

The background of the slide is a circular field filled with a dense pattern of microscopic images of Bacillus thuringiensis spores. The spores are rod-shaped and appear in various orientations, some showing internal structures. The overall color scheme is a mix of olive green and yellowish-green.

Fate and effect of *Bacillus thuringiensis* based insecticides in the human gut

Andrea Wilcks

Department of Microbiological Food Safety

Danish Institute for Food and

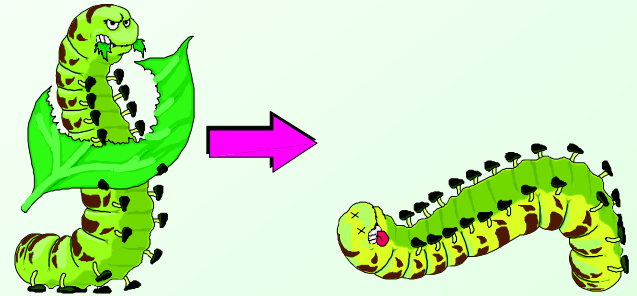
Veterinary Research



MINISTRY OF FAMILY
AND CONSUMER AFFAIRS

Danish Institute for Food
and Veterinary Research

Background



- *Bacillus thuringiensis* (*Bt*) widely used as a bio-insecticide
- Produces enterotoxins that causes diarrhoea in humans
- *Bt* is sprayed as spores on the plants, but unclear whether the spores germinate in the human intestine

B. thuringiensis in food

- Grapes
 - Bidochka et al, 1987; Bae et al, 2004
- Farm bulk tank, creamery silo tank
 - Phillips et al, 1986
- Pasta, bread, milk, fruit, vegetables
 - Damgaard et al, 1996; Rosenquist et al, 2005



MINISTRY OF FAMILY
AND CONSUMER AFFAIRS

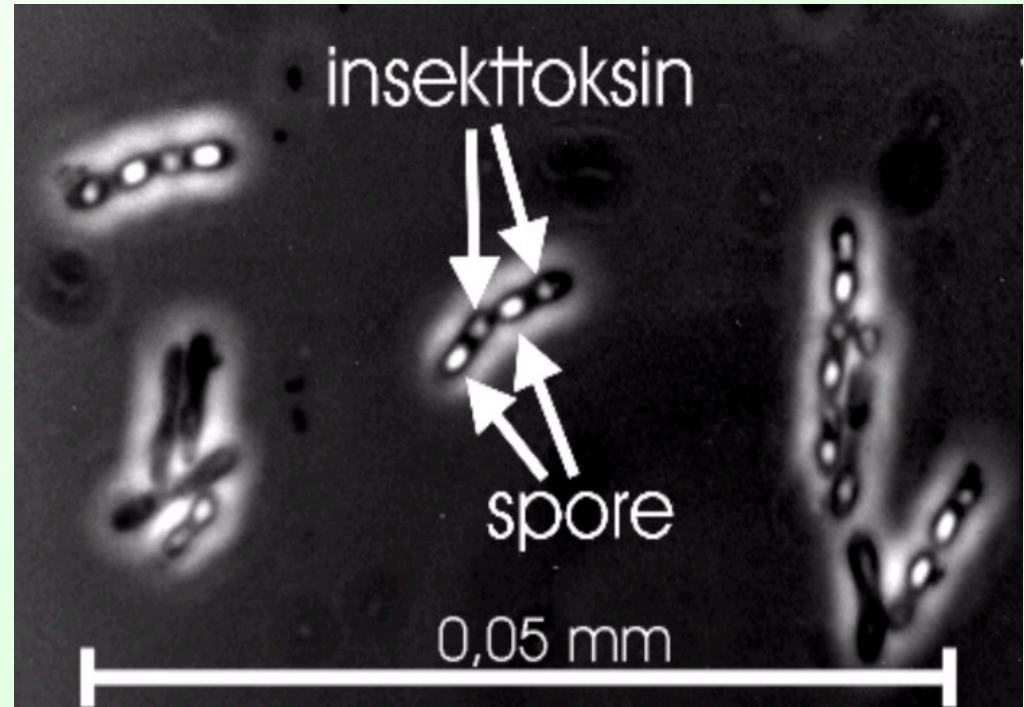
Danish Institute for Food
and Veterinary Research

Objectives

- **Do ingested spores and vegetative cells of *Bt* survive and persist in the gut?**
- **Do ingested spores germinate in the gut?**
- **Does heat-activation of spores have an influence on their survival, persistence and germination?**
- Do ingested *Bt* cells change the composition of the indigenous microflora of the gut?
- Are we able to detect enterotoxins in the gut?

Investigated strains

- *Bacillus cereus*
F4433/73 – RifR
- *B. thuringiensis* subsp.
kurstaki DMU67R –
RifR
- *B. thuringiensis* subsp.
isralensis 4Q1 - StrR



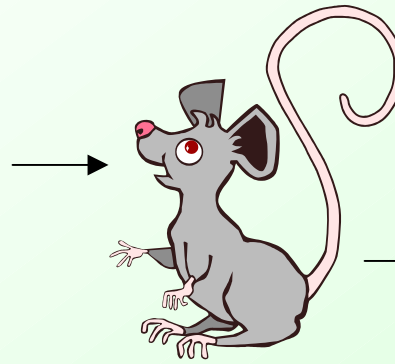
Animal experiments

Group 1: Irradiated spores

Group 2: Untreated spores

Group 3: Heat-activated spores

Group 4: Vegetative cells



*Human flora
associated rats*

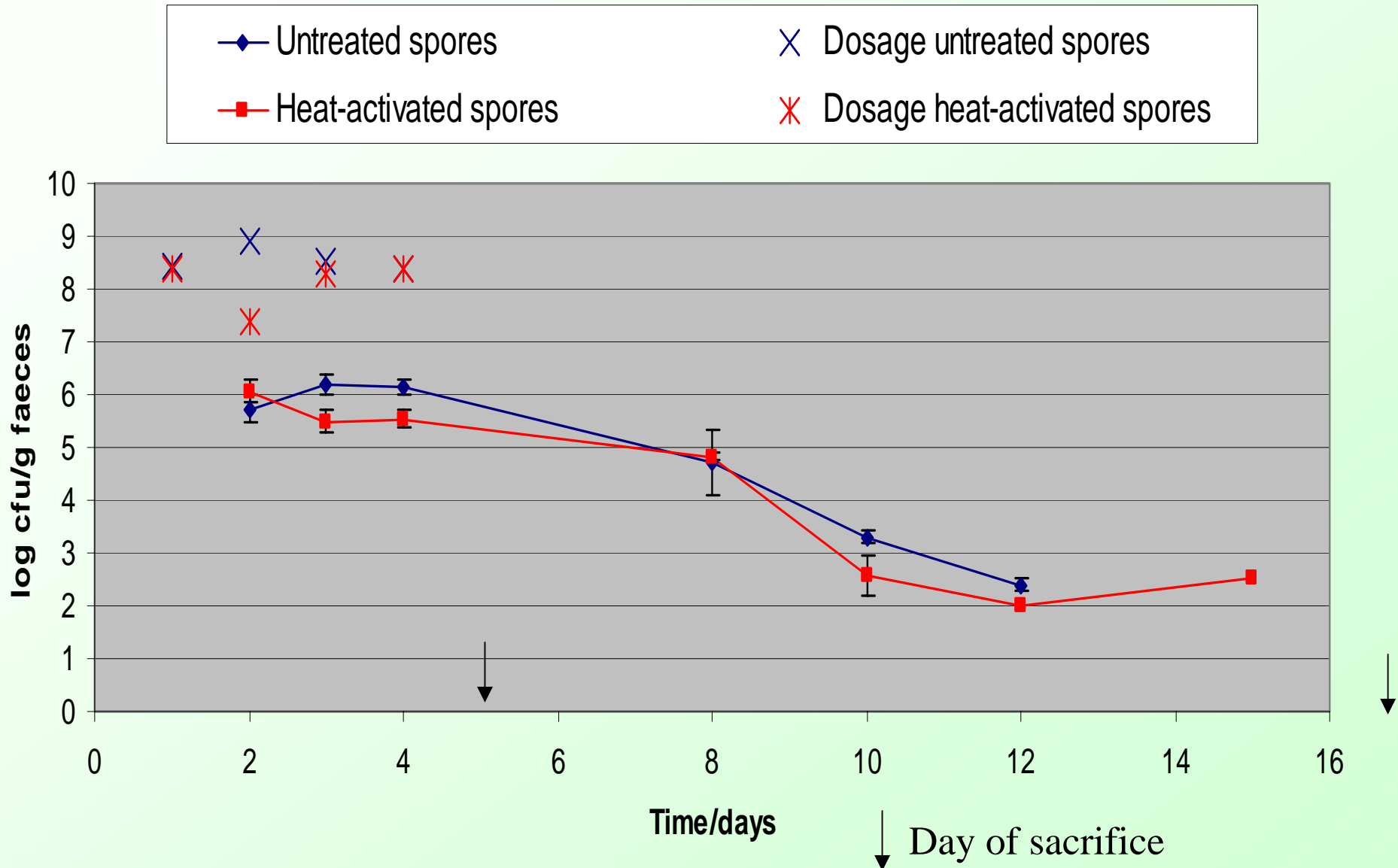
Faecal and
intestinal samples

Plate counts

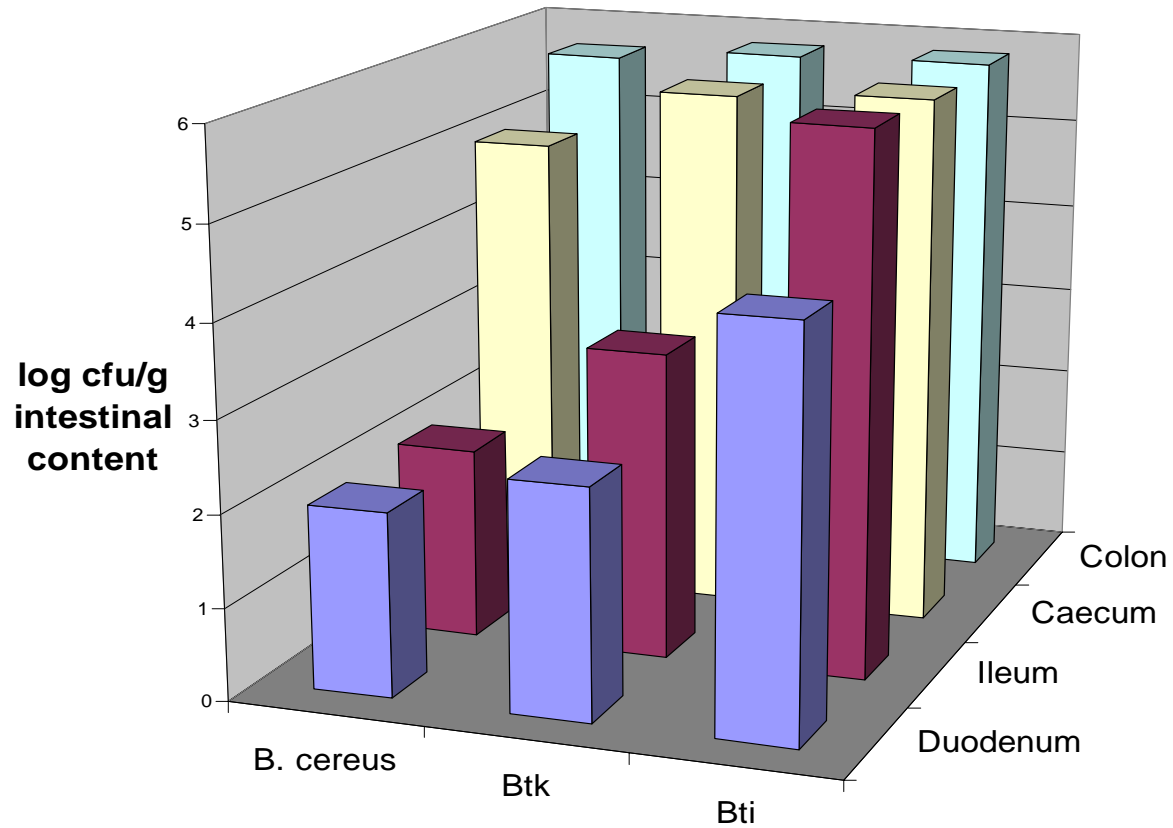
DGGE

Enterotoxins
(NERI)

B. cereus F4433/73 in faecal samples



Bc/Bt counts in intestinal samples at Day 5





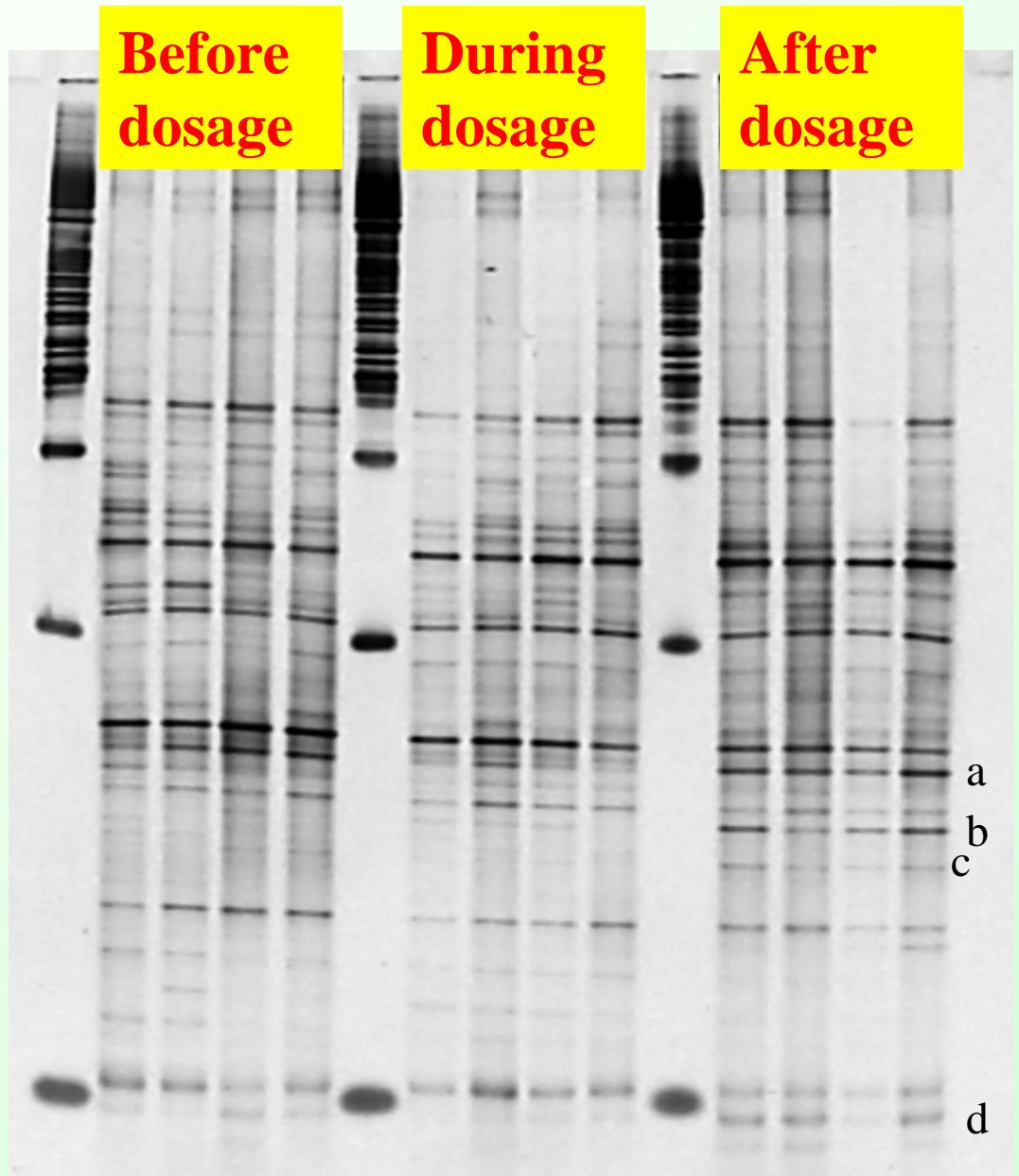
MINISTRY OF FAMILY
AND CONSUMER AFFAIRS

Danish Institute for Food
and Veterinary Research

Objectives

- Do ingested spores and vegetative cells of *Bt* survive and persist in the gut?
- Do ingested spores germinate in the gut?
- Does heat-activation of spores have an influence on their survival, persistence and germination?
- **Do ingested *Bt* cells change the composition of the indigenous microflora of the gut?**
- Are we able to detect enterotoxins in the gut?

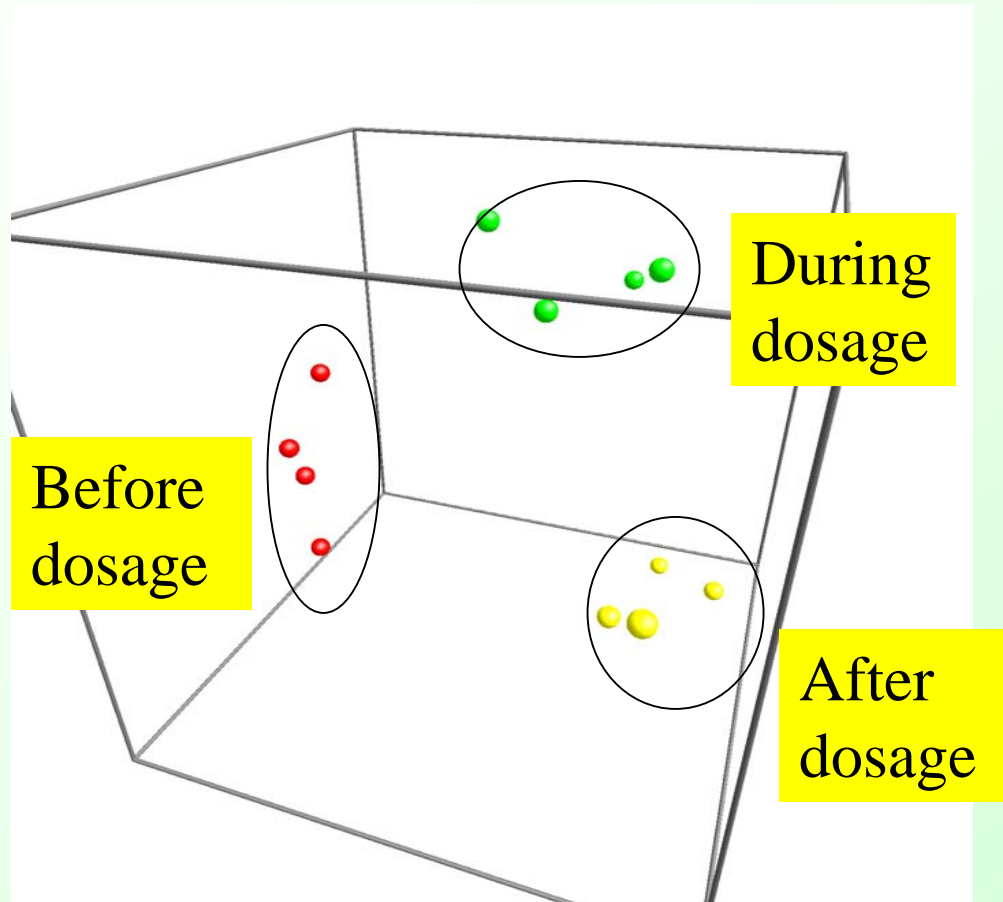
DGGE with
universal primers
on faecal samples
from HFA rats
fed *B. cereus*
F4433/73R



a: *Ruminococcus obeum*
b: *Fusobacterium sp.*
c+d: unculturable bacterium

DGGE – PCA analysis

Rats fed *B. cereus* F4433/73 spores





MINISTRY OF FAMILY
AND CONSUMER AFFAIRS

Danish Institute for Food
and Veterinary Research

Objectives

- Do ingested spores and vegetative cells of *Bt* survive and persist in the gut?
- Do ingested spores germinate in the gut?
- Does heat-activation of spores have an influence on their survival, persistence and germination?
- Do ingested *Bt* cells change the composition of the indigenous microflora of the gut?
- **Are we able to detect enterotoxins in the gut?**



MINISTRY OF FAMILY
AND CONSUMER AFFAIRS

Danish Institute for Food
and Veterinary Research

Summary (1)

- *B. cereus* and *Bt* cells can be detected in faecal and intestinal samples up to two weeks after last dosing (multiplication, attachment)
- One animal fed *Bt* subsp. *kurstaki* had high levels of *Bt* subsp. *kurstaki* in the intestine and faeces after two weeks



MINISTRY OF FAMILY
AND CONSUMER AFFAIRS

Danish Institute for Food
and Veterinary Research

Summary (2)

- Vegetative cells are presumably killed in the stomach, and hence no or only few cells are found in faecal and intestinal samples
- No difference in survival and persistence in the GI tract of heat-activated and untreated spores
- Shift in bacterial population after feeding with *B. cereus* F4433/73



MINISTRY OF FAMILY
AND CONSUMER AFFAIRS

Danish Institute for Food
and Veterinary Research

Conclusion

- *B. thuringiensis* insecticidal strains can be isolated from food products
- *Bt* spores may germinate, grow, re-sporulate in the gut, and some may be able to pass the GI barrier and reach other organs (host factor, immune status, gut microflora)
- Rats are not good models for assessing the virulence of *Bt* for humans

Acknowledgements



MINISTERIET FOR FAMILIE-
OG FORBRUGERANLIGGENDER

Danmarks Fødevarerforskning

*Danish Institute for Food
and Veterinary Research*

Bodil Madsen
Kristine Frederiksen
Hanne Rosenquist
Tine Rask Licht

ami

 arbejds miljø instituttet

*National Institute of
Occupational Health*

Lasse Smidt
Lars Andrup



Danmarks Miljøundersøgelser

*National Environmental
Research Institute*

Bjarne Munk Hansen
Niels Bohse Hendriksen

MILJØMINISTERIET
Miljøetvæsen

*Danish Environmental
Protection Agency*