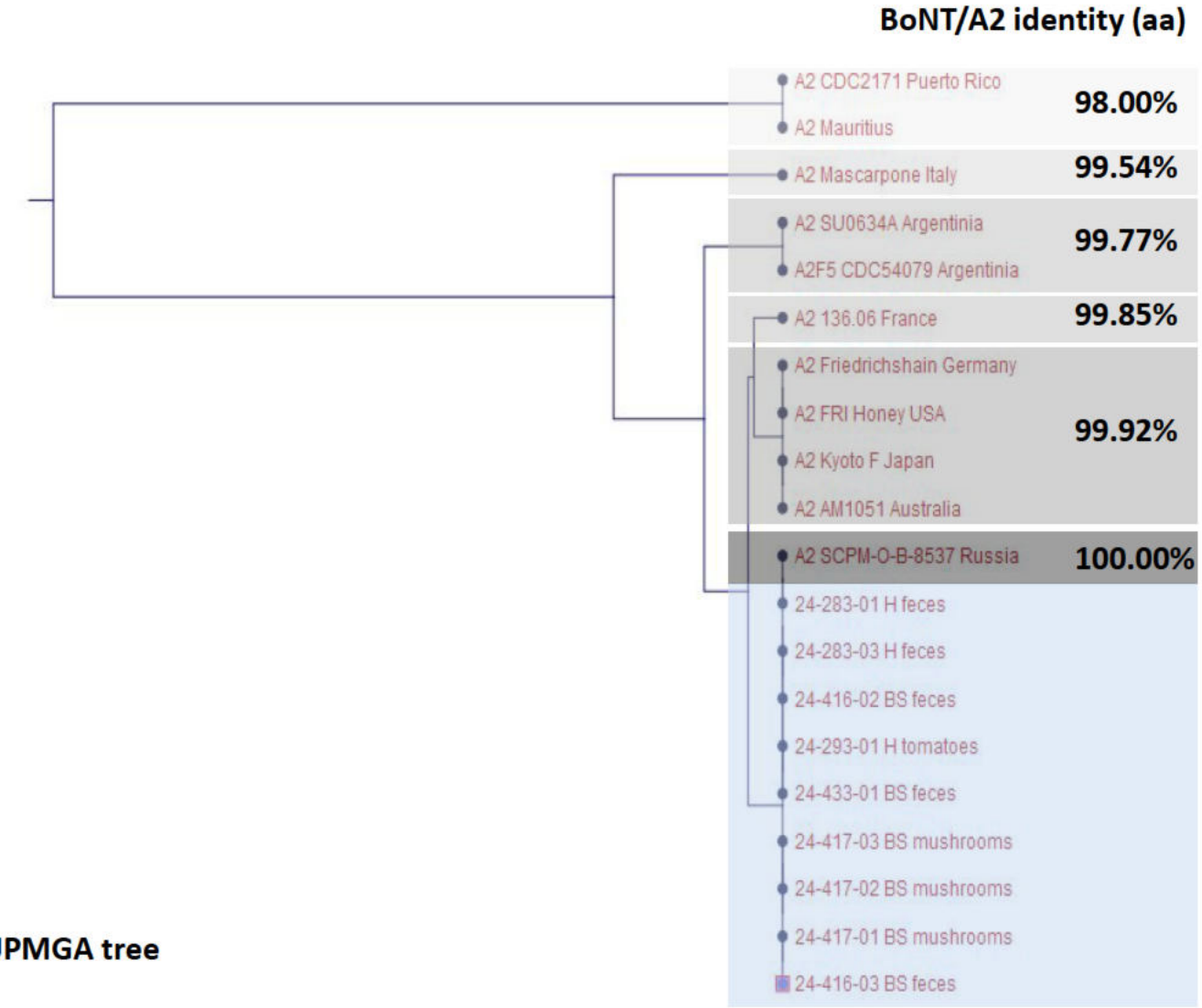
Molecular analysis of BoNT/A2 sequences in the isolates



UPMGA tree

5 isolates from two patients
3 isolates from mushrooms
1 isolate from tomatoes
Total: 9 isolates (all A2)

Molecular analysis of BoNT/A2 sequences in the isolates



UPMGA tree

Identical sequence submitted Nov 2021 from the State Research Center for Applied Biotechnology and Microbiology, Moscow

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Total: 9 isolates (all A2)

Molecular analysis of BoNT/A2 sequences in the isolates

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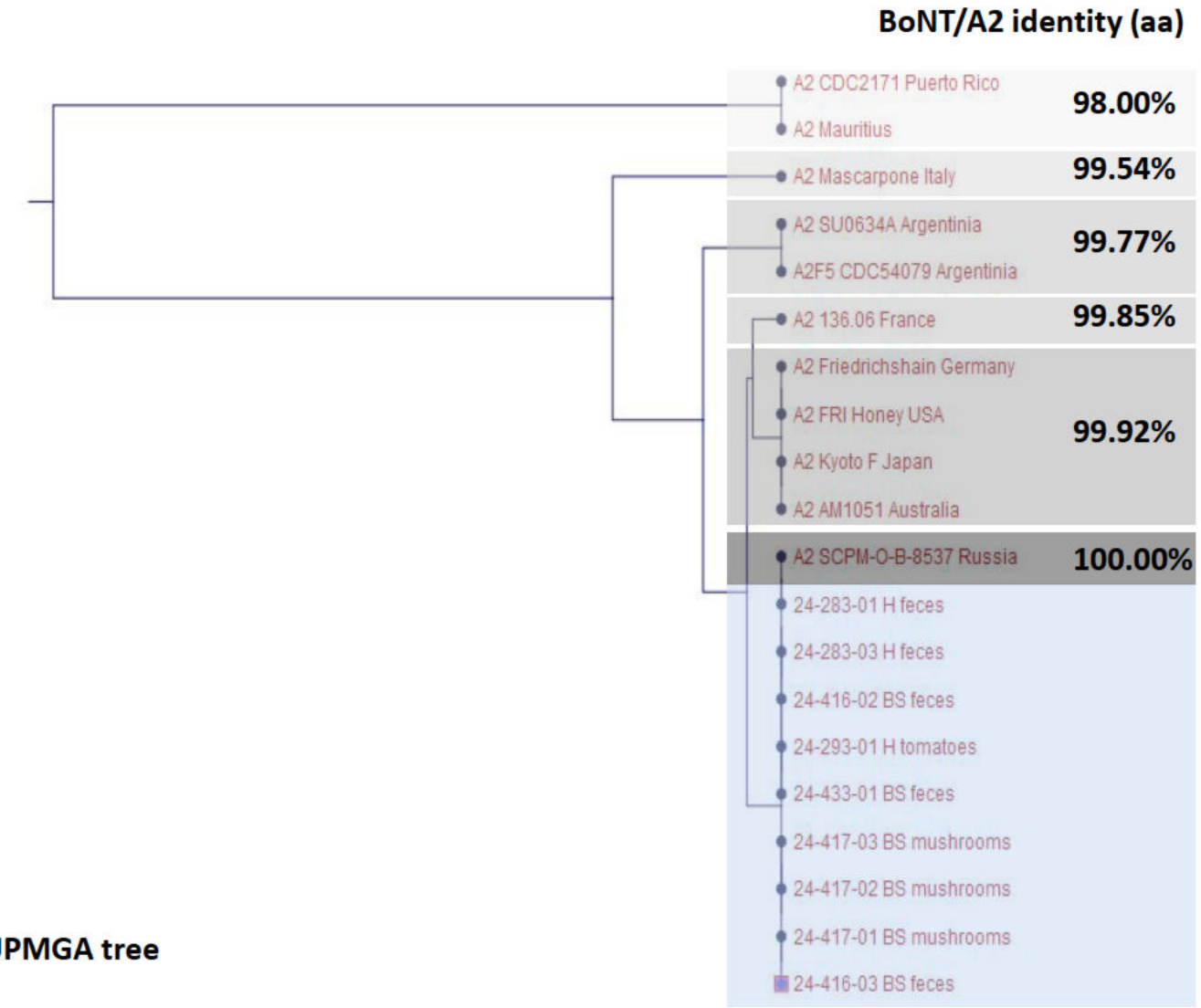

UPMGA tree

1 amino acid exchange (E→K) compared to A2 Kyoto F at position 1156 in the heavy chain

Very rare: Commercial food as source of food-borne botulism

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Food used after best-by-date or after temperature abuse

Rainbow trout 2000 (1 case)
Rainbow trout 2016 (2 cases)
Rainbow trout 2023 (1 cases)
Herring 2023 (1 case)

E1/E3

'Real' outbreaks with contaminated commercial products

Dried roach 2016
(7 cases), E1+E3
Source: NL or LV

Hendrickx et al.
Frontiers Public Health 2023

Almond coconut cream 2021
(2 cases), A2
Source: DE?

Mushrooms 2024
(2 cases), A2
RUS

Some lessons learned – food items

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- **“Russian mushrooms” are a niche product on the German market**
- **Unknown production process (batch size, incubation, etc.)**
- **Unknown quality control for food items with limited production size; “artisanal food product”**
- **Products with long slf-life: Cases can occur over an extended period**

- **3/3 glass jars from one supermarket -> all negative**
- **12/12 glass jars from the German distributor -> all positive**

} **This questions the batch labelling (no lot number)**

- **Same strain (so far) from two unrelated patients and different glasses of mushrooms indicates contamination of a single batch**

Some lessons learned – public health aspects

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- **Botulism patients are often suspected for other neurological diseases delaying diagnosis (14 d in this outbreak)**
- **“Window of opportunity” to corroborate clinical diagnosis in the lab:**
  - **serum (days) *versus* stool (weeks)**
- **Food items can be cross-contaminated within the household (e.g. via cutlery) and mislead investigation**
  - **Food source should contain toxin and organism (intoxication)**
- **Cases can sometimes be identified as connected only retrospectively due to missing information / material**
  - **Cooperation with local health authorities necessary**

- **Political crisis situations imply health risks**
  - **Ukraine war impacts exchange of information between RUS and EU**
  - **EU was not notified on the Russian foodborne botulism outbreak due to mushrooms (March 2024)**

Thank you!

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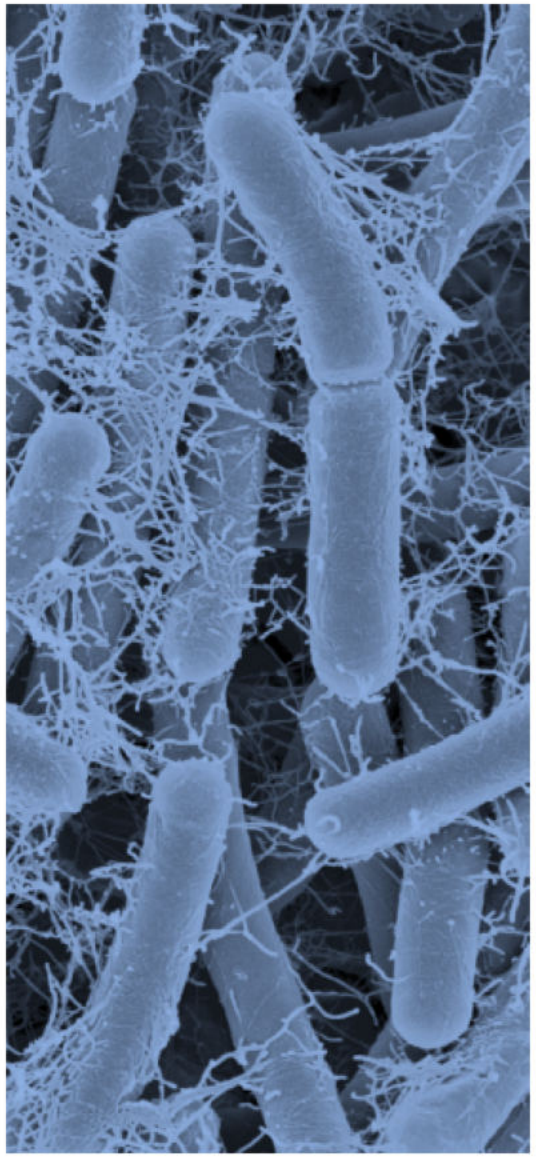

Robert Koch Institute

ZBS3
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Laura von Berg
Laura Wilk
Sylvia Worbs

RKI
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Hendrik Wilking
Klaus Stark

ZBS7
Agatha Mikolajewska

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DTox-Net
Deutsches Toxin-Labornetzwerk

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EQuATox
EuroBioTox
RefBio UNSGM
Germany's Contribution to Strengthen the Reference Laboratories 'Bio' in the UNSGM