Monitoring Human Exposures to *Bacillus thuringiensis* after Aerial Applications

Bio-Weapons, Insects, and Humans

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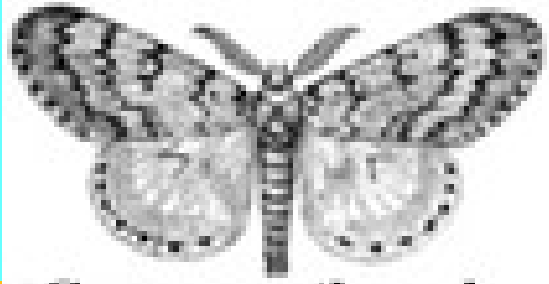
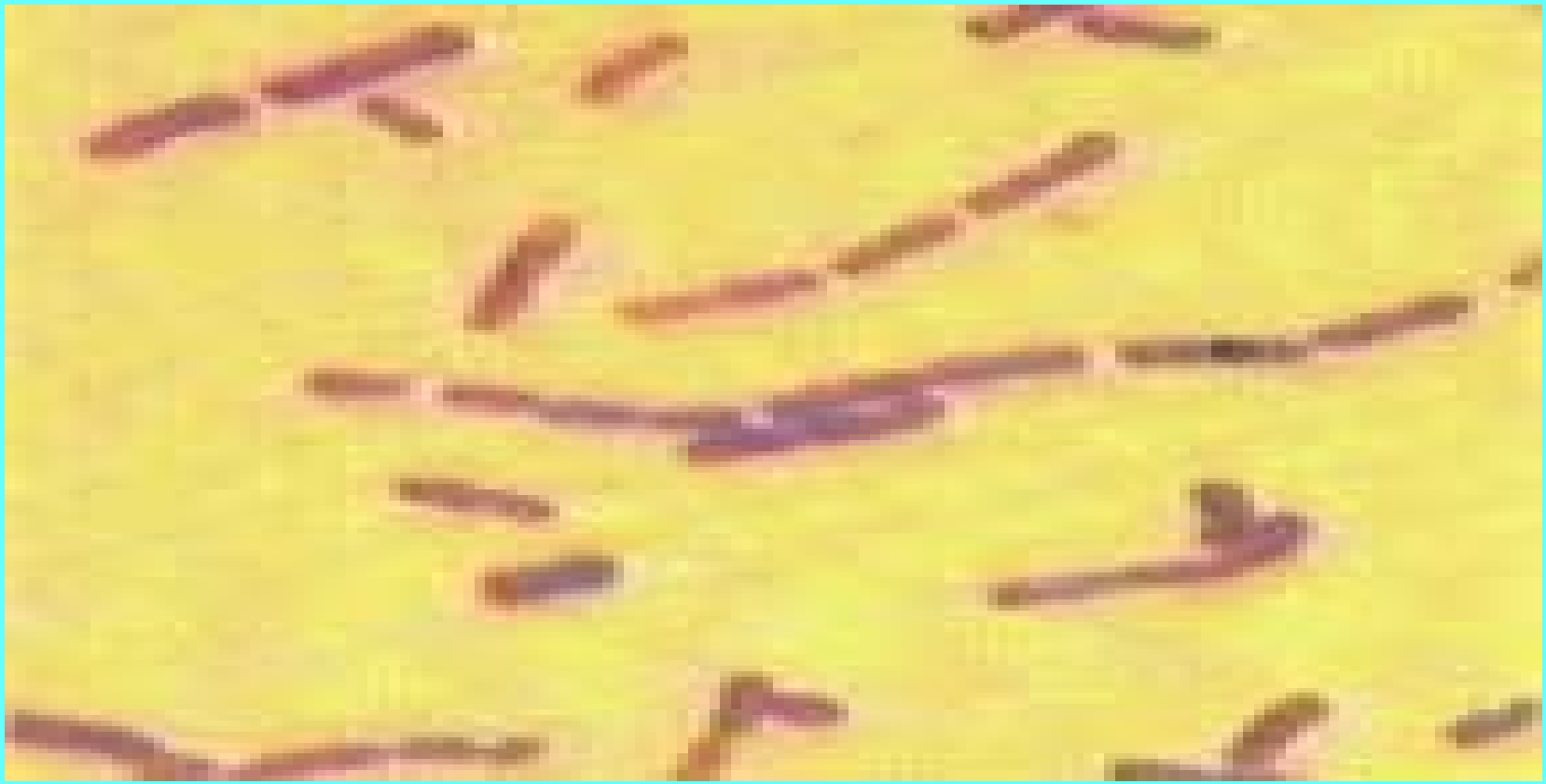
Biological Control of Insects


Gypsy moth male

- **Microbial insecticides for control of insect pest populations: Biological Warfare**
- **Biological weapons of choice:**
  - **Bacteria: *Bacillus thuringiensis***
  - **Viruses: Nucleopolyhedrovirus**

Bacillus thuringiensis (*Bt*)

- **Gram positive, spore-forming bacterium**
- **Ubiquitous in soil**

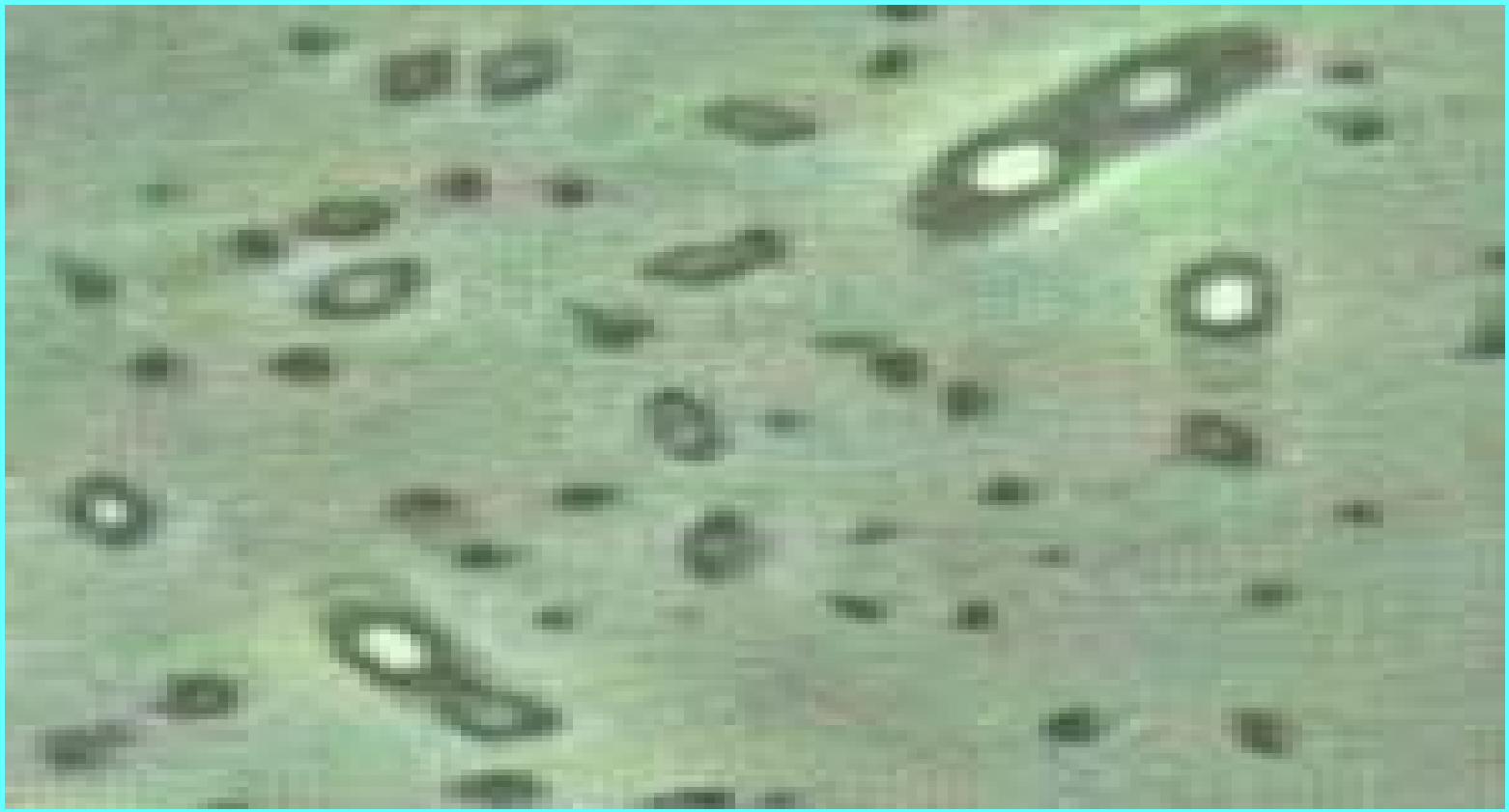


Bacillus thuringiensis (*Bt*)

- **Member of the *Bacillus Cereus*-Group of *Bacillus***
  - ***Bacillus thuringiensis***
  - ***Bacillus cereus***
  - ***Bacillus subtilis***
  - ***Bacillus mycoides***
  - ***Bacillus anthracis***
- **Very similar morphologically and biochemically**
- ***B. cereus* can cause gastroenteritis and diarrhea**
- ***B. anthracis* is highly pathogenic; Anthrax**

Bacillus thuringiensis (*Bt*)

- ***Bt*** **distinguished by presence of parasporal "Crystal Protein" = insect-specific toxin**



Bt Varieties and Strains

- **Many different types of *cry* genes carried by different Varieties or "Subspecies" of *Bt***

  - ***Bt* var. *Kurstaki* (*BtK*) - specific to Lepidoptera**
  - ***Bt* var. *Israeliensis* (*Btl*)- specific to Diptera**

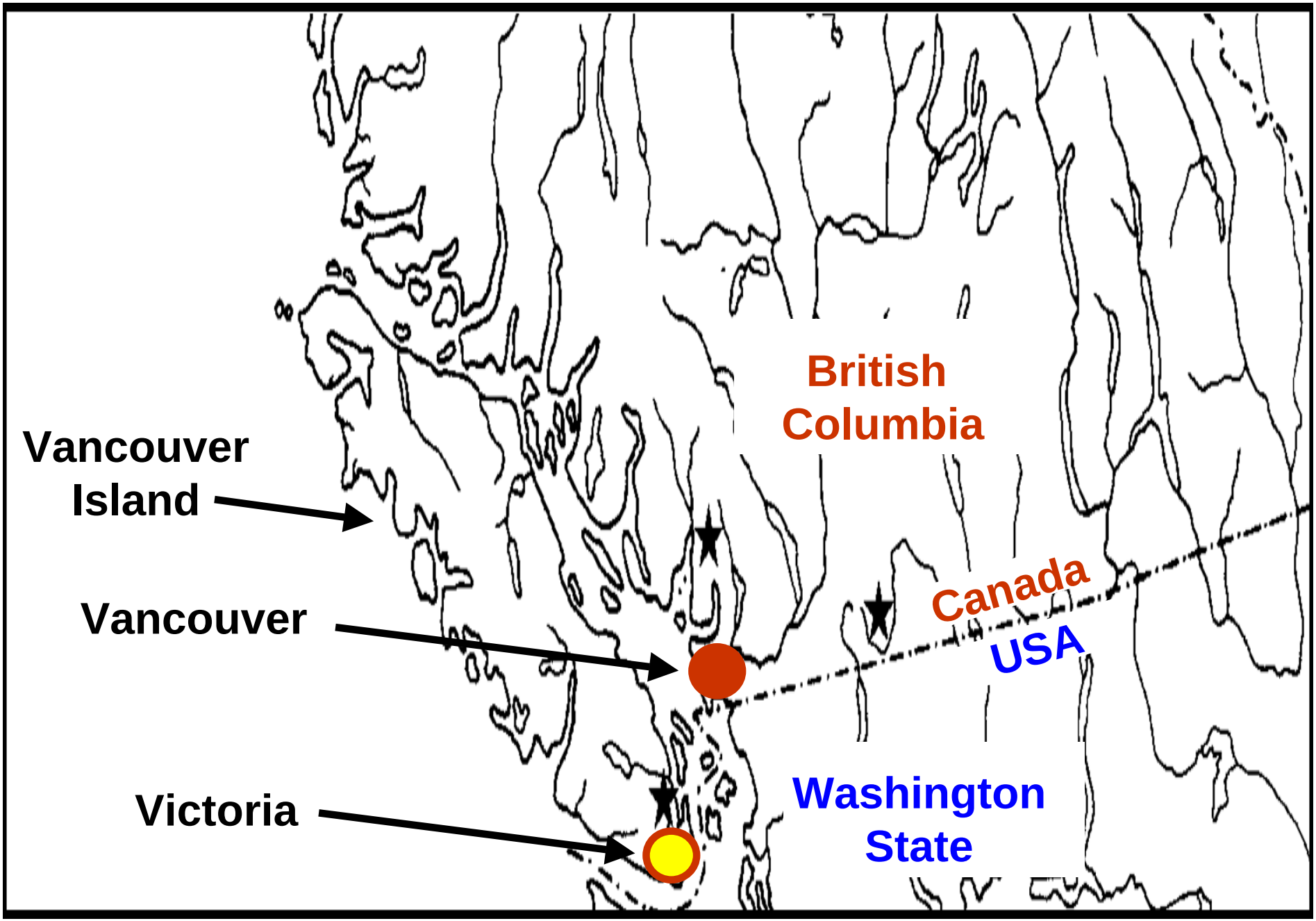
- **Many Strains within Varieties**

  - ***BtK* HD1 encodes a complex of five Lepidoptera-specific toxin genes: *cry* 1Aa, *cry* 1Ab, *cry* 1Ac, *cry* 2A, and *cry*2B**

The Gypsy Moth

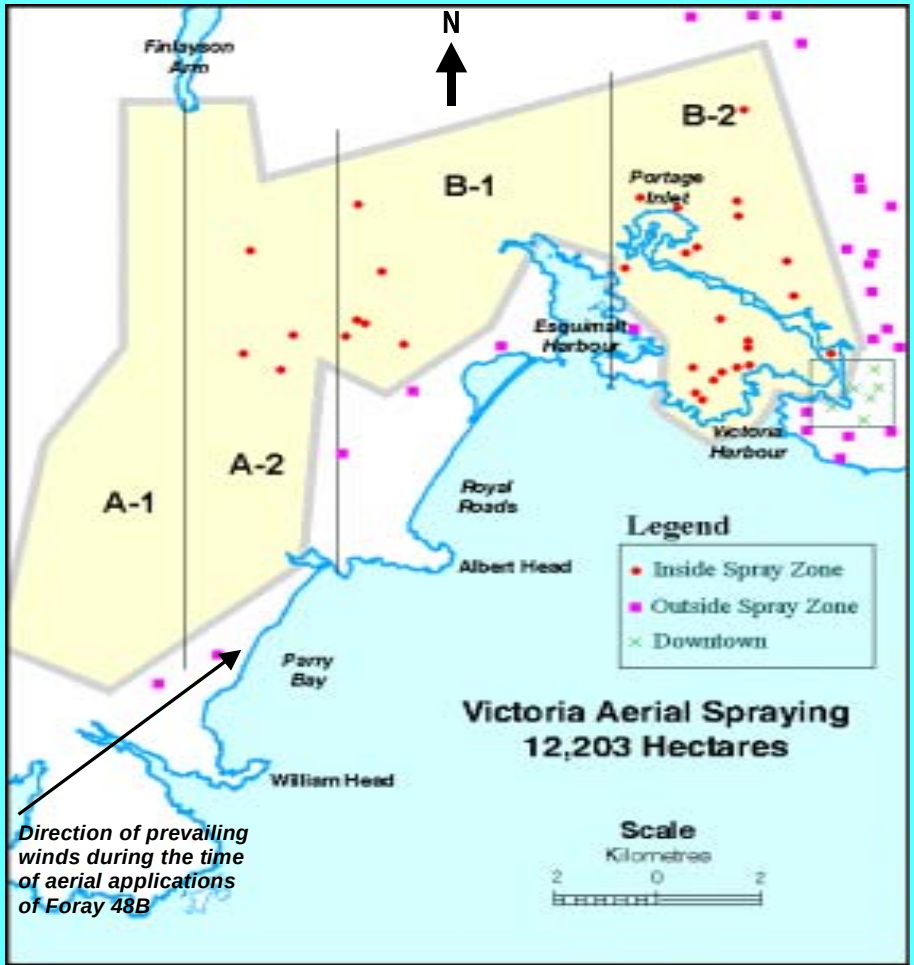

Gypsy moth male

- **The gypsy moth- *Lymantria dispar* :**
  - **Major deciduous tree defoliating insect pest in eastern North America**
  - **Not yet established in British Columbia**
  - **Infestation became problematic, 1998 - 1999**
  - **Sale and export of B.C. lumber products, valued at approximately $2.7 billion dollars, faced embargo if gypsy moth populations were not controlled**


British
Columbia
Vancouver
Island
Vancouver
Canada
USA
Victoria
Washington
State

Gypsy Moth Control in Victoria

- **Population of Victoria is approx. 70,000**



Gypsy Moth Control in Victoria

- **How was spray applied?**

Gypsy Moth Control in Victoria

- **Molasses (20% solution) is used as carrier**

Aerial Application of Foray 48B

- **Microbial insecticide, Foray 48B contains:**
  ***B. thuringiensis* subsp. Kurstaki, strain HD-1**

- **Spray applied by Cessena 188:**

  - **580 L @ 70 L/min (approx. 152 gal @ 20 gal/min)**

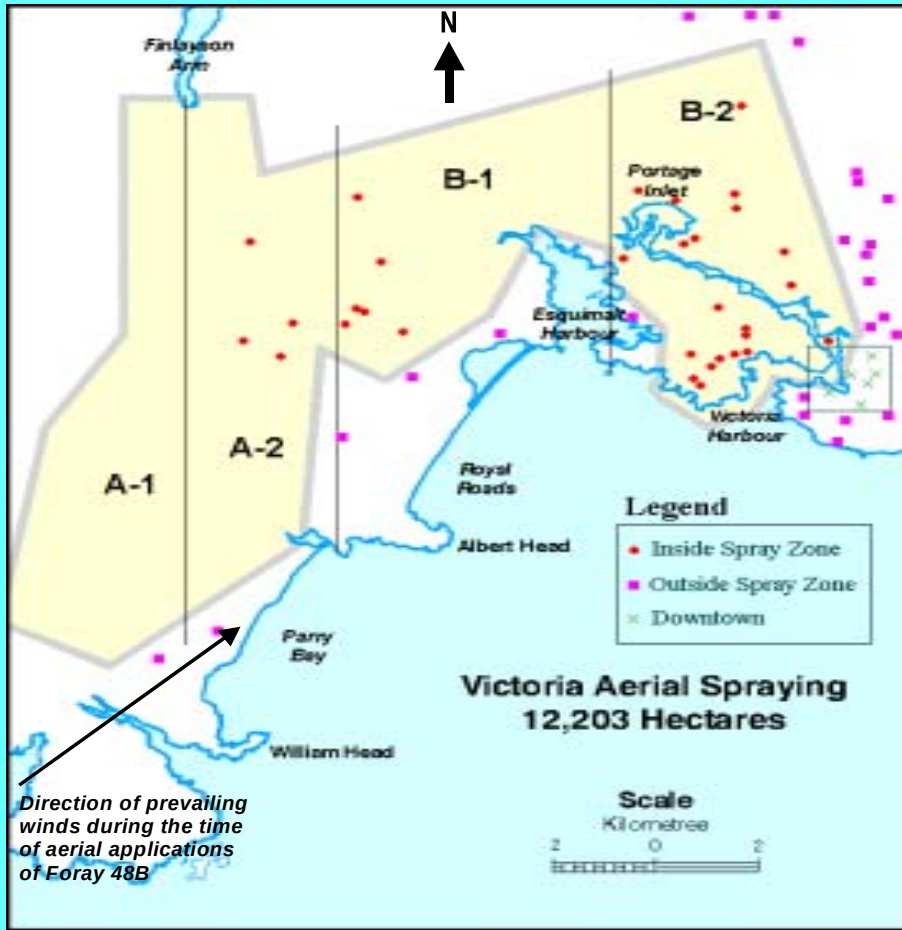
- **Applied at 4 L / hectare**
  (0.25 gal/acre)

- **Droplet size: 110 – 130 μm**

Aerial application of Foray 48B

- **Applied by aircraft in 3 spray periods**

- **1st Spray, May 09, 10**
- **2cd Spray, May 19, 20, 21**
- **3rd Spray, June 08, 09**



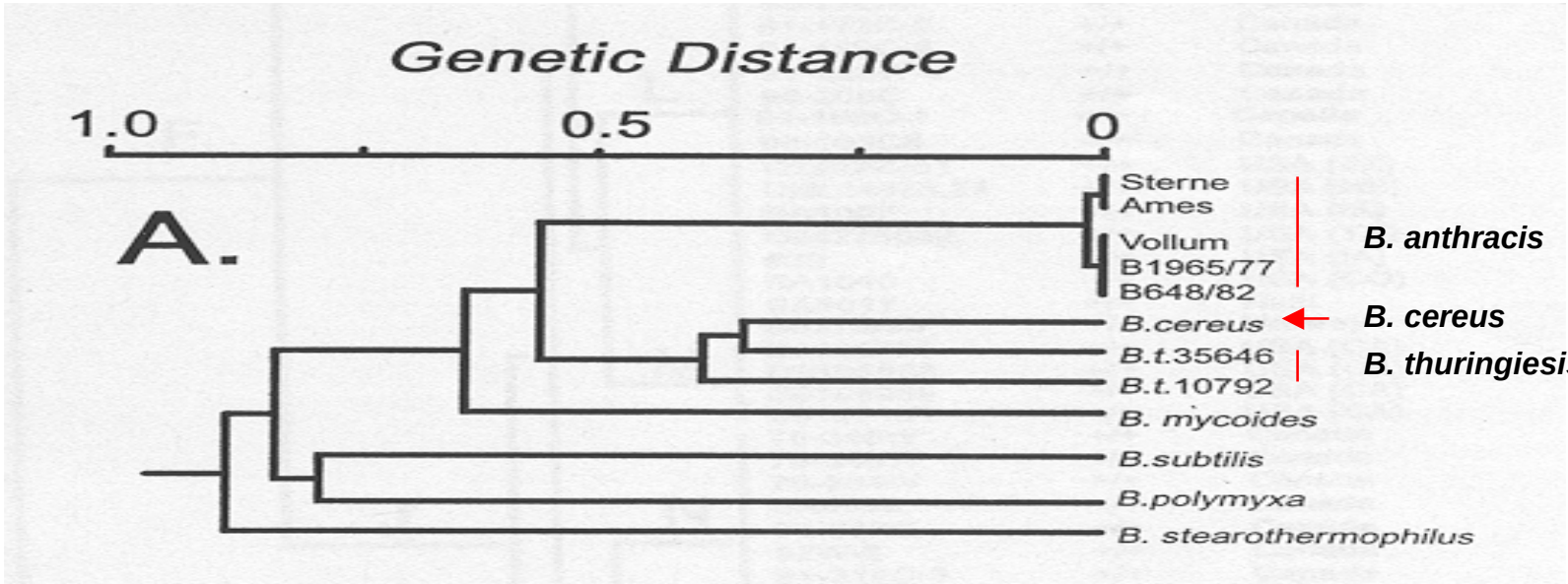
Bt Safety

- ***BtK* is not toxic to mammalian species**
- **Toxin is quickly degraded in the environment by UV-light**
- **Despite safety, concern over possible health impact of aerial spraying mandated public health study**

Health Impact Study

- **To determine if bacteria isolated from air, human, food, and water samples collected pre- and post-spray were *BtK* HD1**
  - **Environmental samples collected pre- and post-spray from Air, water, and grocery produce pre- and post-spray**
  - **Nasal swabs collected from 15 families (approx. 50 people) pre- and post-spray**
  - **Samples collected both inside and outside spray zone**

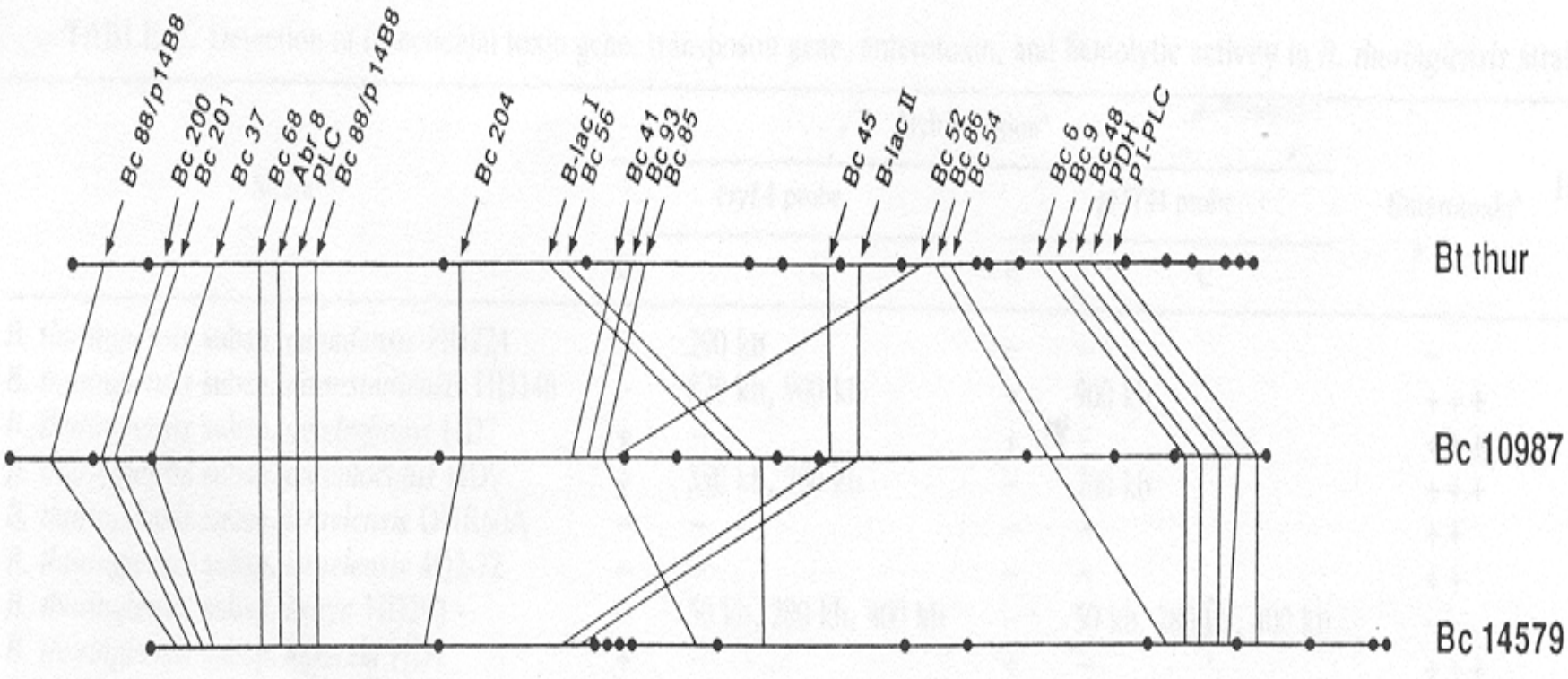
Technical Challenge



- **Bacteria within *B. cereus-group* are very similar biochemically and morphologically**

- ***B. thuringiensis*, *B. cereus*, and *B. anthracis* are closely related at both nucleic acid and amino acid levels**

Technical Challenge



- ***B. thuringiensis and B. cereus*** **have very similar genome organization**

Technical Challenge

- ***B. thuringiensis, B. cereus*, and *B. anthracis* considered to be variants of the same "species" differentiated only by presence of specific plasmids which encode toxins**

- ***B. thuringiensis: cry* gene plasmids**

  - ***BtK* HD1 encodes a complex of five toxin genes encoded on three plasmids:**

    - ***cry* 1Aa, *cry* 1Ac**
    - ***cry* 1Ab**
    - ***cry* 2A, *cry*2B**

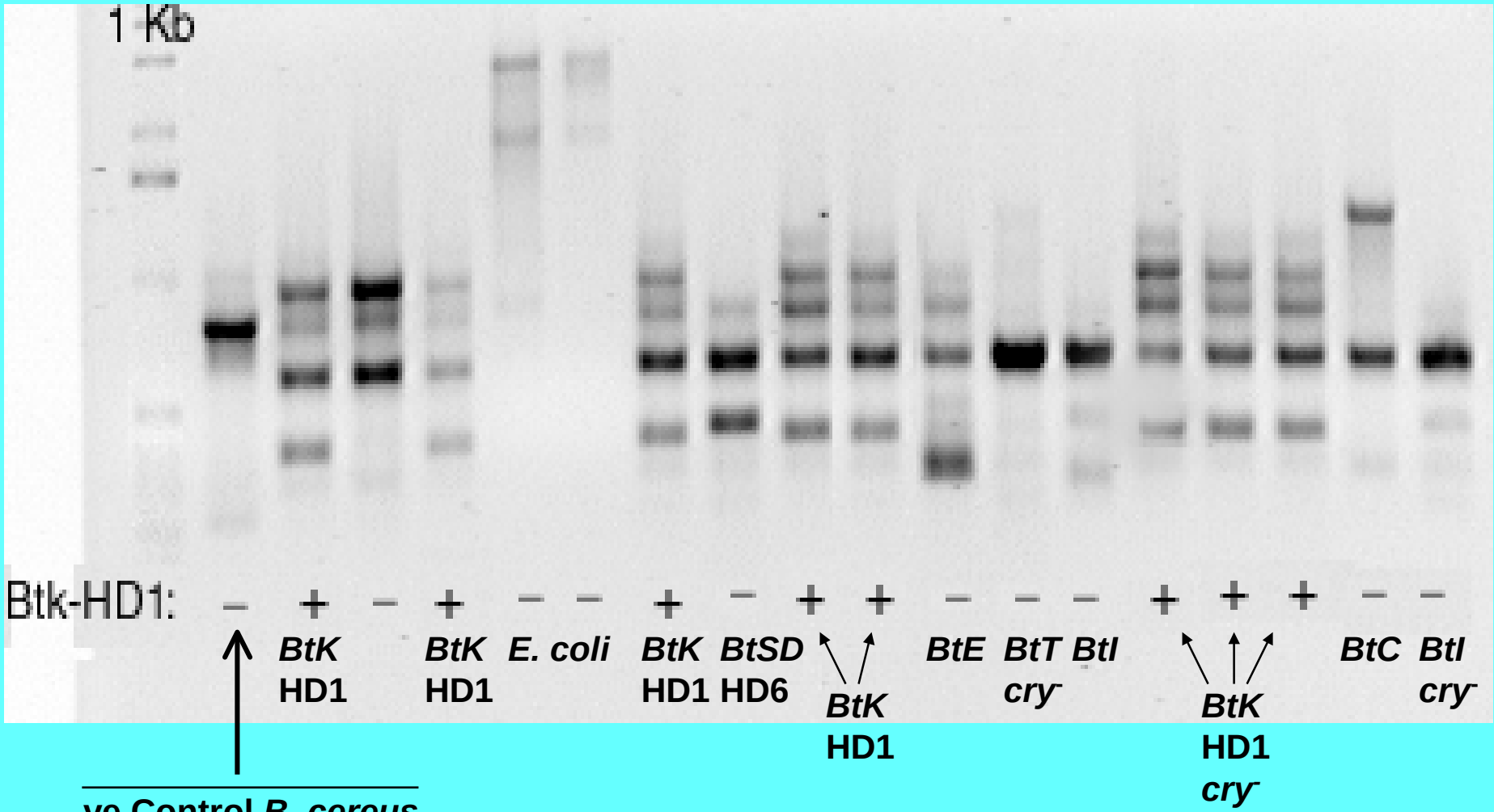
Experimental Approach

- **Combined use of three molecular techniques to identify *BtK* HD1 in exposed and non-exposed individuals**
  - **RAPD- PCR**
    - **Random Amplified Polymorphic DNA- Polymerase Chain Reaction**
  - ***Cry*-gene PCR**
  - **Dot Blot Hybridization**
- **Amplification of genomic DNA from *BtK* HD1 produced four characteristic bands of approximately 1000, 800, 60, and 400 bp**

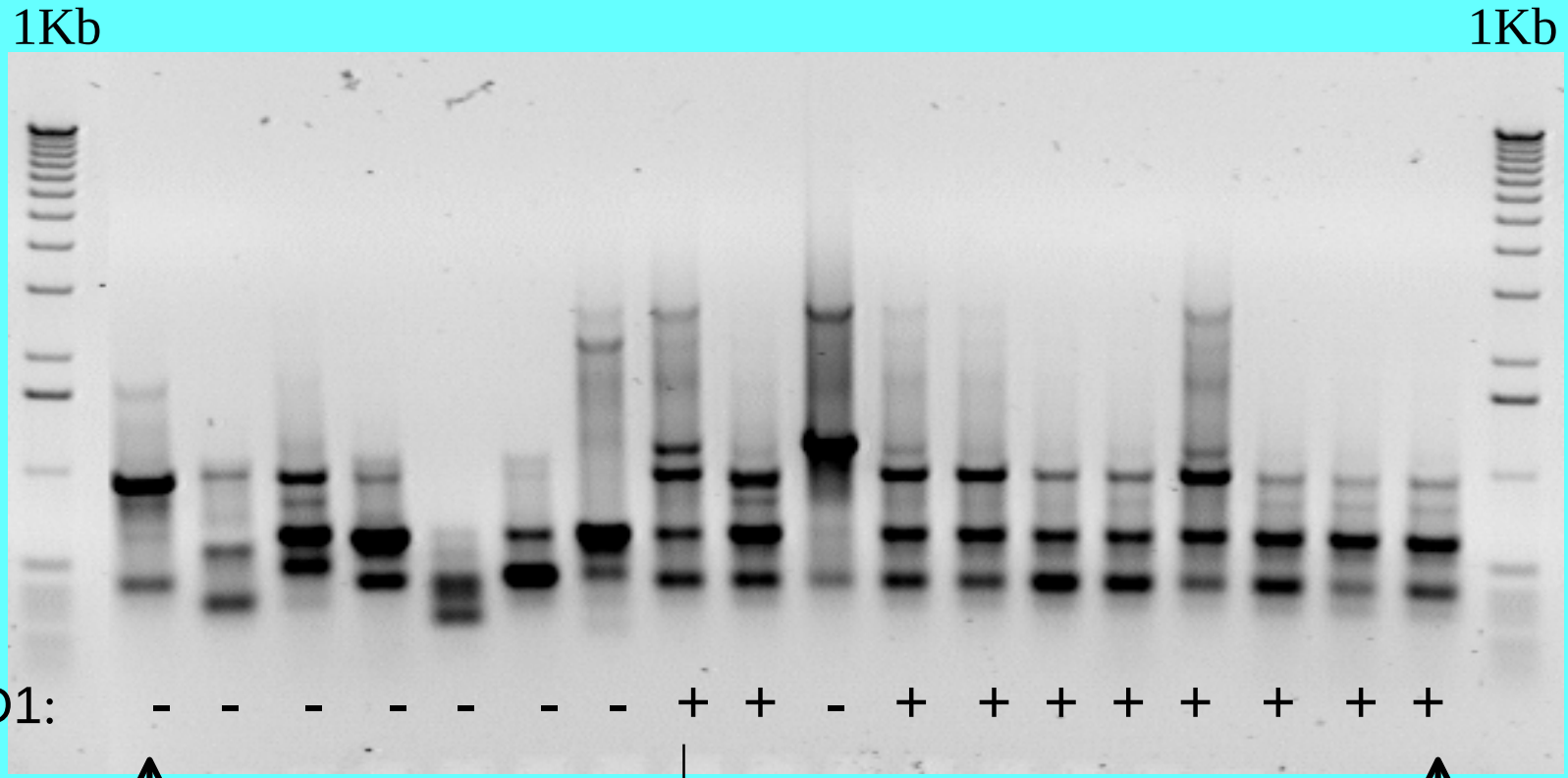
RAPD-PCR

- **Able to distinguish *BtK* from several varieties of *Bt* and between *BtK* and *B. cereus***

RAPD-PCR of Different *Bt* Varieties

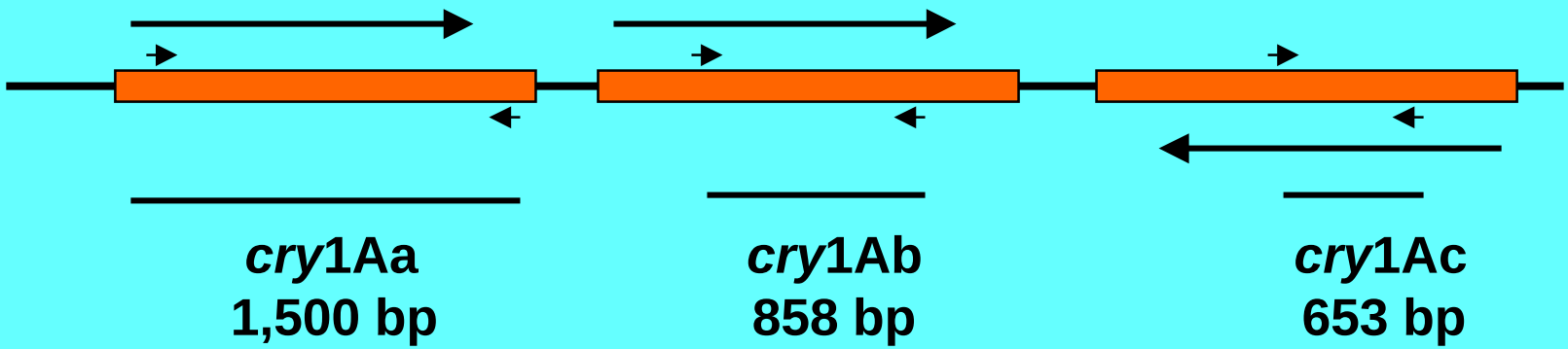


RAPD-PCR From Nasal Swabs

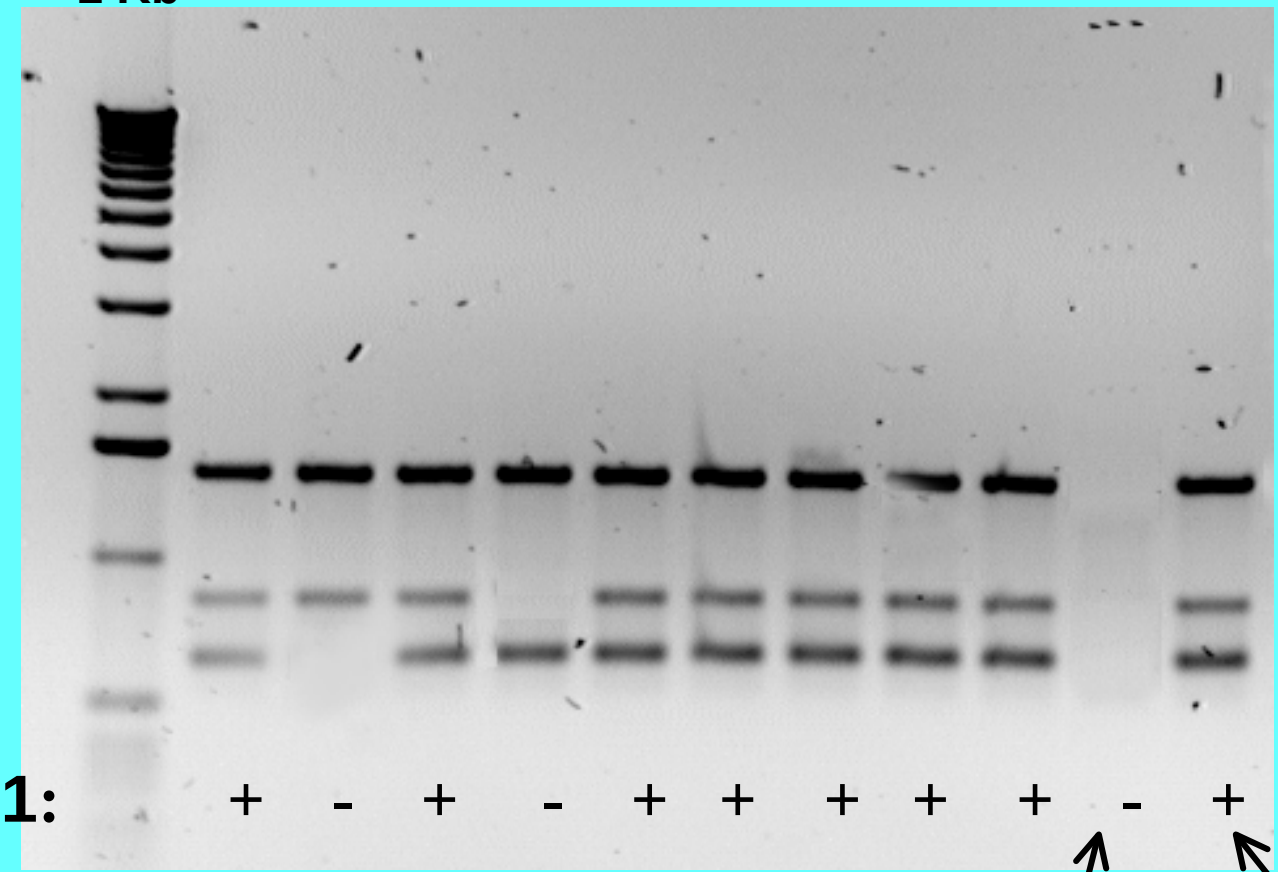


Cry 1 Gene PCR

- ***BtK* HD1 contains 3 plasmids that encode complex of five toxin genes:**
  - ***cry* 1Aa, *cry* 1Ab, *cry* 1Ac,*cry* 2A, and *cry*2B**
  - **Presence of *cry* 1Aa, *cry* 1Ab, *cry* 1Ac is diagnostic of *BtK* HD1**



Cry 1 Gene PCR

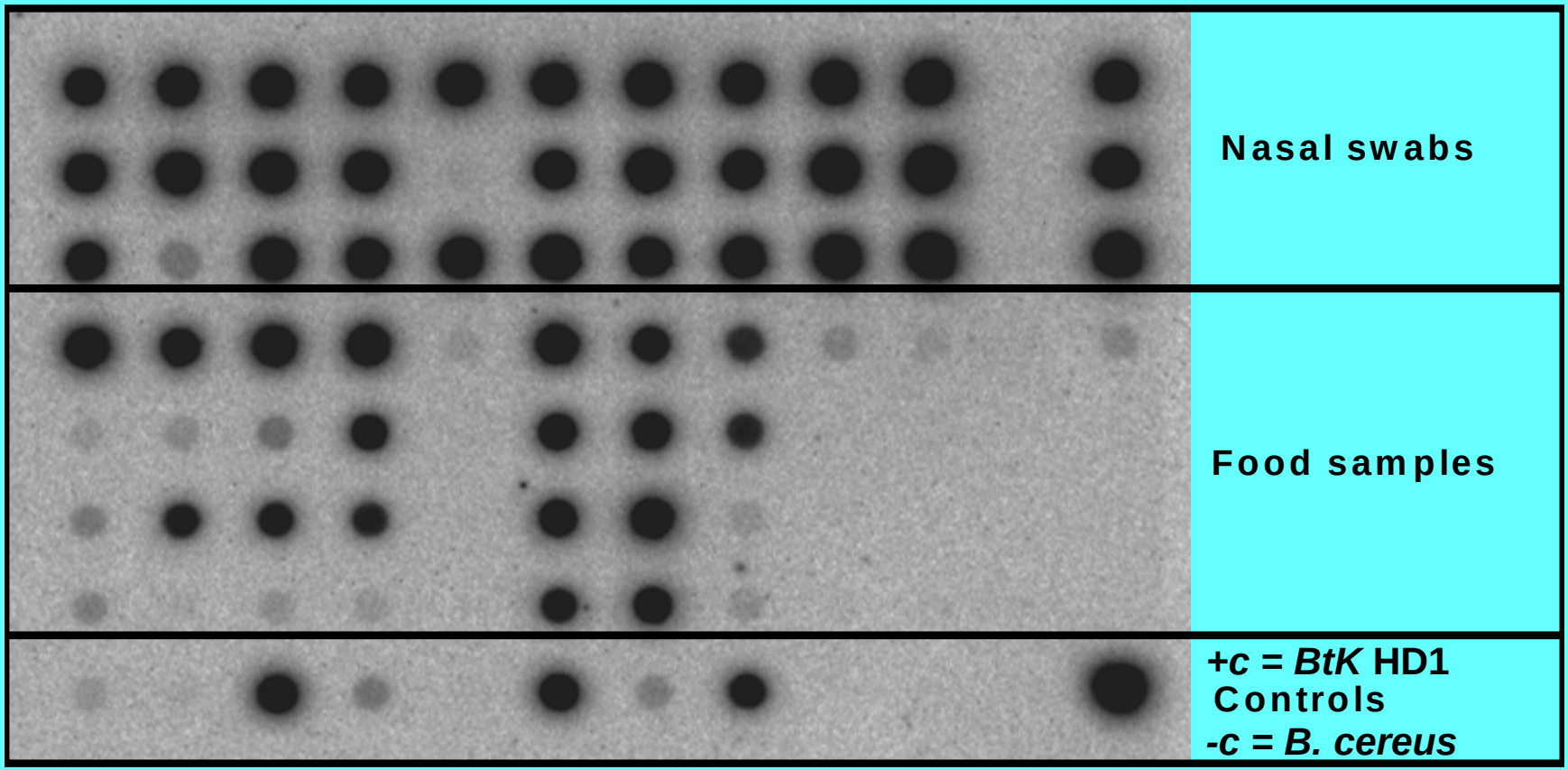


Limitations of PCR-Based Analyses

- **RAPD and *cry* gene PCR are very sensitive**
  - **Extremely low frequency of False Positives**
  - **High frequency of False Negatives (32%)**
- **Confirmed PCR-based results by DNA Hybridization**
  - **Low frequency of False Negatives (< 2%)**
  - **May produce some False Positives**

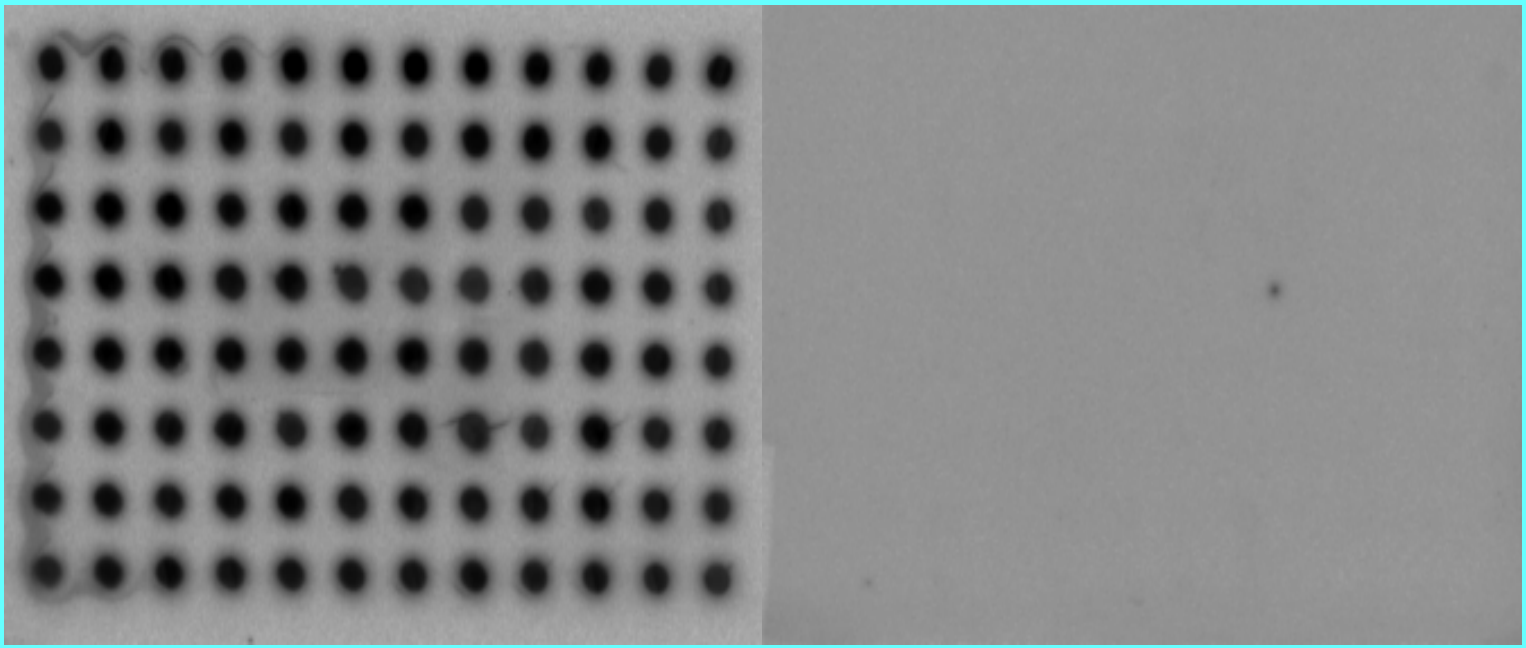
Cry 1 Gene Hybridization

- **Screened 171 isolates of bacteria from nasal swabs and 29 isolates from food samples**



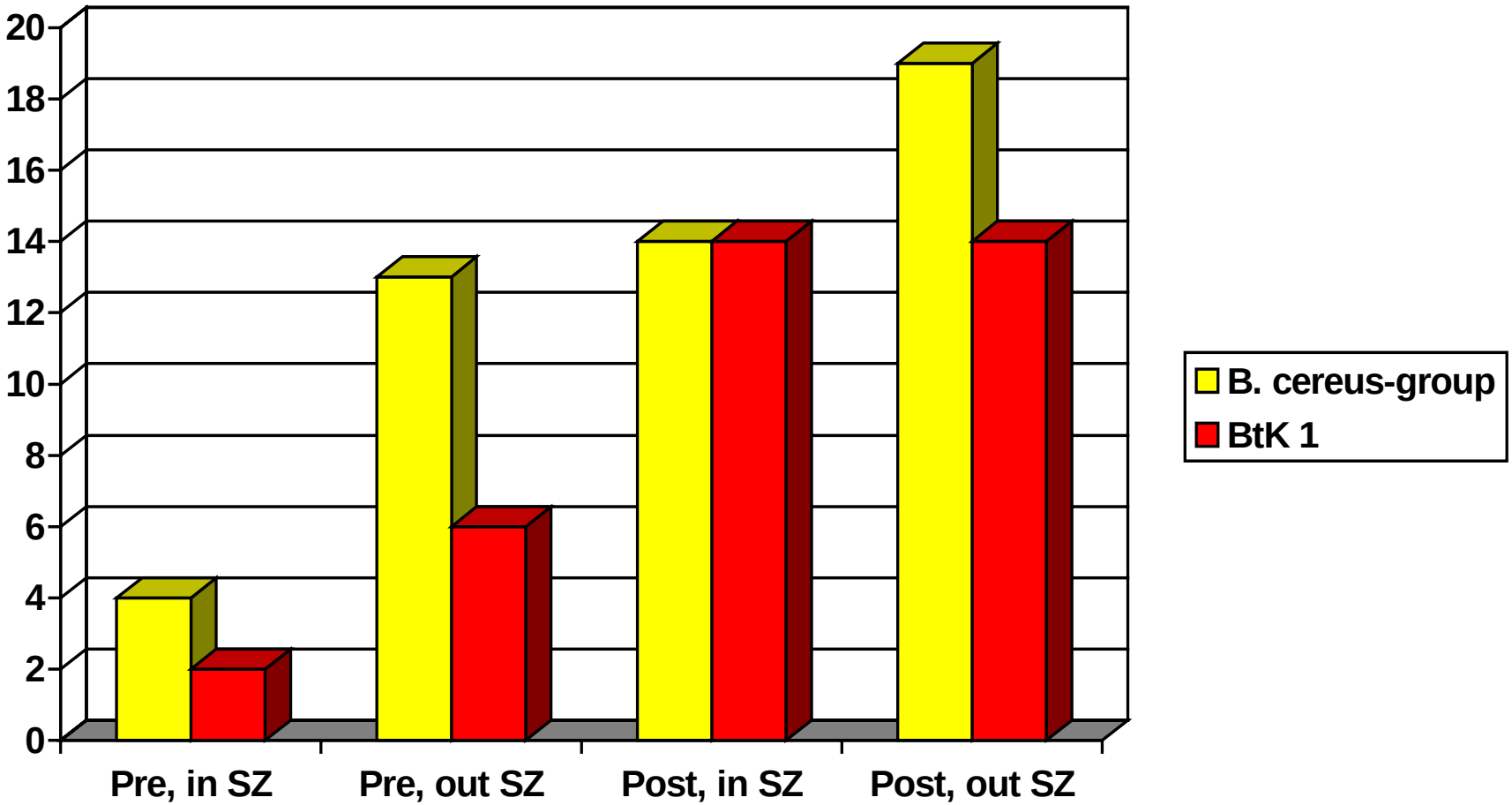
Cry 1 Gene Hybridization

- **Screened over 10,000 isolates of bacteria from air samples: 85.4% *BtK* HD1 Positive**



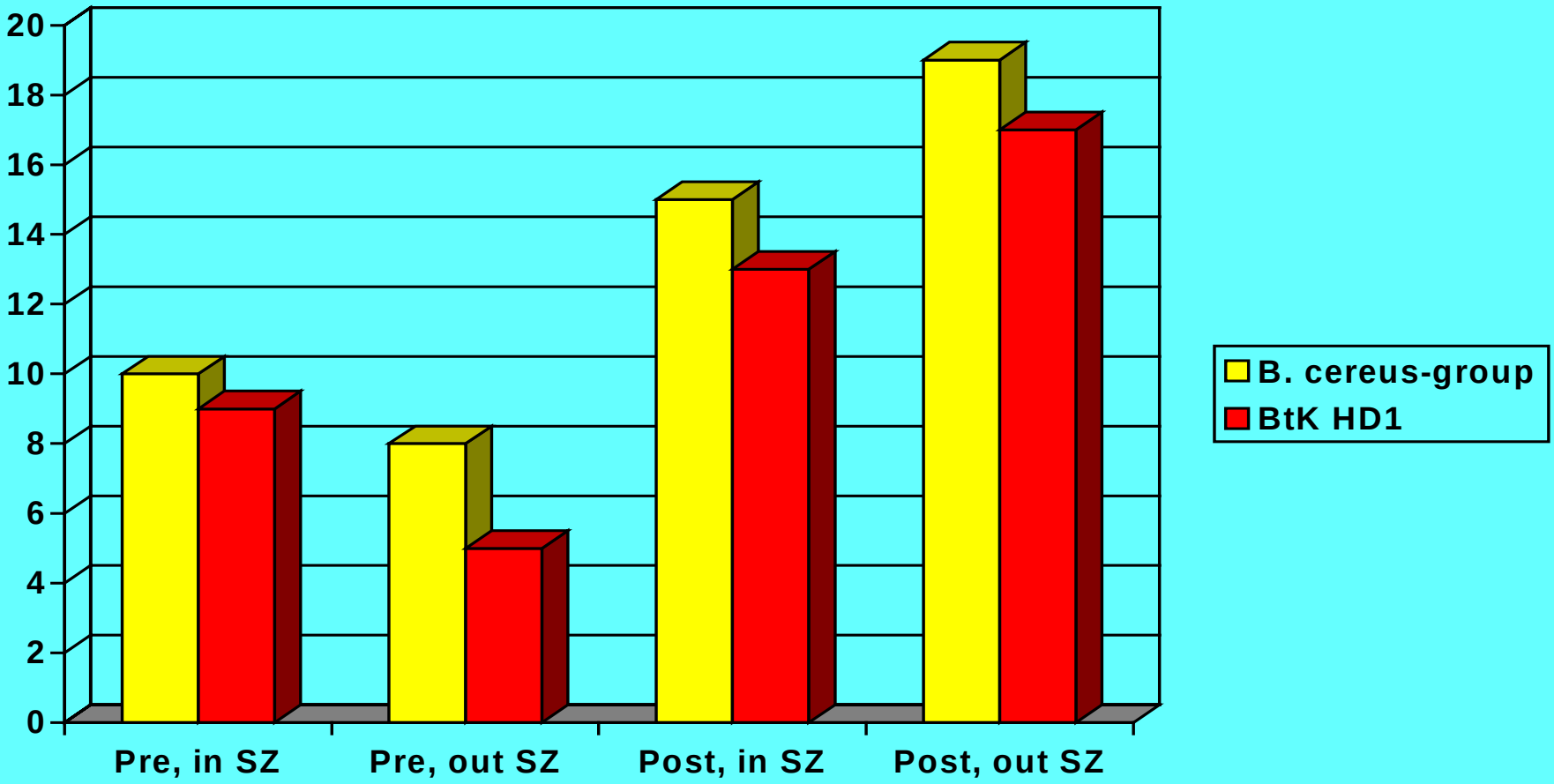
BtK* HD1** ***B. cereus

Combined Analysis
Nasal Swabs- First Spray



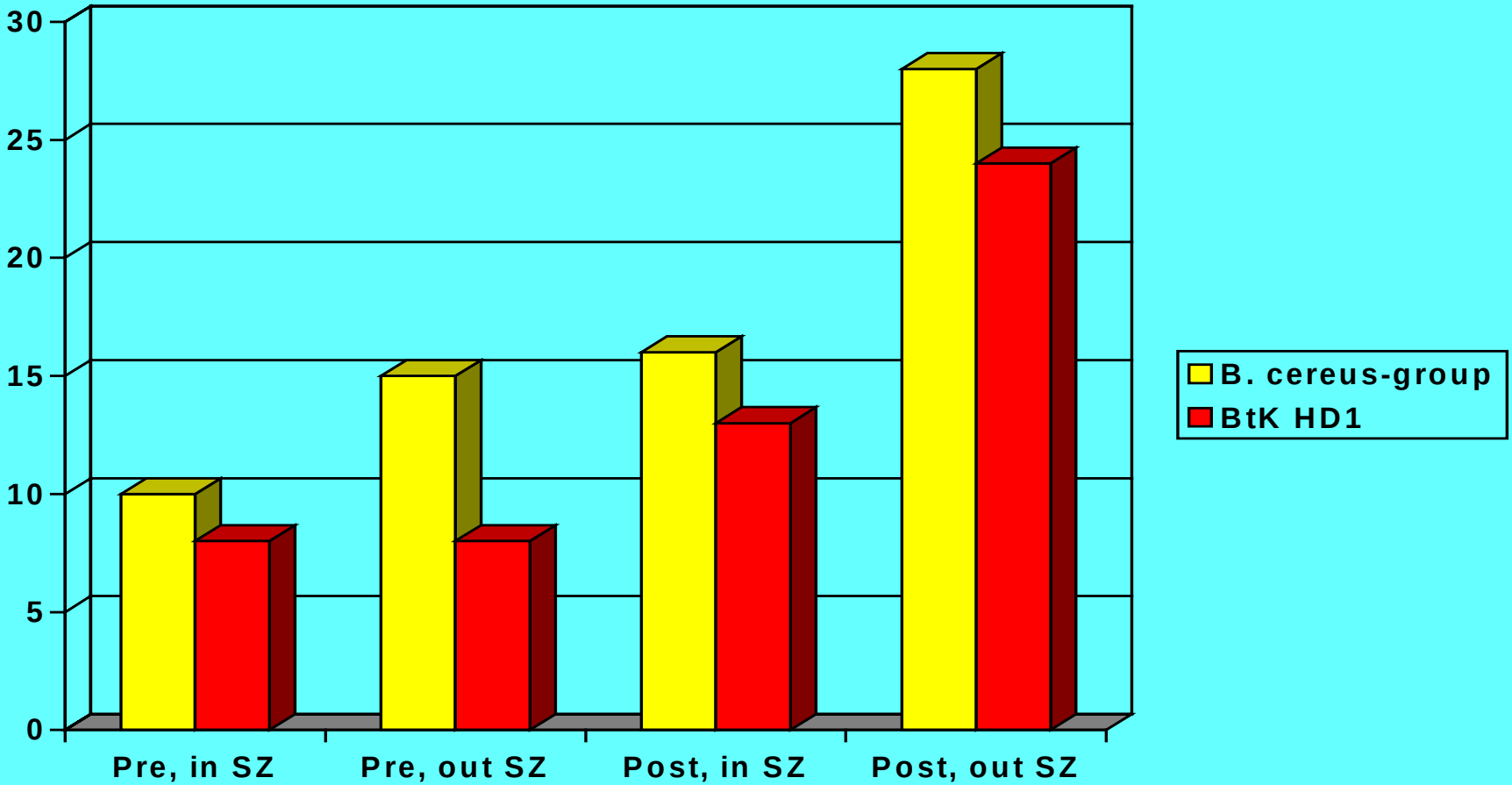
Pre-Spray: 47.0 % *BtK* HD1 Post-Spray: 84.8 % *BtK* HD1

Combined Analysis
Nasal Swabs- Second Spray



Pre-Spray: 77.7 % *BtK* HD1 **Post-Spray: 82.3 % *BtK* HD1**

Combined Analysis
Nasal Swabs- Third Spray



Pre-Spray: 64.0 % *BtK* HD1 **Post-Spray: 84.1 % *BtK* HD1**

Conclusions

- **Positively identified *BtK* HD1**
- **Distinguish *BtK* HD1 from other varieties of *Bt* and from different isolates of *B. cereus***
- ***BtK* HD1 was present in environment and human population of Victoria prior to aerial applications of Foray 48B**
- **Incidence of *BtK* HD1 increased human population even though people were inside houses at time of spray**