Waterborne Bacterial Infections in Georgia:

Pathogens of Concern, Environmental Factors and Potential Biocontrol Strategies

Dr. Marina Tediashvili

Eliava Institute of Bacteriophage, Microbiology and Virology

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Waterborne diseases

- Caused by pathogenic microorganisms (protozoa, viruses, bacteria), transmitted through contaminated water

  - Direct contact or ingestion of water

- Drinking water
- Water used in preparation of food (= foodborne disease)
- Irrigation water (= foodborne disease)
- Recreational waters

  - Bathing water (fresh, brackish or marine)

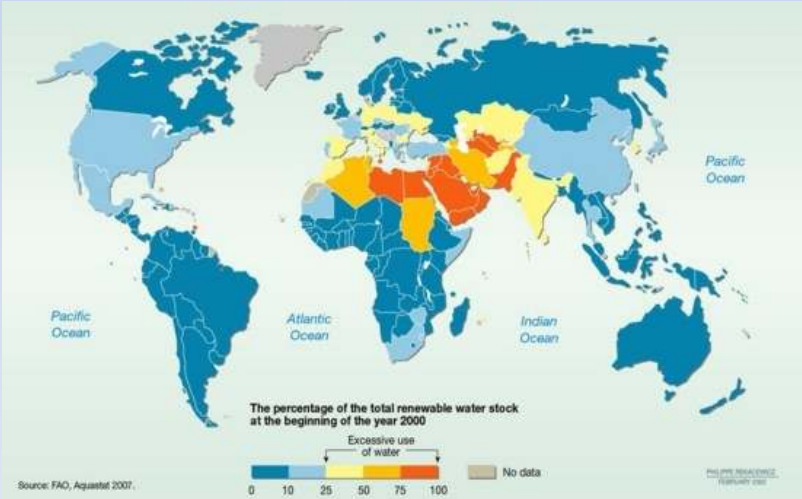
Waterborne infections

Waterborne infections may occur

in the areas /countries experiencing :

- Freshwater deficiency (arid lands, deserts)
- Excess of water (due to flooding)
- Natural catastrophes (earthquakes etc)
- Wars/ethnoconflicts (crowded refugee camps)

Global climate change - triggering factor



Vital Water Graphics 2, 2009

Waterborne diseases

- Major reason of water borne diseases –
  Unsafe water supply, poor sanitation and hygiene
- World-wide around
  - 1.1 billion people lack access to safe water sources
  - 2.4 billion have no basic sanitation.

- Diarrheal disease
  ~ 4.1% of global burden of disease
  - cause of 1.8 million deaths / year
    (4% of all deaths)
- In Southeast Asia and Africa
  - up to 8.5% and 7.7% of all deaths respectively
  - children under 5 year most affected

http://www.who.int/water_sanitation_health/diseases/diseasefact

Waterborne bacterial infections

- **Dysentery (bacillary) – *Shigella spp., Sh. disenteriae***
- **Campylobacteriosis – *C.jujuni , C. coli***
- ***Cholera – V. cholerae O1, O139***
- ***E.coli infections – E.coli species - ETEC, EHEC, EIEC,***
- **Typhoid fever - *S. typhi***
- **Salmonellosis - *Salmonella spp.***
- ***Vibrio* illness – *V. parahaemolyticus, V. vulnificus, V. algynolyticus***
- ***Aeromonas* ilness – *A. hydrophyla, A. caviae, A. sobria* (Toxicoinfection)**
- **Leptospirosis - *Leptospira spp***
- **Legionellosis – *Legionella spp.* (Legionar's and Pontiac's disease)-**
- ***Helicobacter* disease – *Helicobacter pylori (* indirect evidence of water transmission)**
- ***F. tularensis (possibility for Y.pesits)*– Class A bacterial pathogens - high risk of water transmission**

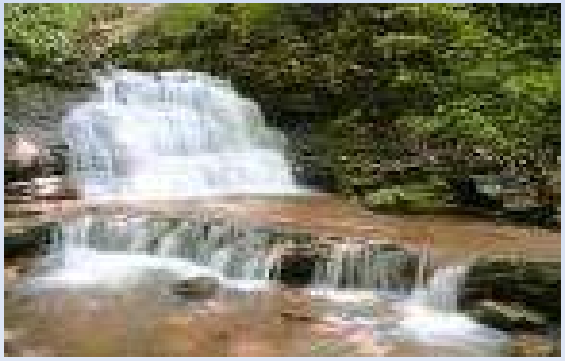
Water borne infections in Georgia

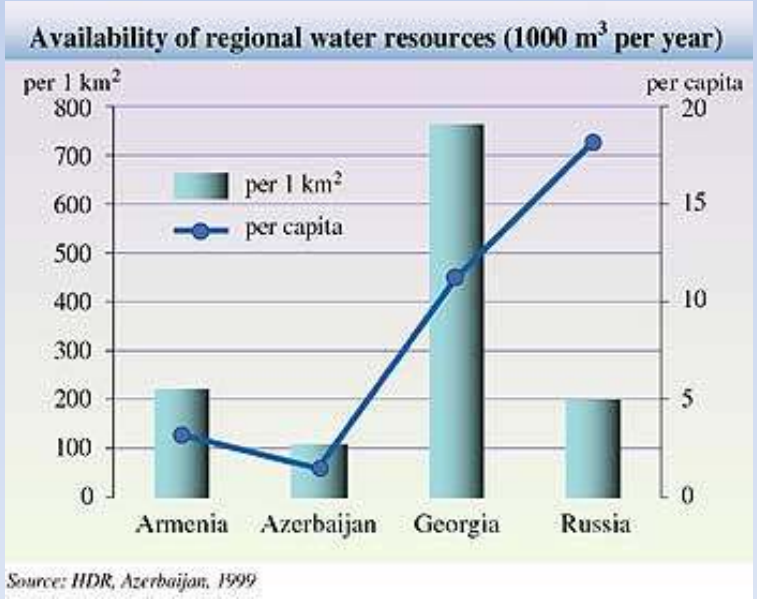
Water resources in Georgia

- *Unequal* natural availability of freshwater resources in the South Caucasus region
- **Georgia** is the richest country in water resources
- **Azerbaijan** suffers from water shortages the most.

➢ **Total renewable water resources (TRWR) per inhabitant- 11 637 m3/yr**

(the international water poverty line of 1,000m3 /yr)





Caucasus Environment Outlook (CEO) 2002

Water borne infections in Georgia

Issues related to safe water supply

- **Potable water supply problems**

  Centralized water supply systems in rapidly growing cities do not meet the requirements (outdated, low technical capabilities)

  - 13.3-17.3% and 15.2-16.0% of samples from centralized water supply systems did not meet existing drinking water quality standards for toxicity and bacteriological quality *(WHO/MoH of Georgia, report 2001).*

  - Tbilisi water Supply system has been significantly improved during last decade and water quality is high

Water borne infections in Georgia

Issues related to safe water supply

Rural areas

- **Many sites do not have central water supply systems**
- **the rural population uses the water from artesian wells and rivers that might be contaminated.**
- **>36% of well water samples didn't meet existing drinking water quality standards *(WHO/MoH of Georgia, report 2001).***

56% rural drinking water samples exceeded drinking water quality standards *(Eliava Lab data, 2006-2010)*

Water borne infections in Georgia

Issues related to safe water supply

Recent ethnic conflicts

- Destroyed sanitation infrastructure, aggravated sanitary-hygienic conditions
- Internally displaced population (IDP) is at risk of acquiring water borne infections due to cramped, crowded and unhygienic living conditions
- Diarrhea is prevalent among children of IDPs.

Intensified international traffic

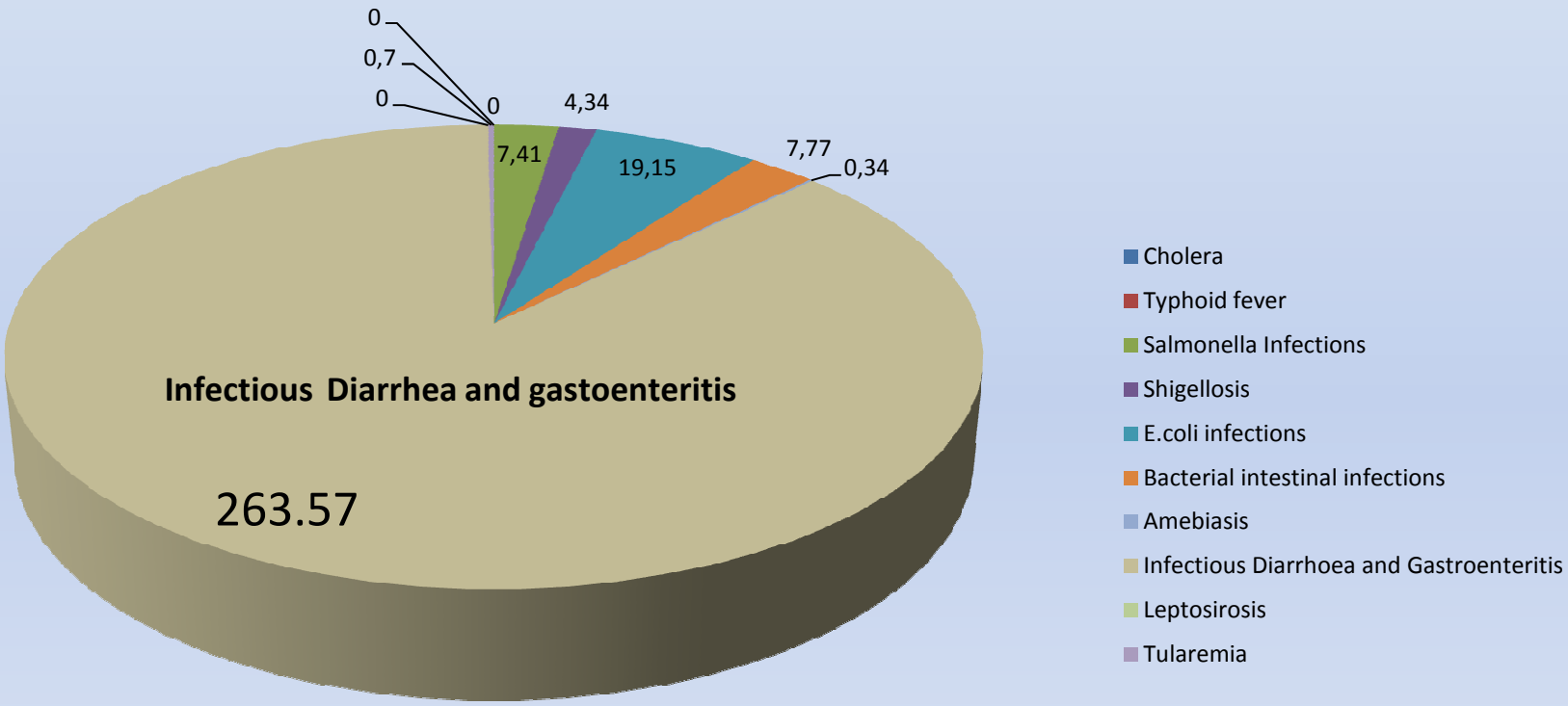
Europe-Caucasus-Asia transport corridor

- *East- West, South- North*
- *Ground transportation (trucks)*
- *international workers*
- *Migration routes*

Possible source of non-endemic infections

Water borne infections in Georgia

Morbidity data of main waterbone infectious diseases (2007)



Indication of Morbidity for 100000 population

*** Prepared f based on NCDC, Annual report 2007-2008**

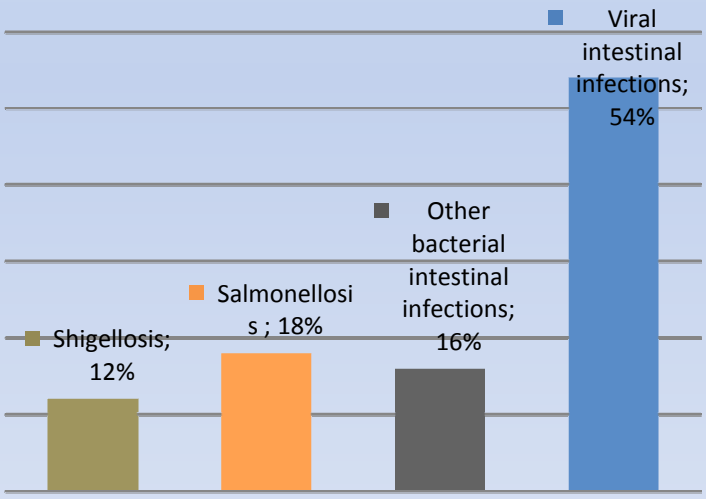
- **High morbidity rate for presumed infectious diahhrea and gastroenteritis (etiologic agents not identified)**

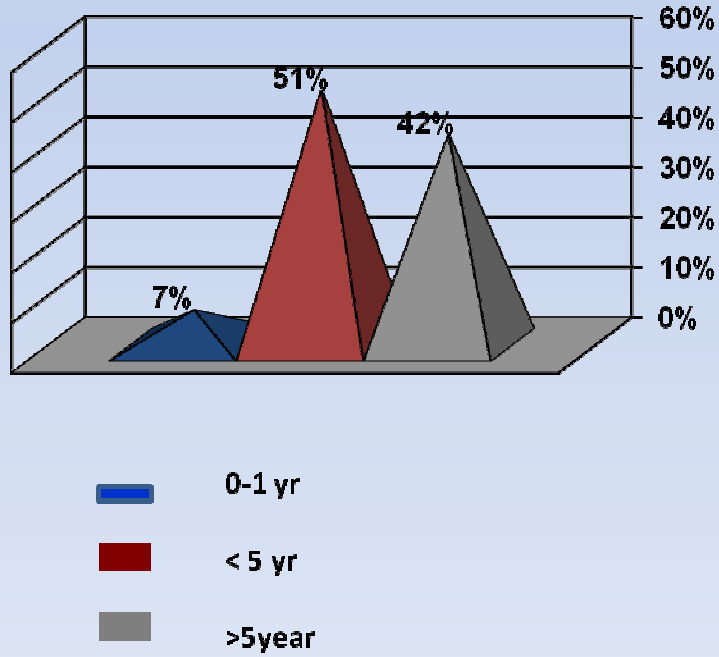
Water borne infections in Georgia

Etiologic structure of Intestinal infections in children

Hospitalized patients, 2004-2008

Children's Infectious Disease Hospital ,Tbilisi



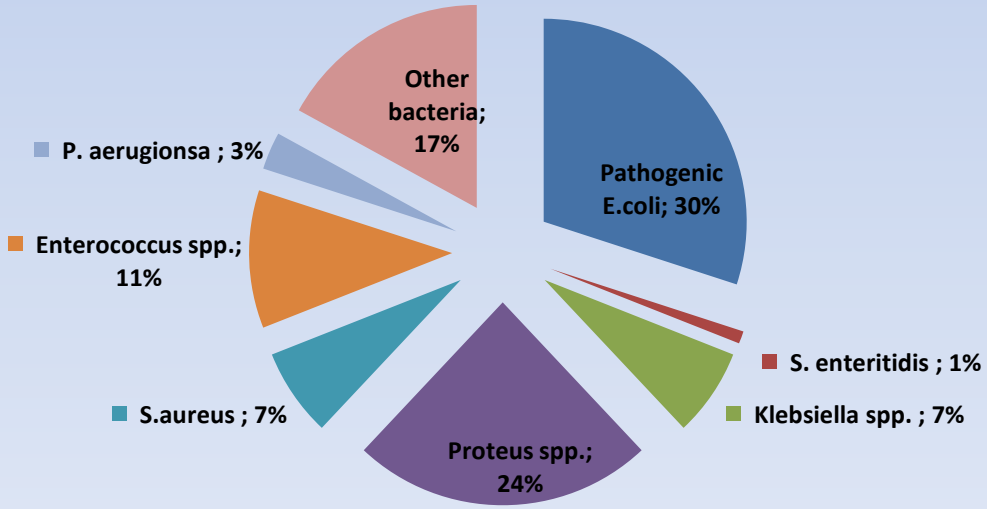


I. Shalamberidze et al, 2009

Water borne infections in Georgia

Etiologic structure of Intestinal infections (Data of the Eliava Analytical- Diagnostic Center, Tbilisi)

- Intestinal bacterial pathogens or opportunistic bacteria are isolated ~ 25% of stool samples from patients with intestinal disorders



n=226,
Sept.- Dec., 2008

N. Topuria, 2009

Water borne infections in Georgia:

Outbreaks of Amebiasis and tularemia

- **In July -September 1998, 177 cases of suspected amebiasis were identified.**
- **52 persons had diagnosed liver abscesses**
- **Drinking water was the source of infection**
- **inadequate treatment or contamination of municipal water distribution system**

R. S. Barwick et al, *Am. J. Trop. Med. Hyg., 67(6), 2002, pp. 623–631*

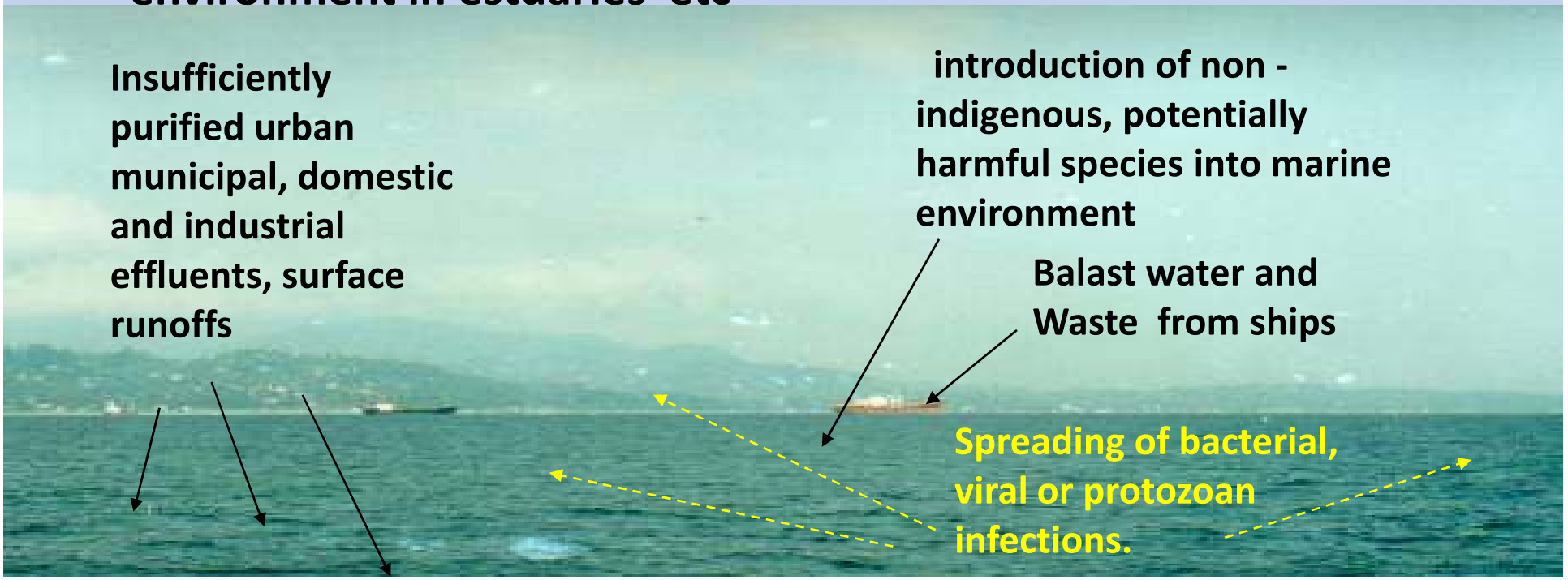
- **In November 2006, an outbreak of waterborne tularemia occurred in an eastern region of Georgia.**
- **26 cases:**
- **21 oropharyngeal cases**
- **5 glandular tularemia cases.**
- **Transmission – through contaminated spring water**

Chitadze et al , Infection. 2009 Dec;37(6):514-21.

Water Borne Infections in Georgia

Recreational waters: Black Sea Coastal Zone of Georgia

- Highly populated cities, recreational/tourist places
- Ports, factories, farms
- Possible health threat: typhoid, cholera, dysentery, hepatitis A, other… exotic diseases
- Favourable conditions: low salinity, warm climate , nutrient rich environment in estuaries etc



Water Borne Infections in Georgia

Vibrio-related infections

- Warm, subtropical climate in Georgia provide favorable conditions for emergence and spread of *Vibrio*- related infections
- In the early 1970's the outbeak of cholera was reported in Georgia (Batumi and Tbilisi), caused by *V. cholerae* O1, biotype El-Tor
- Several Reports on NAG-Vibrio isolation in Coastal waters in 70'-80's
- Two small outbreaks of cholera in 90's (imported cases)

- Periodic outbreaks & sporadic cases in neighboring countries

- No published reports were available before 2006 on detection of toxigenic *V. cholerae* in water reservoirs
- Waterborne diarrheal diseases of unknown etiology in the warm season - possible vibriosis?

Water borne infections in Georgia:

Pathogenic vibrios in Georgian water environment

Project GG-13

Main Goal

Development of strategies for monitoring, prevention, and control of cholera and other Vibrio-related infections

- **Ecology and biodiversity of pathogenic vibrios, especially *V. cholerae,* in different geographical regions**
- **Occurrence and spread of epidemic strains**
- **Ecology and biodiversity of *Vibrio* – specific bacteriophages**

Map of Georgia

Monitoring in the Black Sea Georgian Coastal Zone and freshwater lakes in Tbilisi area

Pathogenic vibrios in Georgian water environment

Sampling in different water environment (2006-2008; 2009)

Black Sea coast of Georgia

- Ports
- Oil terminals
- Sea resorts
- Agriculture
- Fishery

4 Coastal sites

- *Chorokhi,*
- *Batumi Boulevard*
- *Green Cape,*
- *Supsa*

- **Tbilisi Sea** (used for drinking, recreation)
- **Lisi Lake** (recreation, fishing)
- **Kumisi Lake** (recreation, fishing, irrigation)

Pathogenic vibrios in Georgian water environment

Isolation and identification of environmental Vibrio spp

- **11 pathogenic *Vibrio* spp. cultured from marine environment and 8 from freshwater lakes (phenotypical identification)**
- **Prevalence of non- halophylic vibrios in freshwater environment**
- **In total, >2000 primary isolates of *Vibrio* spp. have been studied by phenotypic properties**

  - 800 *Vibrio* spp. isolates identified by ITS -PSR

- ***V. cholerae*– 58,9%**
- ***V. parahaemolyticus* -10,4%**
- ***V. vulnificus*- 5,1%**
- ***V. algynolyticus* – 3,8%**
- ***V. metschnikovii* -3,1 %,**
- ***V. mimicus*- 0,8% etc**

V. parahaemolyticus

V.cholerae

Pathogenic vibrios in Georgian water environment

Direct detection of Vibrio spp. in water

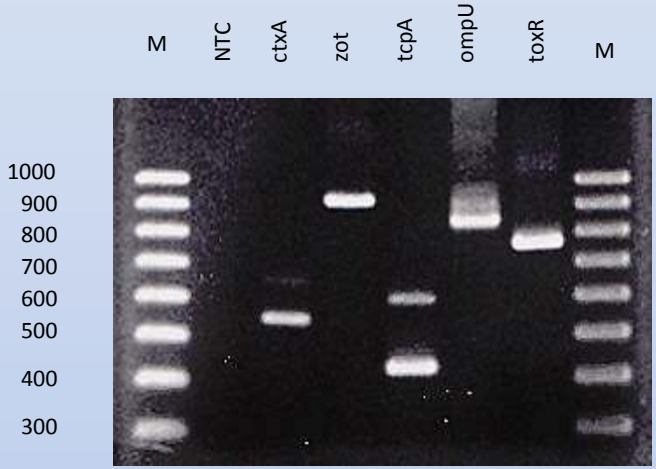
- **Novel pathogen detection system using PCR ionization electro spray mass spectrometry (PCR/ESI-MS)**
  - Determination of the full spectrum of vibrios in water samples (Total DNA's)

- **440 DNA samples from marine and lake water analyzed by PCR/ESI-MS**
  - 67% of water samples positive for *Vibrio spp.*

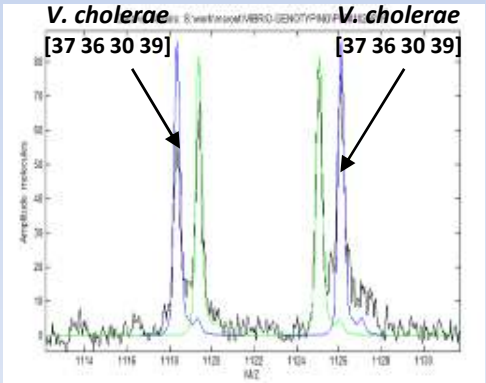
- **9 pathogenic *Vibrio* species detected**

- ***V. cholerae, V. parahaemolyticus, V. vulnificus* were prevalent the Black Sea samples**

  - The lake waters were dominated by *V. cholerae*



Direct Molecular Detection of *Vibrio cholerae* Virulence Factor Genes in the Water Samples from Lisi Lake



Pathogenic vibrios in Georgian water environment

Direct detection of epidemic serotypes of Vibrio cholerae in the environment

- **Direct Fluorescent Antibody (DFA) Assay**

  V. cholerae **O1 and O139 DFA kit (NHD, MD)**
  Epifluoroscent microscope ZEISS Axioscop 40

- **Up to 450 Samples examined**

  85 Samples positive for V.cholerae 01

- **29 - Freshwater samples (mainly Lisi and Kumisi Lakes)**
- **59 – Marine samples (all sites)**

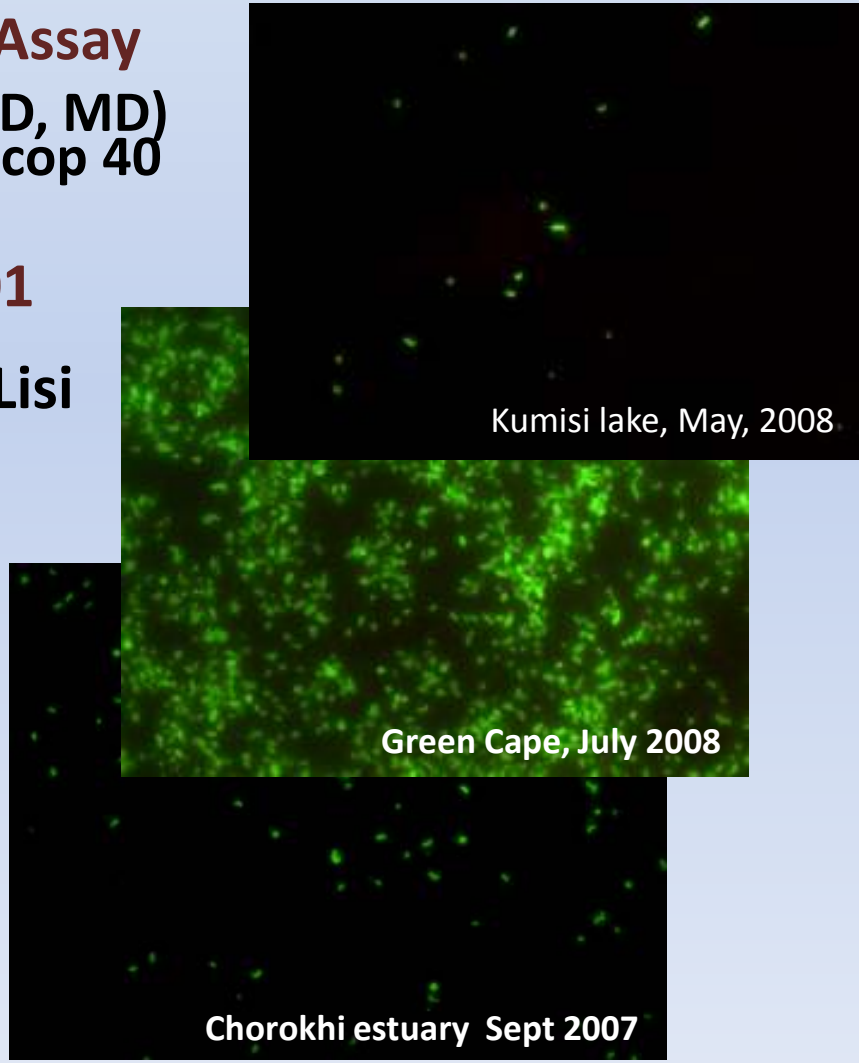
- **Presumptively positive signals for**

  V. cholerae 0139 **in**

  - 7 marine samples

  - 1 sample form Kumisi lake

Percentage DFA- positive samples increased during 2007 - 2009

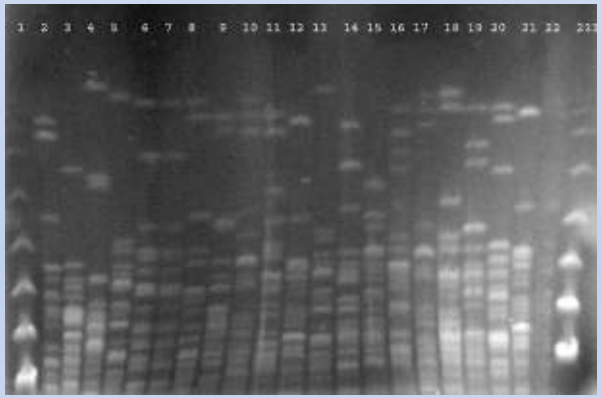


Pathogenic vibrios in Georgian water environment

Study of genetic relatedness of V.cholerae and V. parahaemolyticus isolates

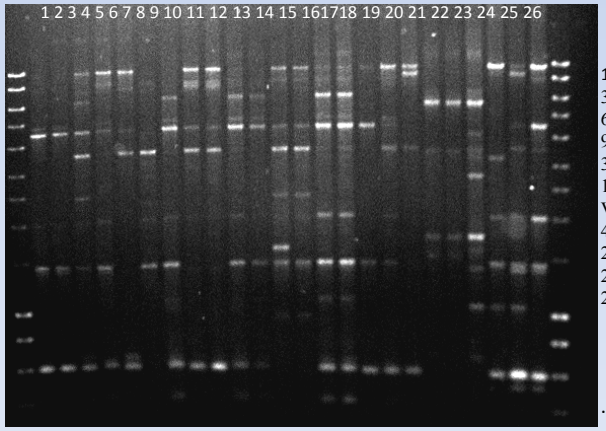
- **210 *V.cholerae* isolates studied by PFGE**
- **500 V.choleare isolates by ERIC- PCR**

***V. parahaemolyticus* isolates**
PFGE (160) , ERC-PCR (70)



1. PFGE marker2.Vchol89; 3.Vchol103;4.Vchol105;5. Vchol111; 6.Vchol226; 7.Vchol267;8.Vchol467;9.Vcho l834;10.Vchol835; 11.Vch975;12.Vchol980; 13.Vchol108; 14.Vchol142; 15.Vchol155;16.Vchol157; 17.Vchol458; 18.Vchol467; 19.Vchol471;20.Vchol979; 21.Vchol980;22.Vchol984; 23.PFGE marker

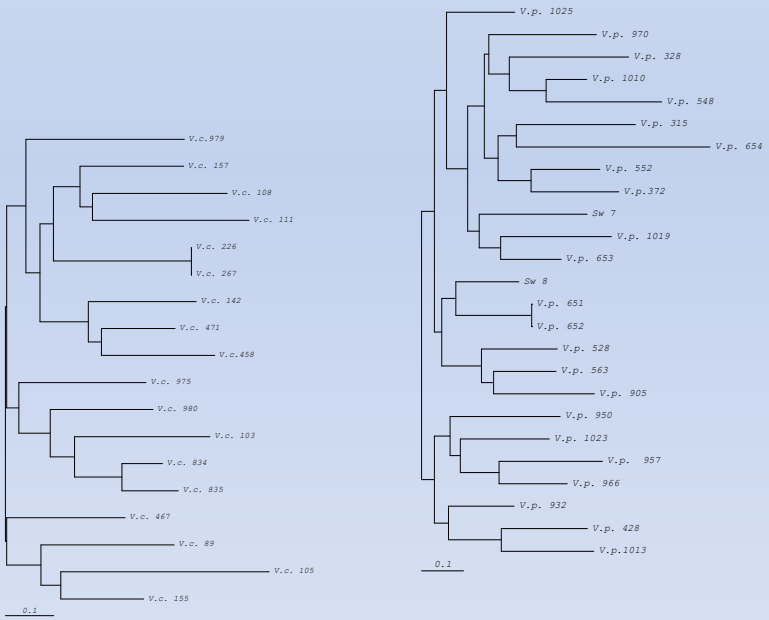
PFGE of *V. cholerae* isolates



1.λDNA marker; 2. Vchol16; 3.Vcho17; 4.Vchol 20; 5.Vch21; 6.Vcho32;7.Vcho89; 8.Vchol95; 9.Vchol 95; 10.Vchol 97; 11.Vchol 300; 12.Vchol 301; 13. Vchol 302; 14. Vchol 304; 15. Vchol 306;16. Vchol 308;17. Vchol 746;18.Vchol 453; 19.Vchol 697; 20. Vchol 687; 21.Vchol684;22.Vchol677; 23.Vchol676;24.Vchol694;; 25.Vchol834; 26.Marker

.

ERIC-PCR of *V.cholerae* isolates



High Genetic diversity of Environmental isolates

Pathogenic vibrios in Georgian water environment

What we have found

- **Data obtained (DFA, PCR/ESI-MS, and culture) provide evidence for environmental reservoirs for toxigenic *V. cholerae* O1 in recreational waters and drinking water sources in Georgia, in freshwater lakes near Tbilisi, and in the Georgian coastal zone of the Black Sea.**
- **Direct detection methods (DFA, PCR/ESI- MS) serve as**

  effective and precise tools for detection and enumeration of selected *Vibrio spp.* in the aquatic environment. It is important that bacteria in VBNC state can be detected.

Pathogenic vibrios in Georgian water environment

What we have found

- **Temperature dependence of pathogenic vibrios might be a triggering factor for their massive propagation in the water environment (even in the considerably clean zones) in the conditions of continuing global warming.**

- **High density of *Vibrio* populations may facilitate genetic exchange between pathogenic and non-pathogenic vibrios and support evolving of potentially pathogenic variants/ serotypes of *Vibrio* species in the aquatic environment.**

Waterborne infections

Current and Future challenge

- **Worsening of environmental conditions (elevated temperature, drought, heavy rainfalls etc), also enforced anthropogenic impact may trigger futher decline in water microbial quality and safety**

***Haiti:** by February 9, 2011, there were 231,070 cases and 4,549 deaths due to cholera.*

- **In the era of natural catastrophes the probability of emergence and spread of water borne infections increases, including the areas non- endemic for a particular pathogen *(Haiti lesson)***

- **Increasing antibiotic resistance of Intestinal pathogens is in many areas of the world**

- ***Waterborne infections may pose a risk, the impact of which reaches <u>beyond local and national borders</u>.***

Waterborne infections

Future strategy (Monitoring and preparedness)

- **Regular monitoring in recreation and drinking water reservoirs for:**

  - Early detection of human pathogenic species , including pathogenic *Vibrio spp*. and other potentially harmful autochotnous microflora

- **Research targeting the predictive models and biological indicators ;**

  - Disease surveillance, collecting clinical data;

- **Necessity for joint collaborative efforts /projects on regional level in environmental monitoring and disease surveillance**

Waterborne infections

Future strategy (Monitoring and preparedness)

- **Elaboration of effective biological control measures to prevent and treat emerging waterborne infections.**
- **Biological measures /preparations – flexible, genetic plasticity, overcome of bacterial resistance**

  - Bacteriophages - alternative ecologically safe biocontrol means (especially for antibiotic resistant water borne pathogens).

Phages and Water borne infections

- Detection/diagnostics
- Infections source tracking
- Subtyping (for Epidemiological studies)
- Prevention
- Therapy

Phages as indicators

- Somatic and male – specific Coliphages - indicators of water microbial pollution

- Specific phages as indicators of presence of particular *pathogenic* bacteria

- Phage amplification assay -- indication of a pathogen in VBNC state

- Correlation between Etiologic structure of water borne infections and phage profile of water samples
- Phages as predictive or retrospective indicators of disease outbreaks

Seasonal monitoring of microbial quality

South - East coastal zone of the Black Sea

Batumi city area (2002-2004)

- Seasonal variability of microbial quality indices
- Highest level of microbial contamination of sea water in summer season
- Abundance of indicator somatic phages in sea water - proportional to total bacterial count (TBC)
- Two distinct (clean and polluted) zones in Batumi aquatoria: Gonio- Kwariati and Bartskhana

Table 1. Indices of Microbial pollution of the Black sea water and sediments in Adjara coastal zone (Winter, 2003)

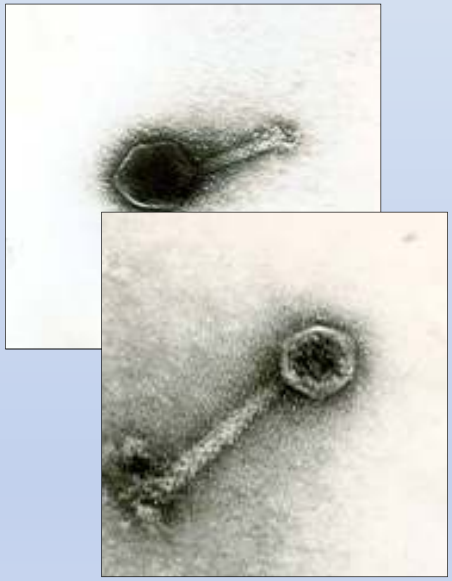
Sampling site No	Sampling site NAME	Depth(m)	Sample type	TBC cfu/ml or cfu/g	SBC cfu/ml or cfu/g	Total coliforms cell/L	E.coli-index cell/L	ENT-Index cell/ L	pathogenic bacteria cfu/ml	Somatic phages pfu/ml
1	Riv. Khoro-listskali 1	8,5-10,0	water	$1,2x10^3$	$1x10^2$	1000	1200	230	0	0
			sediments	$1,5x10^6$	$2,2x10^3$	210	230	-	-	-
2	Riv. Khoro-listskali 2	5,0-6,0	water	-	-	-	-	-	-	-
			sediments	$8,2x10^5$	$8,2x10^3$	256	230	-	-	-
3	Petrol plant BNZ	7,0-8,0	water	$3,2x10^3$	$3x10^2$	2400	1000	620	0	1,2
			sediments	$1,5x10^6$	$5,2x10^3$	256	620	-	-	-
4	Bartskhana 1	8,3- 9,0	water	-	-	-	-	-	-	-
			sediments	$2,5x10^6$	$2,0x10^3$	256	370	-	-	-
5	Bartskhana 2	3,5-4,5	water	$1x10^3$	$1,2x10^2$	1400	700	230	0	0
			sediments	$1,8x10^6$	$1,5x10^3$	210	620	-	-	-
6	Gonio-Kvariati	8,0-9,0	water	$1,5x10^3$	$3,4x10^2$	1000	230	46	0	0
			sediments	$1,6x10^6$	$2,7x10^3$	210	220	-	-	-
7	Kvariati 1	10,0-11,5	water	-	-	-	-	-	-	-
			sediments	$1,1x10^6$	$1,3x10^3$	46	46	-	-	-
8	Kvariati 2	18,0-20.0	water	$5x10^3$	$1x10^2$	60	0	0	0	0
			sediments	$1,9x10^6$	$8x10^3$	60	60	-	-	
9	Kvariati 3	20,0-21,0	water	-	-	-	-	-	-	-
			sediments	$1,6x10^6$	$8x10^3$	60	60	-	-	-
10	Kvariati 4	15,0-16,5	water	$4.3x10^2$	$6,5x10^1$	60	0	0	0	0
			sediments	$1,8x10^6$	$6,8x10^3$	60	46	-	-	-

Table 2 . Indices of Microbial pollution of the Black sea water and sediments in Adjara coastal zone (August, 2003)

Sampling site No	Sampling site NAME	Depth(m)	Sample type	TBC cfu/ml or cfu/g	SBC cfu/ml or cfu/g	Total coliforms cell/L	E.coli-index cell/L	ENT-Index cell/ L	pathogenic bacteria cfu/ml	Somatic phages pfu/ml
1	Riv. Khoro-listskali 1	8,5-10,0	water	$1,0x10^4$	$2x10^3$	70000	2400	1200	4,1	$1,8x10^2$
			sediments	$4x10^6$	$2,2x10^4$	24000	230	-	-	-
2	Riv. Khoro-listskali 2	5,0-6,0	water	-	-	-	-	-	-	-
			sediments	$3,8x10^6$	$8,8x10^4$	24000	630	-	-	-
3	Petrol plant BNZ	7,0-8,0	water	$5x10^3$	$8x10^2$	7000	1000	700	3,2	$2.3x10^1$
			sediments	$3,0x10^6$	$7,7x10^4$	7000	210	-	-	-
4	Bartskhana 1	8,3- 9,0	water	-	-	-	-	-	-	-
			sediments	$2,5x10^6$	$2,0x10^4$	24000	210	-	-	-
5	Bartskhana 2	3,5-4,5	water	$9x10^3$	$6x10^3$	24000	1000	700		8,4
			sediments	$5x10^6$	$9,5x10^4$	24000	620	-	-	-
6	Gonio-Kvariati	8,0-9,0	water	$2,3x10^3$	$5x10^2$	3000	620	96	0	7,3
			sediments	$2,3x10^6$	$1,5x10^4$	620	230	-	-	-
7	Kvariati 1	10,0-11,5	water	-	-	-	-	-	-	-
			sediments	$2,2x10^6$	$1,2x10^4$	256	94	-	-	-
8	Kvariati 2	18,0-20.0	water	$1,8x10^3$	$3x10^2$	1000	620	46	0	4,6
			sediments	$3,4x10^6$	$1,0x10^4$	210	94	-	-	
9	Kvariati 3	20,0-21,0	water	-	-	-	-	-	-	-
			sediments	$3,6x10^6$	$2,0x10^4$	210	60	-	-	-
10	Kvariati 4	15,0-16,5	water	$5x10^2$	$1x10^2$	700	210	0	0	1,8
			sediments	$1,8x10^6$	$6,8x10^3$	60	46	-	-	-

Phages as indicators

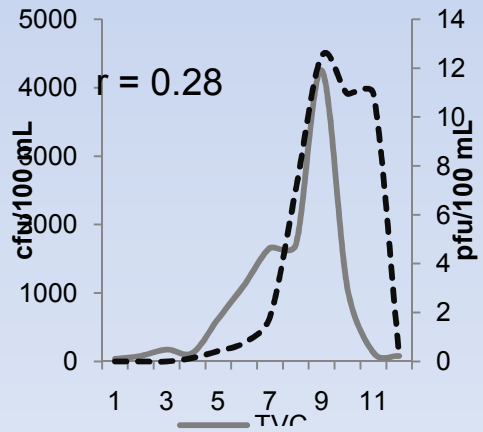
Vibriophages in Georgian water environment (2006-2009)



- Vibriophages were isolated from freshwater and marine sampling sites
- *Vibrio* species vary by their phage potential
- Majority of *V.cholerae* phages- freshwater isolates (72%)
- *V. parahaemolyticus* –specific phages prevailed at the Black Sea sites

➢ Peak of phage isolation summer – autumn period

➢ *Correlation with isolation dynamics of host bacteria*



Correlations between average monthl
vibriophage counts, and TVC

Water borne infection Source tracking
Phage tracing

- **Properties of the tracer phages resemble those of infectious agents (pathogenic bacteria and viruses).**
- **Phage is only harmful to a specific bacterial host(s)**
- **No limitation in the concentration of the initial inoculum**
- **The water course can be traced over long distances (>20 km)**
- **The low detection limit for plating - comparable to that for chemicals**

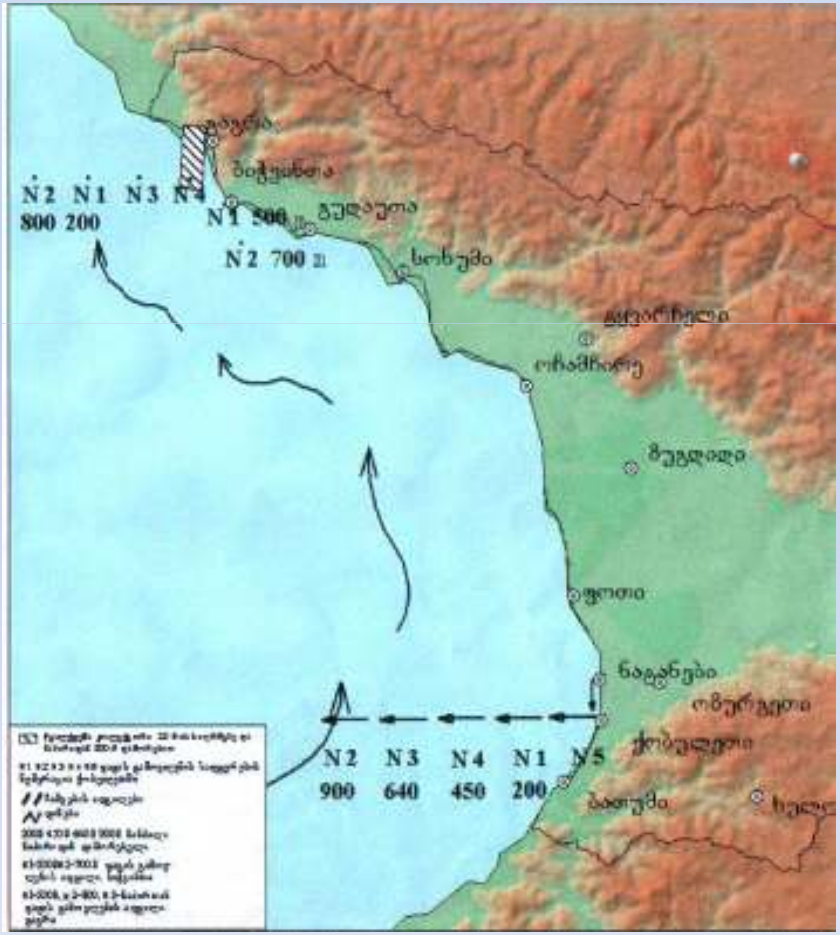
Water borne infection Source tracking

Phage tracing in Black Sea costal Zone

- **Testing of water treatment plant**

- **Impact of ballast waters from ships**

- **Swimming associated infections**



I. Chkonia et al, 1988;1997

Control Water borne infections: Phage therapy and prevention

- **Prevention**

  Prophylactic use of polyvalent phage preparations (e.g. Intesti phage) in the population under the risk of infection spread

- **Therapy**

  Treatment of sick(infected) people with polyvalent or specific phage preparations

  • Phages alone

  • Phages in combination with antibiotics

Bacteriophage preparations for treatment and prophylactics of intestinal disorders / enteric infections

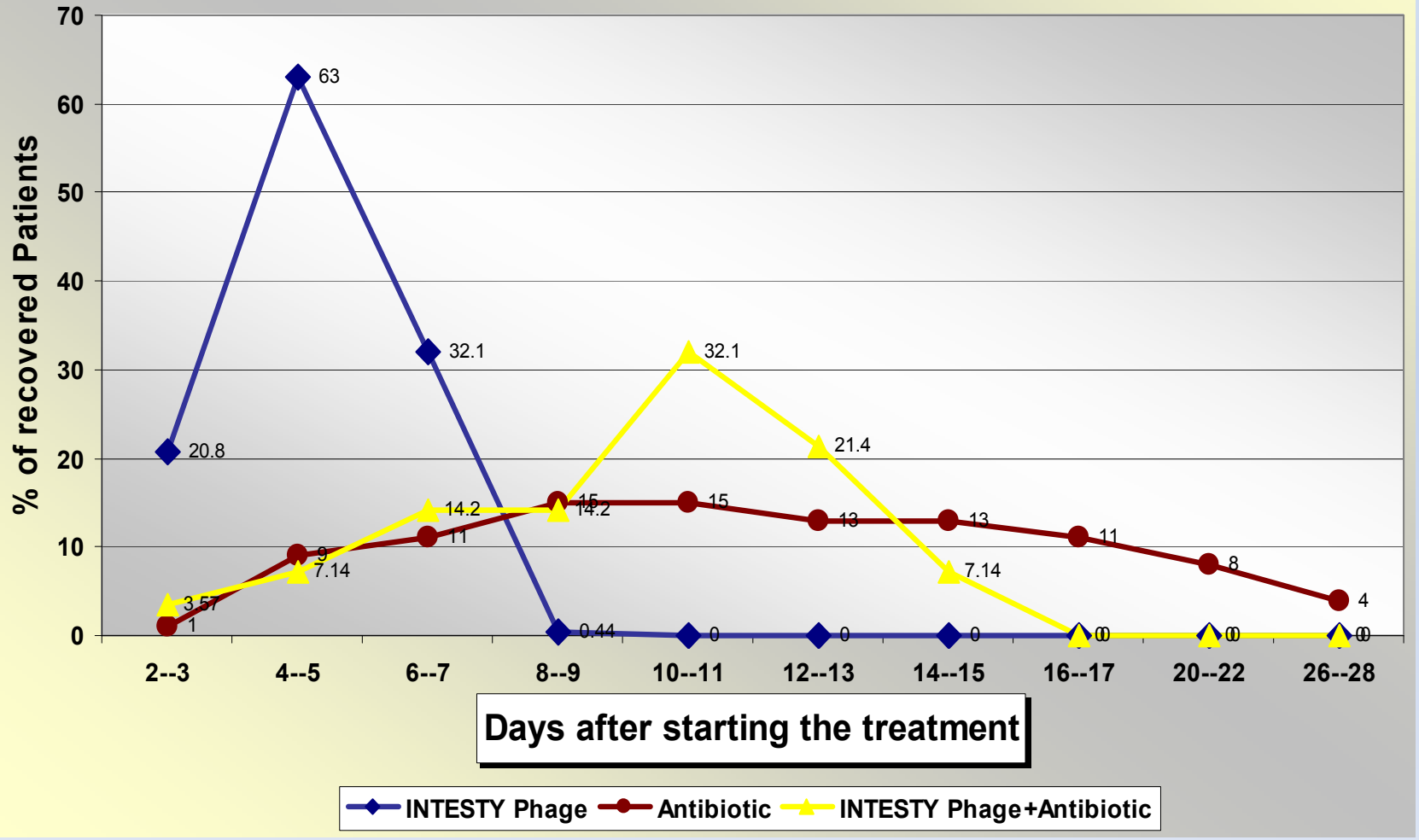
Salmonella phage preparation

- **11 components** , active against
- ***S. typhimurium***
- ***S. cholera suis***
- ***S. dublin,***
- ***S. enteritidis***
- ***S. paratyphi A, B***
- ***S. newport etc***

- **INTESTY-bacteriophage**
- **17 components ,** active against
- ***Shigella* (3 comp.)**
- ***Salmonella* (6 comp.)**
- ***E.coli* (5 comp.)**
- ***Enterococcus* (2 comp)**
- ***Staphylococcus* (2comp)**
- ***Proteus*(2 comp.)**
- ***Pseudomonas aeruginosa***

Phages to be used for waterborne enteric infections

Study of the efficacy of INTESTY Phage and Antibiotics



Prophylactic use Polyvalent Salmonella Bacteriophage

- SALPhage in children of 0-2 year of age
- 1283 children treated with Sal phage
- 1238 children - control group

- 5 days 3-4 times /day
- Children 5-10-15 ml *per os* or *per rectum*
- *Adults* -30-50ml

  or 1-2 tablets 3Xday

Positive prophylactic effect :
decrease of disease manifestation 5,5 times

(E. Tsereteli et al., 1983)

Phages against other water borne pathogens available at the Eliava institute

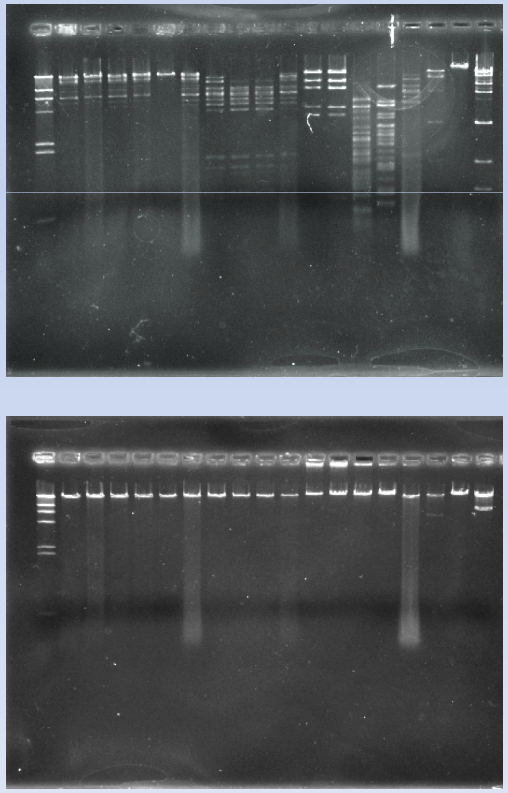
- *Vibrio choleare*
  V.parahaemolyticus
  V.vulnificus
  V.mimicus
  V. algynolyticus
  V. fluvialis

- *Aeromonas hydrophyla*
  A. caviae
  A. sobria
  A. salmonicida

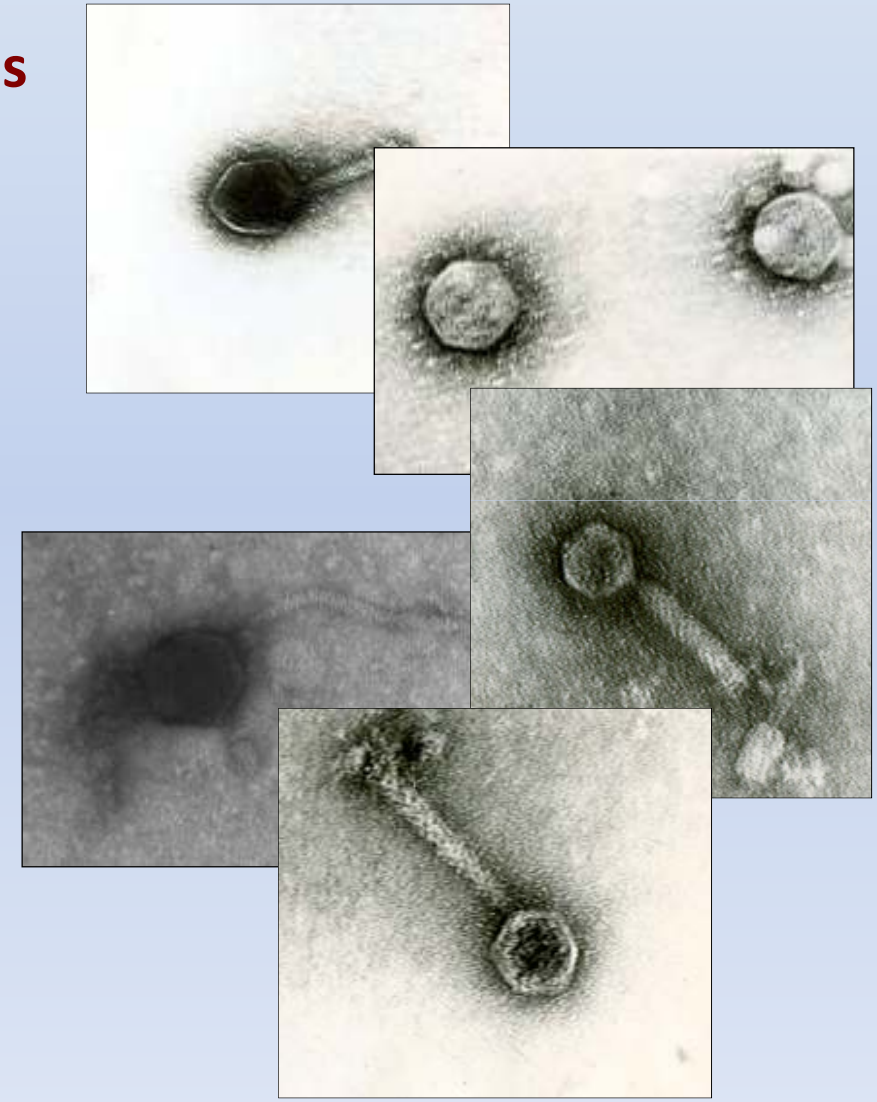
Phenotypic and genetic diversity of Vibriophages

Phage Virion Morphology

- All three types of Tailed phages
- Majority belong to *Myoviridae* family



Restriction analyses of V.cholerae phages
EcoRI and Not I digest



V. cholerae phages

THANK you