Phages for Food Safety: phage application against *Yersinia*

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   1.2. Vibrio parahaemolyticus survey
   1.3. Phage isolation & characterization
   1.4. Phage therapy for pandemic Vibrio
   1.5. Phage prophylaxis for pandemic Vibrio

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   2.2. Yersinia phage characterization
   2.3. Phage application to foods
   2.4. Phage application to kitchen utensils
   2.5. In vivo phage safety test
Part 1

1.1. *Vibrio parahaemolyticus*

**Oyster**

- Pacific oyster, *Crassostrea gigas* (Thunberg, 1793), marine bivalve
- Cultured all over the world including Korea
- Valuable seafood
  1) Fresh taste
  2) Nutritionally perfect
  3) Commercially-important
Part 1

1.1. Vibrio parahaemolyticus

**Infectious Agent**
- Curved rod shape
- Gram-negative
- Single flagellum

**Risk Groups**
- Along coasts
- People who eat shellfish
  - **4,500 cases in US/year**

**Transmission**
- Water-borne
- Fecal-oral
- Food-borne in **shellfish**
- Wound infections after exposure to contaminated water

**Epidemiology**
- Pandemic strains (O3:K6 serotype)
  - India, Russia, Southeast Asia, Japan, Korea, North America

**Symptoms**
- 24 hours incubation period
- Watery diarrhea, nausea, vomiting, abdominal cramps, fever
- Resolves in 72 hours

**Treatment**
- Fluid and electrolyte replacement
- Antibiotics (immunocompromised patients)

**Risk Groups**
- Along coasts
- People who eat shellfish
- **4,500 cases in US/year**
## 1.2. *Vibrio parahaemolyticus* survey

### Study area

![Map of study area](image.png)

### Seafood sampling

<table>
<thead>
<tr>
<th>Seafood samples</th>
<th>Environmental samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black tiger shrimp (<em>Penaeus monodon</em>)</td>
<td>Seafood-related Seawater and Water Sediment</td>
</tr>
<tr>
<td>Corb shell (<em>Cyclina sinensis</em>)</td>
<td></td>
</tr>
<tr>
<td>Kuruma prawn (<em>Marsupenaeus japonicas</em>)</td>
<td></td>
</tr>
<tr>
<td>Razor clam (<em>Solen strictus</em>)</td>
<td></td>
</tr>
<tr>
<td>Short neck clam (<em>Venerupis philippinarum</em>)</td>
<td></td>
</tr>
<tr>
<td>Sea cucumber (<em>Stichopus japonicas</em>)</td>
<td></td>
</tr>
<tr>
<td>Sea mussel (<em>Mytilus coruscus</em>)</td>
<td></td>
</tr>
<tr>
<td>Pacific oyster (<em>Crassostrea gigas</em>)</td>
<td></td>
</tr>
<tr>
<td>Charm abalone (<em>Haliotis discushannahi</em>)</td>
<td></td>
</tr>
<tr>
<td>White leg shrimp (<em>Litopenaeus vannamei</em>)</td>
<td></td>
</tr>
</tbody>
</table>
1.2. *Vibrio parahaemolyticus* survey

**Isolation of strains**

Fisheries wholesale market → Seafood Samples → Stomacher homogenizer → Pure cultures → CHROMagar™ Vibrio → TCBS agar → APW containing 3% NaCl

*Jun et al., 2012. Foodborne Pathogens and Disease*
1.2. *Vibrio parahaemolyticus* survey

**Molecular analysis**

- **Multiplex-PCR**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Target gene</th>
<th>Expected product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vp. flaE</em></td>
<td><em>flaE</em> sequence of <em>V. parahaemolyticus</em></td>
<td>897 bp</td>
<td>Tarr et al., 2007. <em>J. Clin. Microbiol.</em></td>
</tr>
<tr>
<td><em>tl</em></td>
<td>thermolabile hemolysin</td>
<td>450 bp</td>
<td>Bej et al., 1999. <em>J. Microbiol. Methods</em></td>
</tr>
<tr>
<td><em>tdh</em></td>
<td>thermostable direct hemolysin</td>
<td>269 bp</td>
<td>Bej et al., 1999. <em>J. Microbiol. Methods</em></td>
</tr>
<tr>
<td><em>trh</em></td>
<td>thermostable direct hemolysin-related hemolysin</td>
<td>500 bp</td>
<td>Bej et al., 1999. <em>J. Microbiol. Methods</em></td>
</tr>
</tbody>
</table>

- **16S rRNA gene sequencing analysis**
1.2. *Vibrio parahaemolyticus* survey

**Antibiotic susceptibility test**

- Antibiotic susceptibility test via the standard disk diffusion method using 22 antibiotic disks

![Antibiotic Susceptibility Test Image]

CLSI (Clinical and Laboratory Standards Institute), 2005

**Multiplex-PCR to detect antibiotic resistant genes**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Expected product</th>
<th>Primer</th>
<th>Expected product</th>
</tr>
</thead>
<tbody>
<tr>
<td>SXT integrase</td>
<td>1035 bp</td>
<td><em>sul2</em></td>
<td>625 bp</td>
</tr>
<tr>
<td><em>floR</em></td>
<td>526 bp</td>
<td><em>dfir18</em></td>
<td>389 bp</td>
</tr>
<tr>
<td><em>TetA</em></td>
<td>950 bp</td>
<td><em>strB</em></td>
<td>470 bp</td>
</tr>
<tr>
<td><em>dfirA1</em></td>
<td>372 bp</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Part 1 1.2. *Vibrio parahaemolyticus* survey

**Isolation/characterization of strains**

<table>
<thead>
<tr>
<th>Strains (Number)</th>
<th>Positive sample of PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ORF8</td>
</tr>
<tr>
<td><strong>Reference strains</strong></td>
<td></td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em> (3)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Seafood isolates</strong></td>
<td></td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em> (19)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Environmental isolates</strong></td>
<td></td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em> (3)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Clinical isolates</strong></td>
<td></td>
</tr>
<tr>
<td><em>V. parahaemolyticus</em> (2)</td>
<td>2/2</td>
</tr>
<tr>
<td><strong>Positive sample/total</strong></td>
<td>2/27</td>
</tr>
</tbody>
</table>
Part 1

1.2. *Vibrio parahaemolyticus* survey

**Antibiotic susceptibility profile**

- The antibiotic resistance patterns of 22 commercial antibiotics

- All isolates evidenced **multiple resistance** (resistance to > 2 antibiotics) and were resistant to more than 4 antibiotics.
Part 1

1.2. *Vibrio parahaemolyticus* survey

**Publication**

FOODBORNE PATHOGENS AND DISEASE
Volume 9, Number 3, 2012
© Mary Ann Liebert, Inc.
DOI: 10.1089/fpd.2011.1018

**Isolation, Molecular Characterization, and Antibiotic Susceptibility of *Vibrio parahaemolyticus* in Korean Seafood**

Jin Woo Jun¹, Ji Hyung Kim¹, Casiano H. Choresca, Jr.¹, Sang Phil Shin¹, Jee Eun Han¹, Sang Yoon Han¹, Ji Young Chai², and Se Chang Park¹

**Abstract**

The principal objective of this study was to investigate the incidence, risk assessment, antibiotic resistance, and genotyping of *Vibrio parahaemolyticus* in Korean seafood. The incidence of *V. parahaemolyticus* in seafood obtained from several fish markets in Korea was investigated from May to December of 2009, except between July and September. Two selective mediums (TCBS [thiosulfate, citrate, bile salts, and sucrose] agar and CHROMagar™ Vibrio) were used, and the *V. parahaemolyticus* strains were identified via polymerase chain reaction (PCR) amplification (*Vp. flaE, ll*, and *toxR*). 16S rRNA gene sequencing and their virulence were analyzed via the detection of *tdh, trh, ORF8, toxRS/old*, and *toxRS/new* genes. We collected 24 strains of *V. parahaemolyticus*: 19 seafood isolates, three environmental isolates, and two clinical (human) isolates. Among these strains, two *tdh+* strains, two ORF8+ strains, 16 toxRS/old+ strains, and one toxRS/new strain were isolated. Twenty-two
**Part 1**

1.3. Phage isolation & characterization

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**Vibrio phage pVp-1**

- **Morphology**
  - *Siphovirus*
  - T5-like phage
  - No pathogenic factor

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**Genome of pVp-1**

- **Siphovirus**
- **T5-like phage**
- No pathogenic factor

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[Diagram showing the genome of pVp-1 with labeled proteins and genomic regions]
Complete Genome Sequence of a Novel Marine Siphovirus, pVP-1, Infecting *Vibrio parahaemolyticus*

Ji Hyung Kim,\(^a\)\(^b\) Jin Woo Jun,\(^a\) Casiano H. Choresca,\(^a\) Sang Phil Shin,\(^a\) Jee Eun Han,\(^a\) and Se Chang Park\(^a\)

Laboratory of Aquatic Animal Medicine, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea,\(^a\) and Korea Ocean Research & Development Institute, Ansan, Republic of Korea\(^b\)

Among the abundant bacteriophages that belong to the order *Caudovirales* in the ocean, the genome sequences of marine siphoviruses are poorly investigated in comparison to those of myoviruses or podoviruses. Here we report the complete genome sequence of *Vibrio* phage pVP-1, which belongs to the family *Siphoviridae* and infects *Vibrio parahaemolyticus* ATCC 33844.

Marine viruses are the most abundant biological entities in the ocean \(^{10}\), which makes the analysis of their genomes essential for a better understanding of their enormous genetic diversity \(^{1}\). Most of the marine viruses reported to date are bacteriophages that belong to the order *Caudovirales*, which is divided into three families: *Myoviridae*, *Podoviridae*, and *Siphoviridae* \(^{10}\). Among the marine phages whose genomes have been sequenced, siphoviruses are relatively poorly investigated \(^{9}\) and only two of them, including phiHSIC \(^{7}\) and SIO-2 \(^{1}\), were studied and orf156, and orf157), and lytic properties (orf73, orf82, and orf83). Interestingly, most of the ORFs containing DNA metabolism and viral morphogenesis genes were clustered together at each end of the sequenced genome by functional roles and were similar \((\leq 79\%)\) to those of T5 \(^{11}\) or T5-like \(^{3,4}\) phages, thus indicating a close genetic relatedness between pVP-1 and those phages.

In contrast, there were no sequence similarities to marine *Vibrio* phages belonging to the family *Siphoviridae* (phiHSIC and SIO-2), and a large proportion of the genes in pVP-1 were not
1.4. Phage therapy for pandemic *Vibrio*

1 h post treatment

*Jun et al., 2014. Journal of Infectious Diseases*
Part 1

1.4. Phage therapy for pandemic *Vibrio*

3 h post treatment
Part 1

1.4. Phage therapy for pandemic *Vibrio*

12 h post treatment
Part 1

1.4. Phage therapy for pandemic *Vibrio*

24 h post treatment
Histopathology

Control

Experiment

Treated
Bacteriophage Therapy of a *Vibrio parahaemolyticus* Infection Caused by a Multiple-Antibiotic–Resistant O3:K6 Pandemic Clinical Strain

**Jin Woo Jun,1,a Tae Hoon Shin,1 Ji Hyung Kim,2,a Sang Phil Shin,1 Jee Eun Han,1 Gang Joon Heo,3 Mahanama De Zoyea,1 Gee Wook Shin,5 Ji Young Chai,6 and Se Chang Park1**

1College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul; 2Korea Institute of Ocean Science and Technology, Ansan; 3College of Veterinary Medicine, Chungbuk National University, Cheongju; 4College of Veterinary Medicine, Chungnam National University, Daejeon; 5Bio-safety Research Institute and College of Veterinary Medicine, Chonbuk National University, Jeonju; and 6Department of Rheumatology, Bundang Jesang Hospital, Seongnam, Republic of Korea

**Background.** Recently isolated *Vibrio parahaemolyticus* strains have displayed multiple antibiotic resistance. Alternatives to conventional antibiotics are needed, especially for the multiple-antibiotic–resistant *V. parahaemolyticus* pandemic strain.

**Methods.** A bacteriophage, designated pVp-1, showed effective infectivity for multiple-antibiotic–resistant *V. parahaemolyticus* and *V. vulnificus*, including *V. parahaemolyticus* pandemic strains. The therapeutic potential of the phage was studied in a mouse model of experimental infection using a multiple-antibiotic–resistant *V. parahaemolyticus* pandemic strain. We monitored the survivability and histopathological changes, quantified the bacte-
1.5. Phage prophylaxis for pandemic *Vibrio*

**Application to oyster processing**

Infection method: bath immersion ($10^6$CFU/ml)

Treatment method: bath immersion ($10^7$PFU/ml)

Control
- No phage treated

Treatment
- Phage treated

40 oysters/group

Temp.: $18 \pm 0.3 \, ^\circ C$

Volume of water: 35 L (artificial seawater)
Part 1

1.5. Phage prophylaxis for pandemic *Vibrio*

Application to oyster processing

![Graph showing Log10 CFU/g and Log10 PFU/g over time for Control and Treated samples.](image)

*Jun et al., 2014. International Journal of Food Microbiology*
1.5. Phage prophylaxis for pandemic *Vibrio*

**Application to oyster surface**

**Inoculation:** bacteria spread on surface  
(10^6 CFU/oyster)

**Treatment:** phage application on surface  
(10^7 PFU/oyster)
Part 1

1.5. Phage prophylaxis for pandemic *Vibrio*

Application to oyster surface

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td><img src="Image" alt="Control" /></td>
<td><img src="Image" alt="Treated" /></td>
</tr>
<tr>
<td>0,5</td>
<td><img src="Image" alt="Control" /></td>
<td><img src="Image" alt="Treated" /></td>
</tr>
<tr>
<td>1</td>
<td><img src="Image" alt="Control" /></td>
<td><img src="Image" alt="Treated" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="Image" alt="Control" /></td>
<td><img src="Image" alt="Treated" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="Image" alt="Control" /></td>
<td><img src="Image" alt="Treated" /></td>
</tr>
<tr>
<td>6</td>
<td><img src="Image" alt="Control" /></td>
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</tr>
<tr>
<td>9</td>
<td><img src="Image" alt="Control" /></td>
<td><img src="Image" alt="Treated" /></td>
</tr>
<tr>
<td>12</td>
<td><img src="Image" alt="Control" /></td>
<td><img src="Image" alt="Treated" /></td>
</tr>
</tbody>
</table>

*Jun et al., 2014. International Journal of Food Microbiology*
1.5. Phage prophylaxis for pandemic Vibrio

Publication

Eating oysters without risk of vibriosis: Application of a bacteriophage against *Vibrio parahaemolyticus* in oysters

Jin Woo Jun a,b, Hyoun Joong Kim b, Sae Kil Yun b, Ji Young Chai c, Se Chang Park b,*

a Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research (former American Base of Gournes), Heralikon 71003, Crete, Greece
b Laboratory of Aquatic Biomedicine, College of Veterinary Medicine, Research Institute for Veterinary Science, Seoul National University, Seoul 151-742, Republic of Korea
c Departments of Rheumatology, Bundang Jesaeng Hospital, Seongnam 255-2, Republic of Korea

**ABSTRACT**

*Vibrio parahaemolyticus* is a major cause of foodborne illness and related with the consumption of raw contaminated seafood, especially oysters. To evaluate the effectiveness of various applications of a bacteriophage (phage), pVp-1, against a multiple-antibiotic-resistant *V. parahaemolyticus* pandemic strain (CRS 09-17), we designed artificial contamination models that are most likely to be encountered during oyster processing. When live oysters were treated with bath immersion with pVp-1 after CRS 09-17 challenge, the growth of bacterial strain was significantly reduced. After 72 h of phage application with bath immersion, bacterial growth reduction was observed to be $8.9 \times 10^6$ CFU/ml (control group) to $1.4 \times 10^6$ CFU/ml (treatment group). When pVp-1 was surface-applied on the flesh of oysters after CRS 09-17 inoculation, bacterial growth was properly inhibited.
Part 2

2.1. *Yersinia enterocolitica*

University of Helsinki
Part 2

2.1. *Yersinia enterocolitica*

**Infectious Agent**
- Rod shape
- Gram-negative
- Motile

**Risk Groups**
- Infants, young children
- Older children, young adults

**Transmission**
- Raw or undercooked contaminated pork
- Contaminated raw milk
- Untreated water
- Person-to-person

**Symptoms**
- Fever, abdominal pain
- Diarrhea (often bloody)

**Epidemiology**
- Important zoonotic pathogen in developed countries
- 3rd most common foodborne disease in EU
- 117,000 illnesses and 35 deaths in US/yearly

**Treatment**
- Fluid and electrolyte replacement
- Antibiotics
Part 2

2.2. Yersinia phage characterization

Summer in 2016

Jun et al., 2018. International Journal of Food Microbiology
2.2. *Yersinia* phage characterization

**Phage morphology**

- **Podoviridae**: fHe-Yen3-01 (A)
- **Myoviridae**: fHe-Yen9-01 (B), fHe-Yen9-02 (C), fHe-Yen9-03 (D)
2.2. *Yersinia* phage characterization

Host range

- Infectivity of the four *Yersinia* phages (fHe-Yen3-01, fHe-Yen9-01, fHe-Yen9-02, and fHe-Yen9-03)

- 106 *Yersinia* strains, 10 *Yersinia* species
  
  - *Y. enterocolitica* ($n = 81$), *Y. frederiksenii* ($n = 6$), *Y. kristensenii* ($n = 4$),
  
  - *Y. pseudotuberculosis* ($n = 4$), *Y. bercovieri* ($n = 3$), *Y. aleksiciae* ($n = 2$),
  
  - *Y. intermedia* ($n = 2$), *Y. mollaretii* ($n = 2$), *Y. nurmii* ($n = 1$), and *Y. pekkanenii* ($n = 1$)
2.2. *Yersinia* phage characterization

### Host range

- **fHe-Yen9-01** had the broadest host range, infecting 61.3% (65/106 strains).
- **fHe-Yen9-02** exhibited the second broadest host range, infecting 42.5% (45/106).
- Followed by **fHe-Yen3-01** (29.2%, 31/106), and **fHe-Yen9-03** (25.5%, 27/106)
Part 2

2.2. *Yersinia* phage characterization

**Bacteriolytic effect (in vitro)**

**A**

- **fHe-Yen3-01**
  - Phage infection
  - Control
  - MOI: 0.1
  - MOI: 1
  - MOI: 10

**B**

- **fHe-Yen9-01**
  - Phage infection
  - Control
  - MOI: 0.1
  - MOI: 1
  - MOI: 10

**C**

- **fHe-Yen9-02**
  - Phage infection
  - Control
  - MOI: 0.1
  - MOI: 1
  - MOI: 10

**D**

- **fHe-Yen9-03**
  - Phage infection
  - Control
  - MOI: 0.1
  - MOI: 1
  - MOI: 10
2.2. *Yersinia* phage characterization

Green gene: shared with vB_YenM_ϕR1-RT
Red gene: shared with vB_YenM_TG1

Jun et al., 2018. International Journal of Food Microbiology
2.2. *Yersinia* phage characterization

**Phylogeny of fHe-Yen9-01**

- Edwardsiella phage PEi20
- Edwardsiella phage PEi26
- Pectobacterium bacteriophage PM2
- Serratia phage CHI14
- Serratia phage X20
- Yersinia phage φR1-RT
- Yersinia phage TG1
- Yersinia phage fHe-Yen9-01
- Enterobacteria phage JS10
- Escherichia phage JS98
- Enterobacteria phage T4

Genes related to lysogeny or virulence factors were not found in the genome.
2.3. Phage application to foods

Jun et al., 2018. International Journal of Food Microbiology
2.3. Phage application to foods

Food samples

Inoculation: *Yersinia* contamination \( (10^3 \text{CFU/g or ml}) \)

Treatment: phage application \( (10^8 \text{PFU/g or ml}) \)

<table>
<thead>
<tr>
<th>Control</th>
<th>Inoculation</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>No phage treated</td>
<td></td>
<td>phage treated</td>
</tr>
<tr>
<td>RTE pork</td>
<td>15 min air-dry</td>
<td>RTE pork</td>
</tr>
<tr>
<td>Raw pork</td>
<td>Treatment</td>
<td>Raw pork</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td>Milk</td>
</tr>
</tbody>
</table>

In duplicate. 72 h (raw pork and milk), 12 h (RTE pork)

Temp.: 26 ± 1 °C (RTE pork). 4 °C (raw pork and milk)

CFU count by selective (CIN) agar; PFU count by double-layer agar method
2.3. Phage application to foods

Food samples

CFU, left; PFU, right
Control (non-treated) group: open circle
Phage-treated group: filled circle

Bacterial counts decreased by 1-3 logs from the original levels.
2.4. Phage application to kitchen utensils

Artificial hands (surgical gloves)

Cutting board (plastic)

Cutting board (wood)

Knife
Part 2

2.4. Phage application to kitchen utensils
2.4. Phage application to kitchen utensils

Jun et al., 2018. International Journal of Food Microbiology
Dry vs. humid

Dry condition: biosafety level-2 laminar flow hood

Humid condition: biosafety level-2 laminar flow hood

(relative humidity, 81 ± 3 %)
### 2.4. Phage application to kitchen utensils

#### Kitchen utensils

Inoculation: *Yersinia* contamination ($10^3$CFU/ cm² or ml )

Treatment: phage application ($10^8$PFU/ cm² or ml )

<table>
<thead>
<tr>
<th>Dry condition</th>
<th>Humid condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>No phage treated</td>
</tr>
<tr>
<td>Wooden cutting board</td>
<td>Wooden cutting board</td>
</tr>
<tr>
<td>Plastic cutting board</td>
<td>Plastic cutting board</td>
</tr>
<tr>
<td>Knives</td>
<td>Knives</td>
</tr>
<tr>
<td>Artificial hands</td>
<td>Artificial hands</td>
</tr>
<tr>
<td><strong>Treated</strong></td>
<td>Phage treated</td>
</tr>
<tr>
<td>Wooden cutting board</td>
<td>Wooden cutting board</td>
</tr>
<tr>
<td>Plastic cutting board</td>
<td>Plastic cutting board</td>
</tr>
<tr>
<td>Knives</td>
<td>Knives</td>
</tr>
<tr>
<td>Artificial hands</td>
<td>Artificial hands</td>
</tr>
</tbody>
</table>

For up to 2 h
Part 2

2.4. Phage application to kitchen utensils

Kitchen utensils

CFU, left; PFU, right
Treated group, filled symbols; control, open symbols
Dry, triangles; humid, circles

Bacterial counts decreased by 1-2 logs from the original levels.
2.4. Phage application to kitchen utensils

**Kitchen utensils**

CFU, left; PFU, right

Treated group, filled symbols; control, open symbols

Dry, triangles; humid, circles

Bacterial counts decreased by 1-2 logs from the original levels.
Phage safety assessment in mice

- Ethics statement, SNU-170417-7

- Six-week-old SPF BALB/c mice
- Five groups of six mice
- Crude phage lysate, $10^{10}$ PFU/ml; highly purified preparation, $10^{12}$ PFU/ml
- Intragastrically administration
- The health of the mice was monitored for 28 days.

Both phage preparations did not affect the physical condition or survival of the mice over the 28 days of observation.
Keeping kitchen utensils under humid conditions were not recommended.

It would be desirable to dry kitchen utensils as soon as possible.

This is hard to achieve in the kitchen environment.

The presence of food residues may increase the risk of *Yersinia* spreading.

Certain foods pose a yersiniosis risk.

*Mett* is a preparation of minced raw pork that is popular in Germany.
Thank you for your attention.