Vibrios, Hepatitis A & Norovirus

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Food and Water Microbiology Proficiency Testing (PT)

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# Microbial risks in the marine environment

A wide variety of pathogen risks are present in the marine environment. For example, faecal contamination of water can introduce and variety of pathogens, including bacterial, viral, protozoan agents.

Introduced agents include viruses such as enteroviruses, poliovirus, hepatitis A, norovirus and rotavirus. Bacterial pathogens include *Salmonella, Campylobacter, Shigella, Vibrio cholerae* O1/O139 and *E. coli.*

Naturally occurring agents include certain *Vibrio* species as well as harmful algal biotoxin (HABs) producing organisms. Many are also widespread in marine waters worldwide.
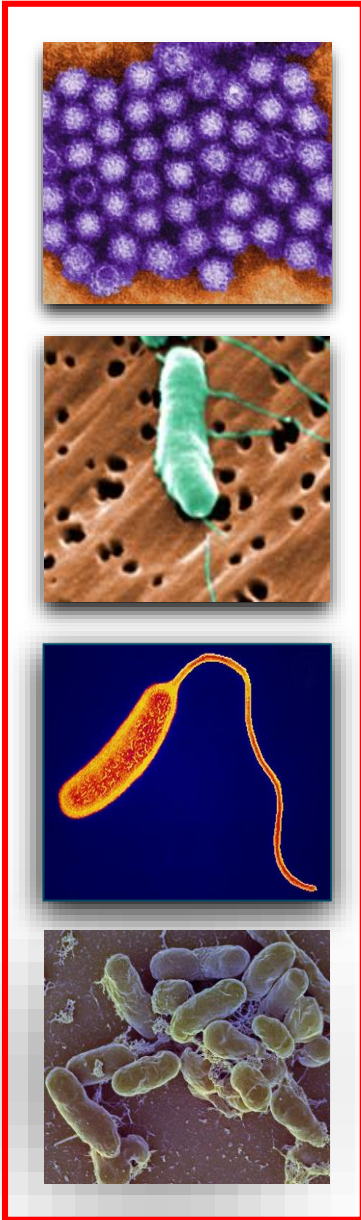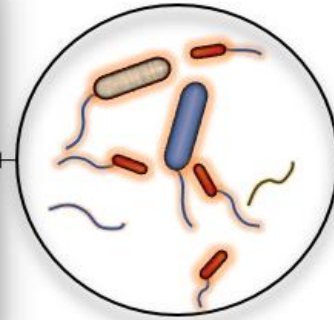
Vibrio cholerae – the causative agent of cholera
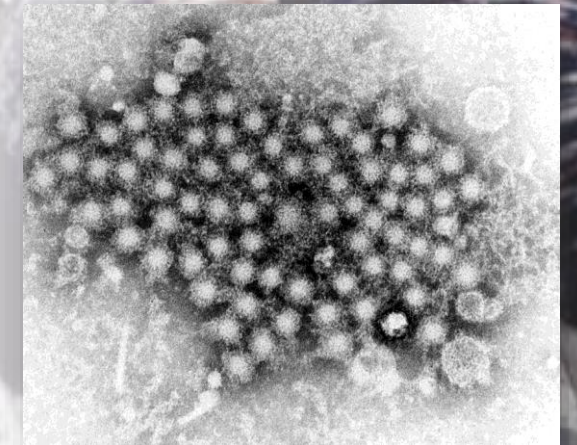
Hepatitis A virus

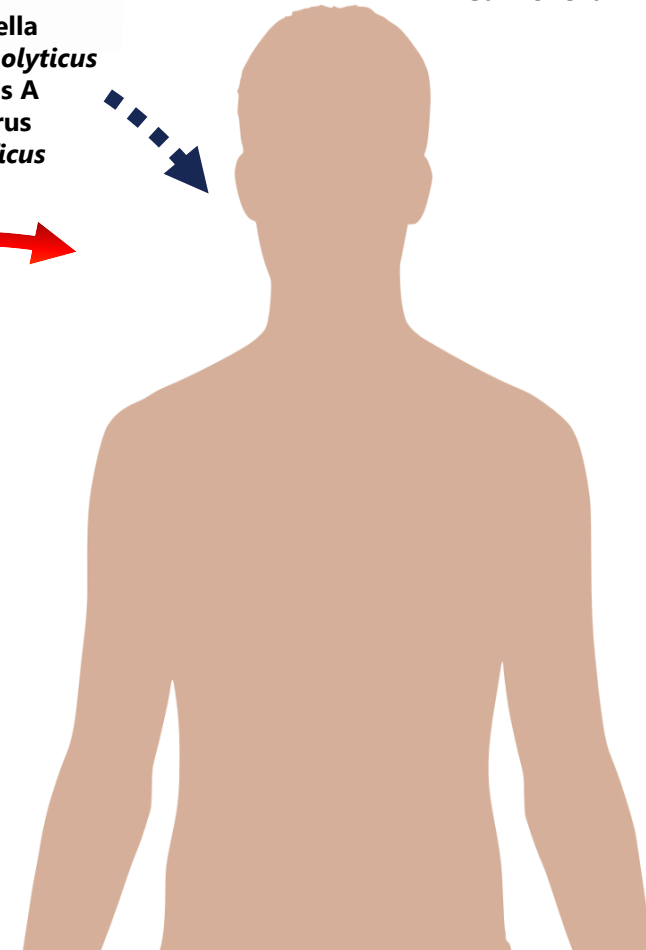# Shellfish (and water) are an effective vehicle in disease transmission

Salmonella
V. parahaemolyticus
Hepatitis A
Norovirus
V. vulnificus

V. cholerae
Hepatitis A
Norovirus
Salmonella

V. cholerae
Norovirus
Hepatitis A
Rotavirus
Astrovirus
Salmonella
E. coli
Shigella

Shellfish and disease risk

Shellfish represent an established route of transmission for a range of important human pathogens. First outbreaks epidemiologically-linked to shellfish consumption emerged in the 19th Century.

Bivalve filter feeding shellfish species such as oysters, mussels and clams very efficient at filtering and concentrating bacterial, viral and algal toxins. Studies have shown that bivalve shellfish can concentrate microbial pathogens >100 times the concentrations found in overlying waters.

Outbreaks often not trivial: Hepatitis A outbreak in China in the early 1990s was responsible for almost 300,000 cases – one of the largest foodborne outbreaks ever reported.
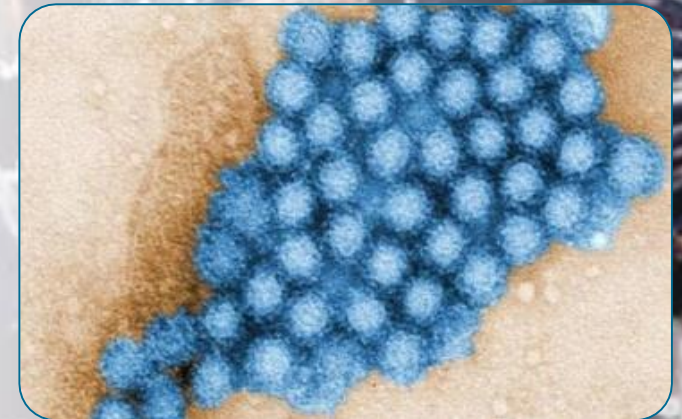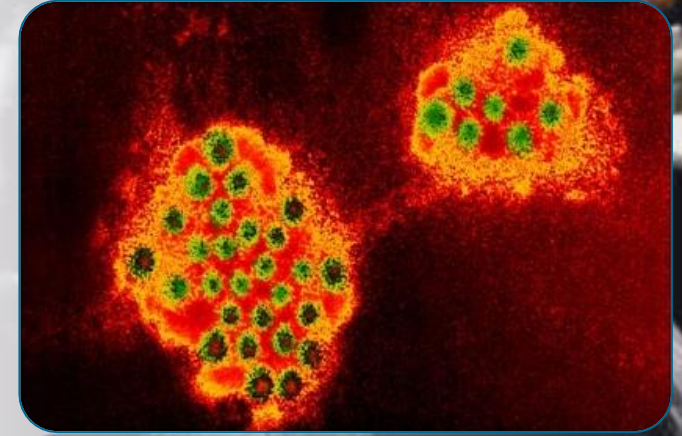
Viruses in shellfish - the problems

- Viruses (esp. norovirus and hepatitis A virus; HAV) recognised as principle cause of illness following shellfish consumption worldwide.

- Several outbreaks of norovirus from shellfish reported in UK annually. Incidents with HAV rarer but can occur.

- Current risk assessment and management world-wide relies on faecal indicators (*E. coli* in EU).

- Bivalve shellfish causing outbreaks are frequently compliant with regulatory standards. Methods for virus detection are an important risk management tool.
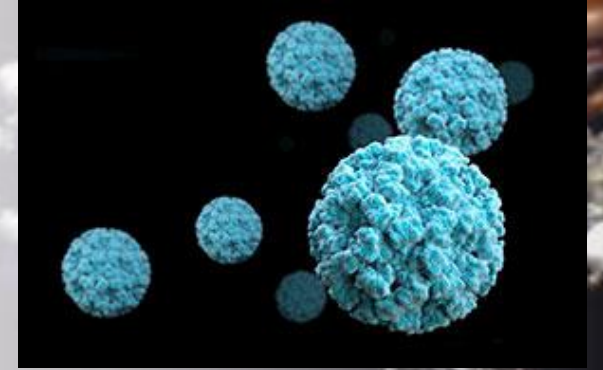
Detection of viruses in shellfish

- A number of publications of culture of norovirus in last 10 years. Methods difficult, expensive, require very high starting levels. Propagation of HAV from shellfish also problematic.

- No realistic short- to mid-term prospect of culture methods for shellfish.

- Detection of virus particles using microscopy or virus coat proteins using immunology (e.g. ELISA) currently lacks sensitivity required for low virus levels in shellfish – no reports of successful application to "real" food samples.

- Identification of virus in shellfish currently only possible through "molecular methods" - specific detection of viral nucleic acid (PCR). Current methodology uses quantitative real-time RT-PCR.
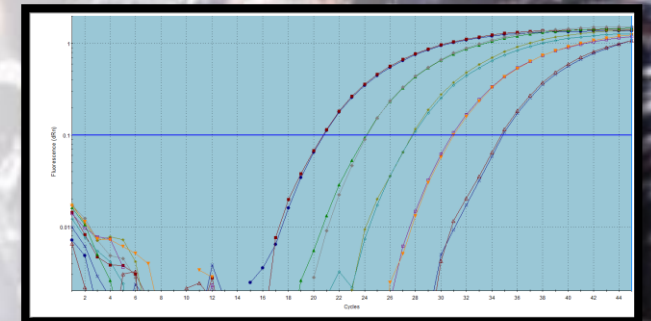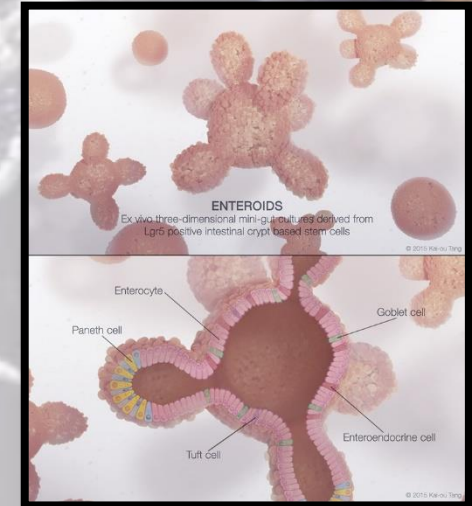
Norovirus: ISO 15216

- Norovirus is the most important cause of illness associated with seafood consumption. Globally most common pathogen associated with seafood-associated illness.

- International standard method for determination of viruses in foods (incl. shellfish) using RT-qPCR developed by expert WG led by Cefas scientists.

- ISO 15216 published as a two-part Technical Specification in 2013.

- International method validation programme led by Cefas, 2012-2015. Fully ISO published 2019.

- First virus standard method of its kind in the world.

INTERNATIONAL STANDARD

ISO 15216-1

First edition
2017-03

Microbiology of the food chain —
Horizontal method for determination
of hepatitis A virus and norovirus
using real-time RT-PCR —

Part 1:
Method for quantification

# Viruses – a standardisation story, from 30 to 1 method
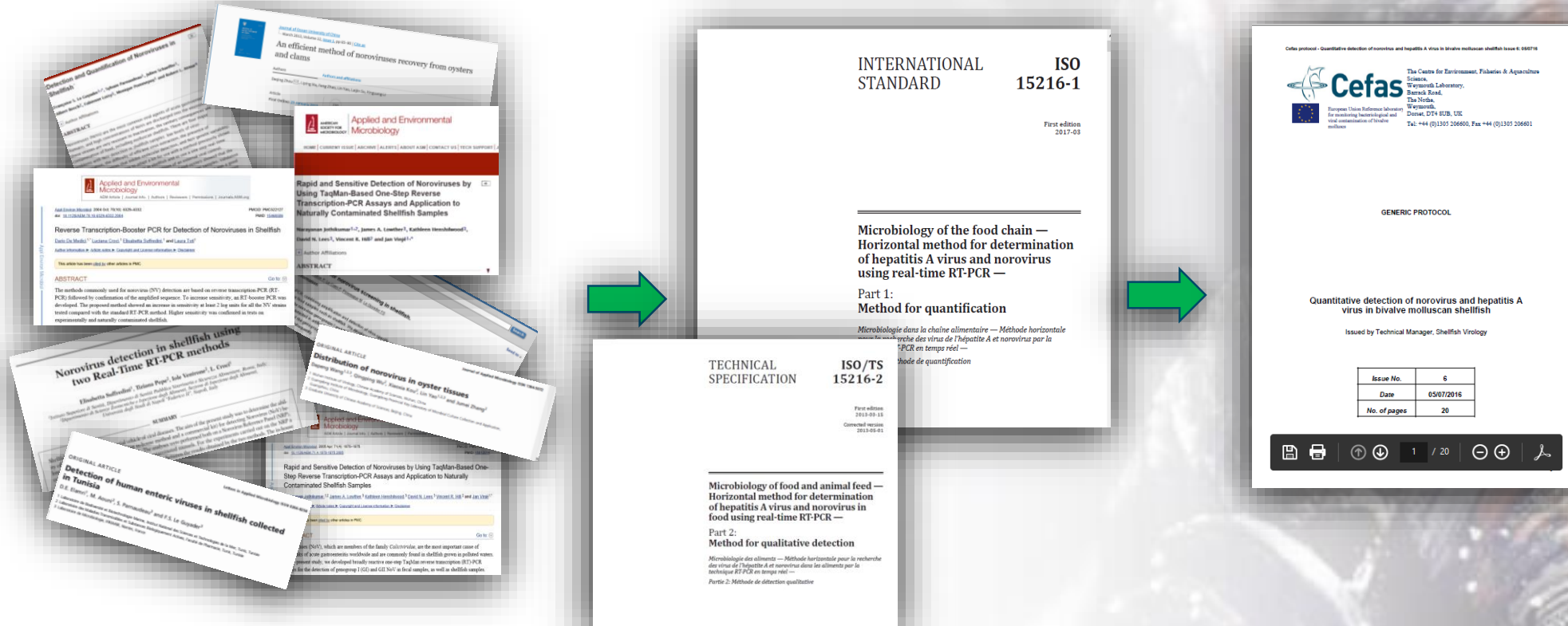
TAG 4, 30+ participants, at least 13 countries, 10 years, 1 ISO!

1) 30+ published studies on method   2) ISO standard   3) Lab protocol

This work took over a decade, and involved massive international efforts

Survey impacts

- Recent surveys led by Cefas (or as a international partner have shown):

  - 11% (European dispatch centres)

  - 35% (European production areas)

  - 69% (UK retail sites)

  - 76% (UK production areas)

- Fit for purpose method now applicable for environmental and food testing.

- **Impact**: First and only virus method of its kind, with direct potential ramifications for risk assessment and future legislation, possible impacts on trade.
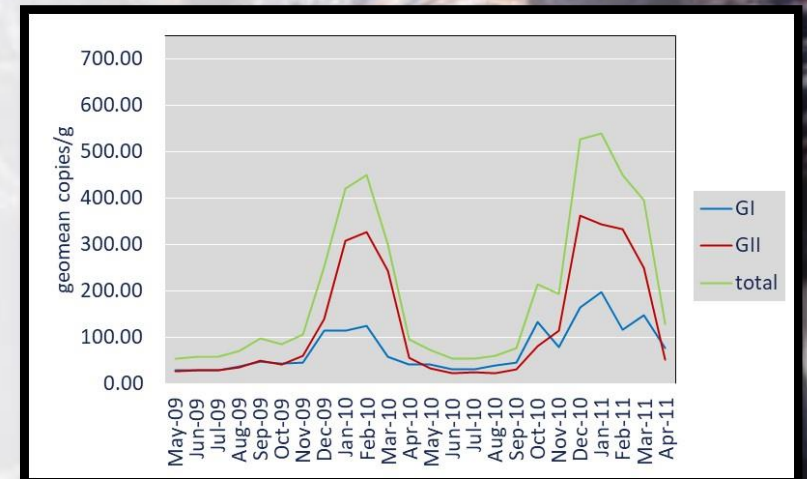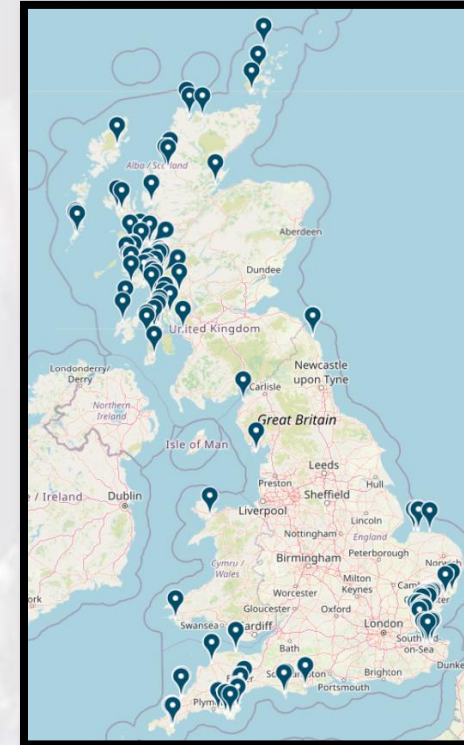
HAV and shellfish – a rare issue in the UK?

- HAV is transmitted by the faecal-oral route.

- HAV is an uncommon infection in the UK and other high-income countries. In 2018, Public Health England reported 452 laboratory-confirmed cases of hepatitis A infection in England and Wales.

- HAV rarely detected in UK shellfish.

- In 2012 we became aware of a cluster of cases of HAV in the Netherlands and UK linked by mussels. Likely cause was an infected individual(s) shedding HAV into mussel production area, which led to outbreak.

Vibrios

Widespread distribution: Globally vibrios are one of the leading groups of human pathogens responsible for shellfish and waterborne-associated illness. ~ dozen species cause disease in humans.

Climate change: These pathogenic bacteria are especially sensitive to climate change – outbreaks are now being observed in temperate areas.

Pandemic potential: Certain strains of vibrios are more pathogenic than others, and capable of rapid pandemic spread.

Changing demographics: Various factors including more at risk/vulnerable groups and greater potential for exposure may increase risks in the future.
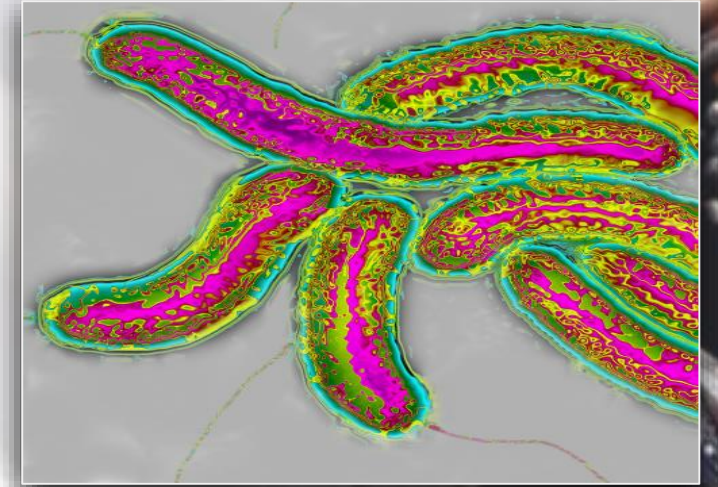
Vibrio parahaemolyticus
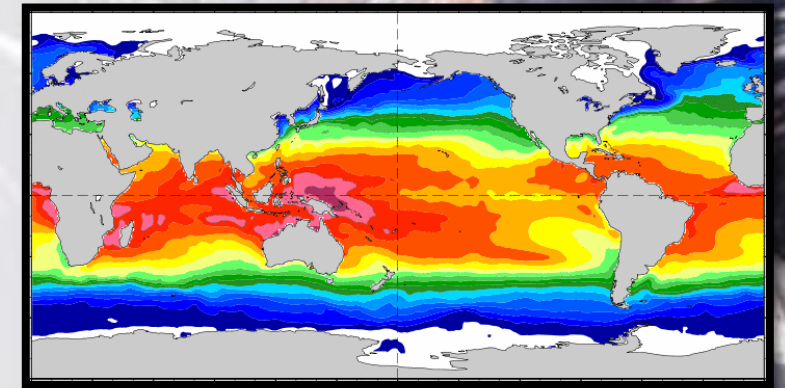
Vibrio parahaemolyticus (*Vp*)

• *Vp* is a common and naturally occurring marine and estuarine bacterium. *Vp* concentrates in bivalve shellfish such as oysters and clams. Globally the most common bacterial infection associated with the consumption of seafood, and often associated with large foodborne outbreaks. We find this pathogen in UK shellfish and water.

• In 2012 a new variant of *Vp* – a highly pathogenic strain normally restricted to Pacific NW region of the USA emerged – jumping to the East Coast of the USA and then Europe. This particular strain (PNW) is of huge concern. Caused major shutdown of US shellfish in 2012 and 2013.

• Pandemic spread of *Vp* is a major global food safety and economic issue.
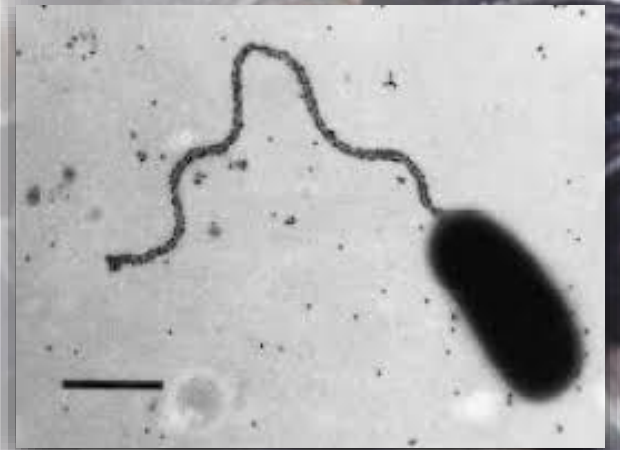
Pandemic spread of *Vp*

Vp outbreaks are currently undergoing a global process of expansion with infections emerging in areas where these diseases have not been previously observed.

Only pathogen (alongside *V. cholerae* which causes cholera) to spread pandemically from the marine environment. O3:K6 pandemic strain emerged in Asia.

Predicted dose response of Alaska Vp strains compared to current risk assessments (**Source**: Martinez-Urtaza *et al.* 2010).

PNW strains

Legend:
- Beta-Poisson fit to clinical trials data
- Beta-Poisson shifted to match CDC data
- Exponential fit to AK outbreak data
- ▲ AK data

risk (y-axis: 0% to 100%)
dose (# tdh+ ingested) (x-axis: 1 to 1E+08)

Russia 1997
1986–1987
Japan 1997
V. c. V. p.
1975–1985
Indonesia 1997
1975–1985
1975–1985
1938–1961
1967–1971
2002
1997
que 2004

O3:K6 strain complex – mid 1990s onwards

Vibrio vulnificus (Vv)

• *Vv* is a bacterium common in warm, low salinity marine waters. Found where surface seawater temperatures exceed 15 ℃. We now find this bacterium in UK water and shellfish.

• *Vv* occurs in high numbers in seafood, such as bivalve shellfish, particularly in warmer months - an important opportunistic pathogen that can cause both wound infections and sepsis, typically via exposure to seawater or through consumption of seafood.

• **Vv carries the highest case fatality rate of any foodborne pathogen.** Infections mostly involve males (nearly 90% of cases) the elderly, and at-risk persons. **Increase in reported infections in Europe and USA recently observed. Regularly reported infection in Asia.**
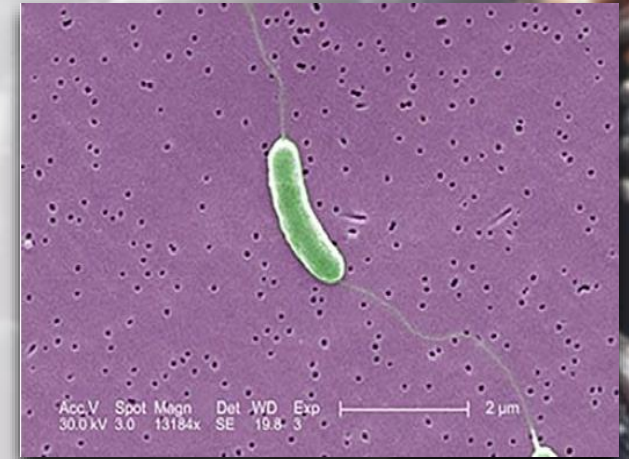
Baker-Austin and Oliver (2019), *Vibrio vulnificus, Trends in Microbiology.*


Vibrio vulnificus wound infection. Baker-Austin *et al. Nature Disease Reviews*, 2018.

Risk prediction tools: (*Vp*)

• Remote sensing makes use of satellites to detect infrared radiation emitted from the Earth's surface.

• Several satellites (from Europe and the USA) have been carrying out global studies of sea surface temperature (SST) for the last 3 decades. These datasets allow you to look at any region on the surface of Earth with a high degree of resolution.

• Some case studies presented here that I'll show you suggest that it may be possible to predict *Vibrio* outbreaks using remote data from retrospective studies.

• **We believe that there are often discernible environmental 'signatures' prior to outbreaks.**
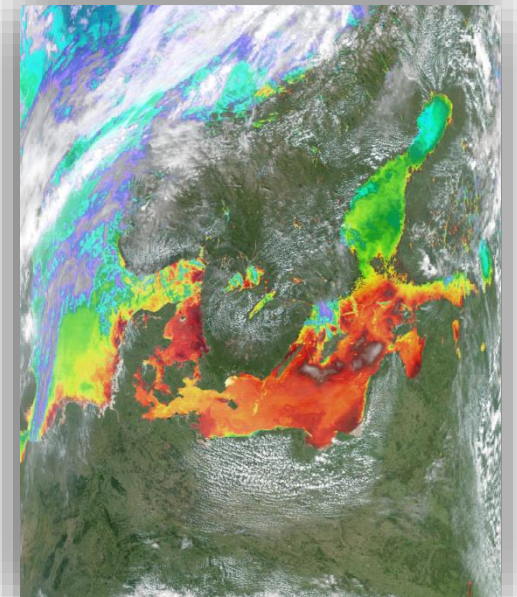
Risk prediction tools: (*Vp*)

- During the late summer of 1999, 35 cases of shellfish-associated illness were reported in Northern Spain. All infections caused by *Vibrio parahaemolyticus*. At the time the largest foodborne incident with *Vp* in Europe.

- As you can see - surface seawater temperature appears to play a significant role in when and where these infections were reported.

- One of the 1st times using satellite data has been used to look at the cause of a disease in this way.
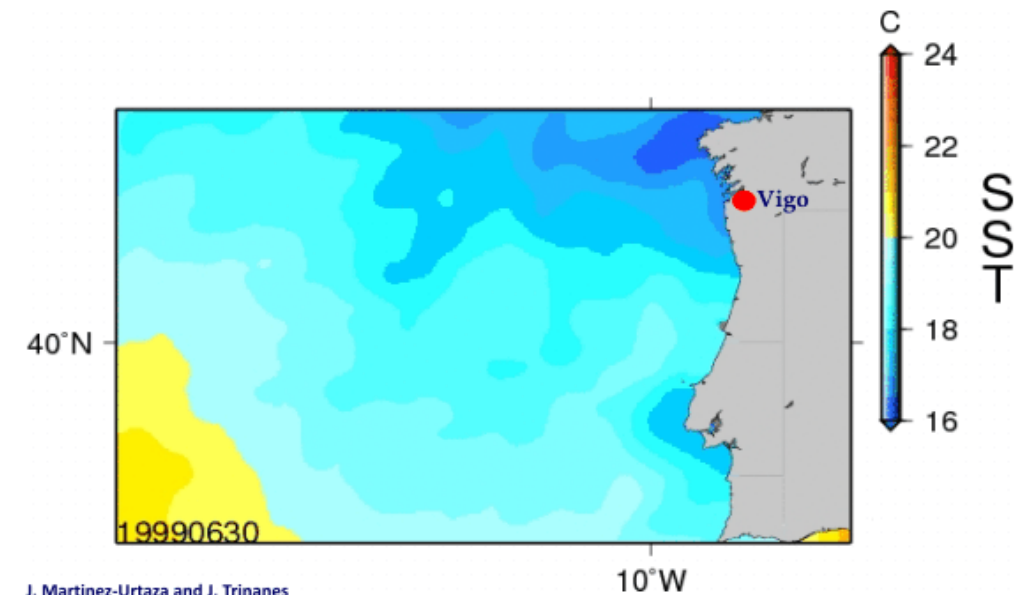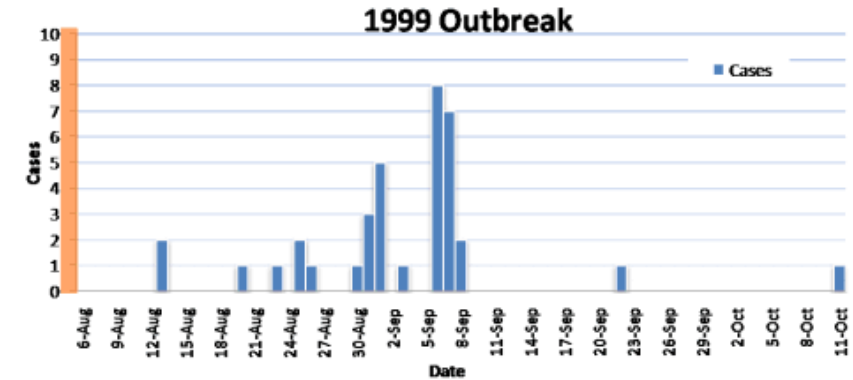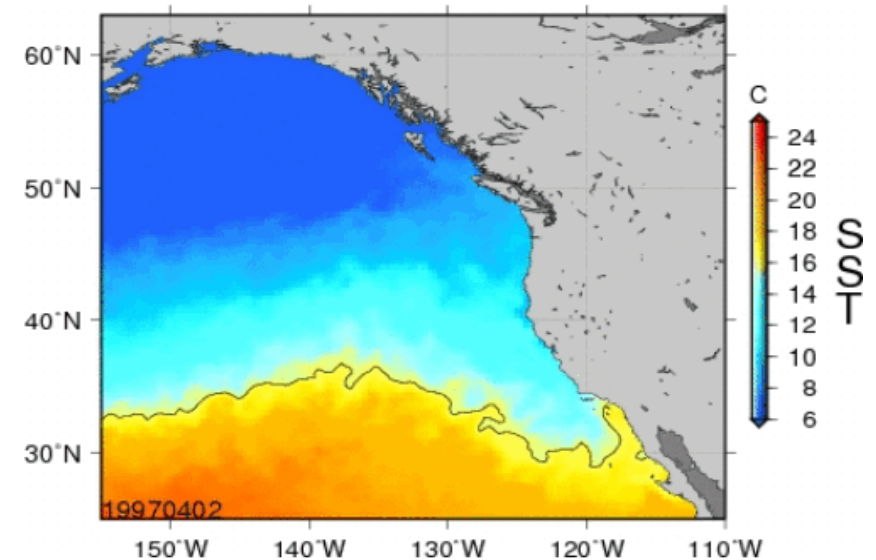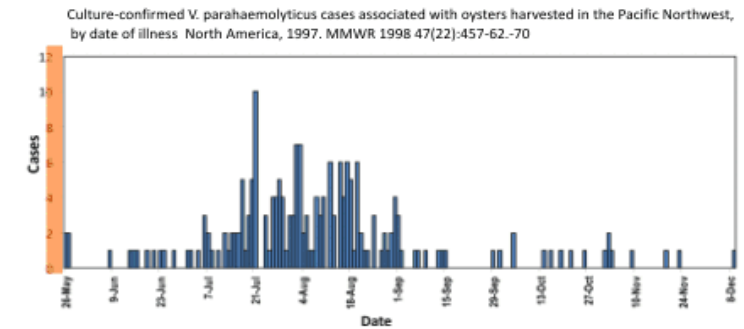

J. Martinez-Urtaza and J. Trinanes
University of Santiago de Compostela, Spain

Source: **Baker-Austin, C.,** L. Stockley, R. Rangdale, and J. Martinez-Urtaza. Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European perspective. *Environmental Microbiology Reports,* 2010.
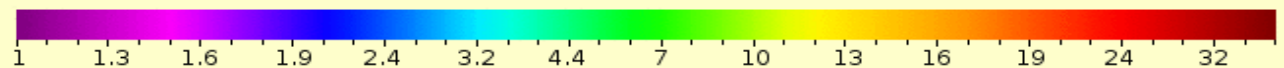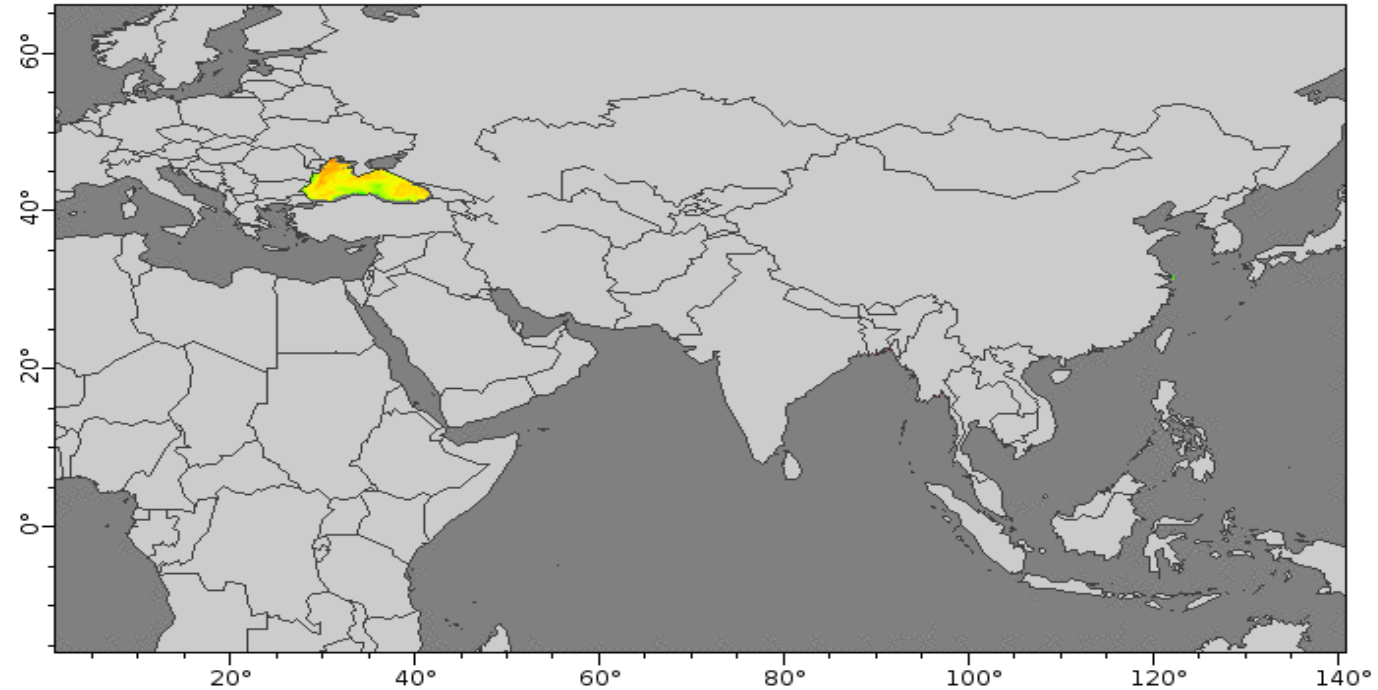
Risk prediction tools: (*Vp*)

- During the summer of 1997, the largest reported outbreak in North America of culture-confirmed *Vp* infections occurred.

- 209 infections reported, all associated with eating seafood harvested from California, Oregon, and Washington in the United States and from British Columbia (BC) in Canada; one person died.

- Mean Pacific coastal sea surface temperatures were significantly higher (typically 3-5 °C) in areas where cases were subsequently reported.

Culture-confirmed V. parahaemolyticus cases associated with oysters harvested in the Pacific Northwest, by date of illness North America, 1997. MMWR 1998 47(22):457-62.-70

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Risk prediction tools for vibrios

- Consequently, we have helped develop risk prediction tools using remote sensing data.

- Most of these approaches use temperature and salinity data and are updated daily models can also incorporate other variables.

- Extremely useful, and can produce risk-based mapping in near real-time. Some limitations e.g. granularity near coastal regions.

Daily Vibrio Risk (Risk Level)
USDOC/NOAA/NESDIS ECDC USC Vibrio Risk Analysis
(2013-06-12T12:00:00Z)
Data courtesy of CoastWatch Caribb-NOAA AOML ECDC USC/IIT

Vibrio **risk prediction tool**
**https://cwcgom.aoml.noaa.gov/cgom/OceanViewer**

# High-resolution tools: Genomic analysis of *Vp*

Recent sequencing and bioinformatic approaches can reconstruct the emergence, dispersal, and evolution of microbial pathogens.

The unparalleled resolution of sequencing methods has enormous practical applications such as inferring mechanisms of transmission, unravelling the evolution of strains, as well as pinpointing outbreaks for risk management purposes.

Can be applied to bacteria and viral pathogens e.g. vibrios and NoV, HAV.
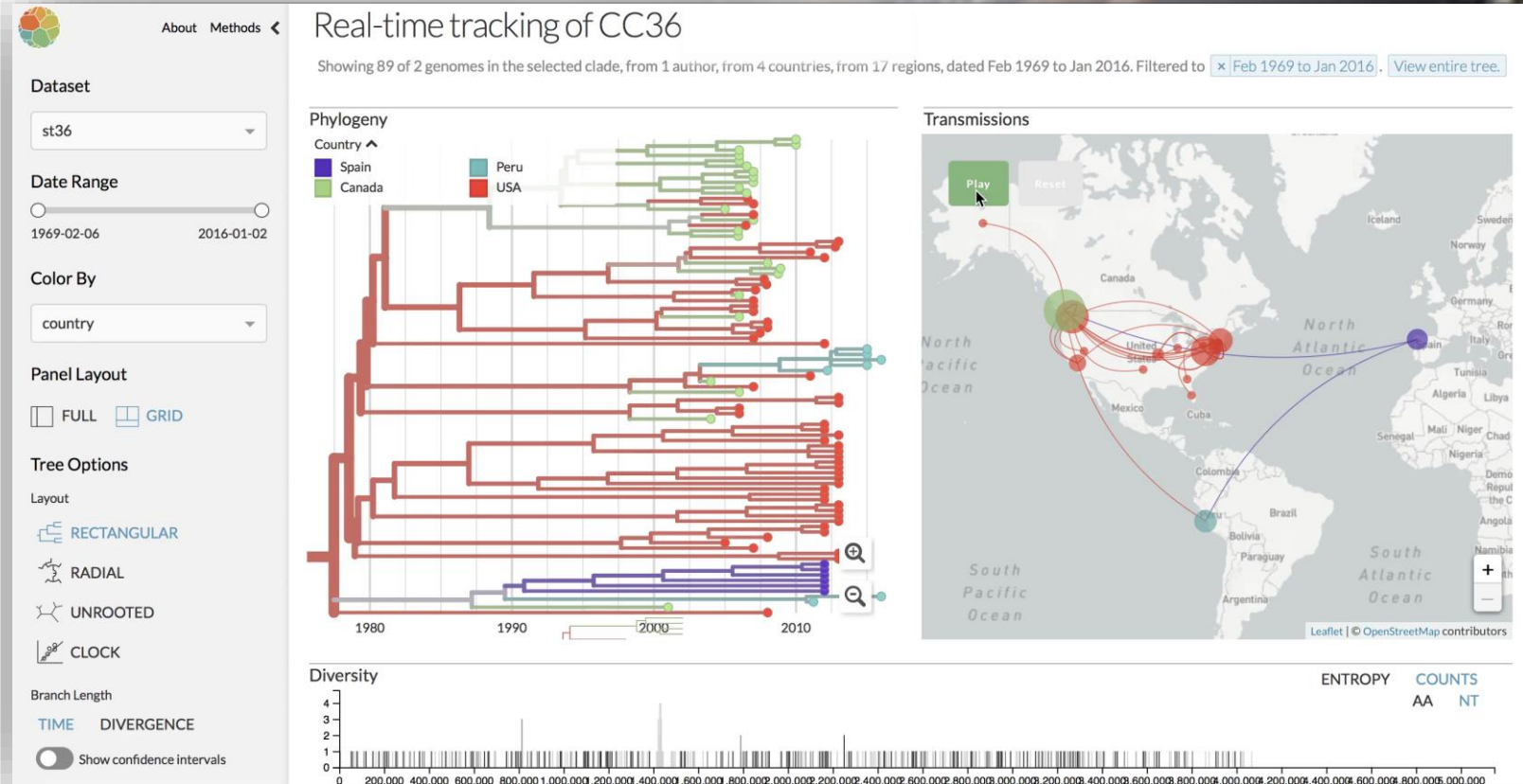
Where is the science (and the methods) going?
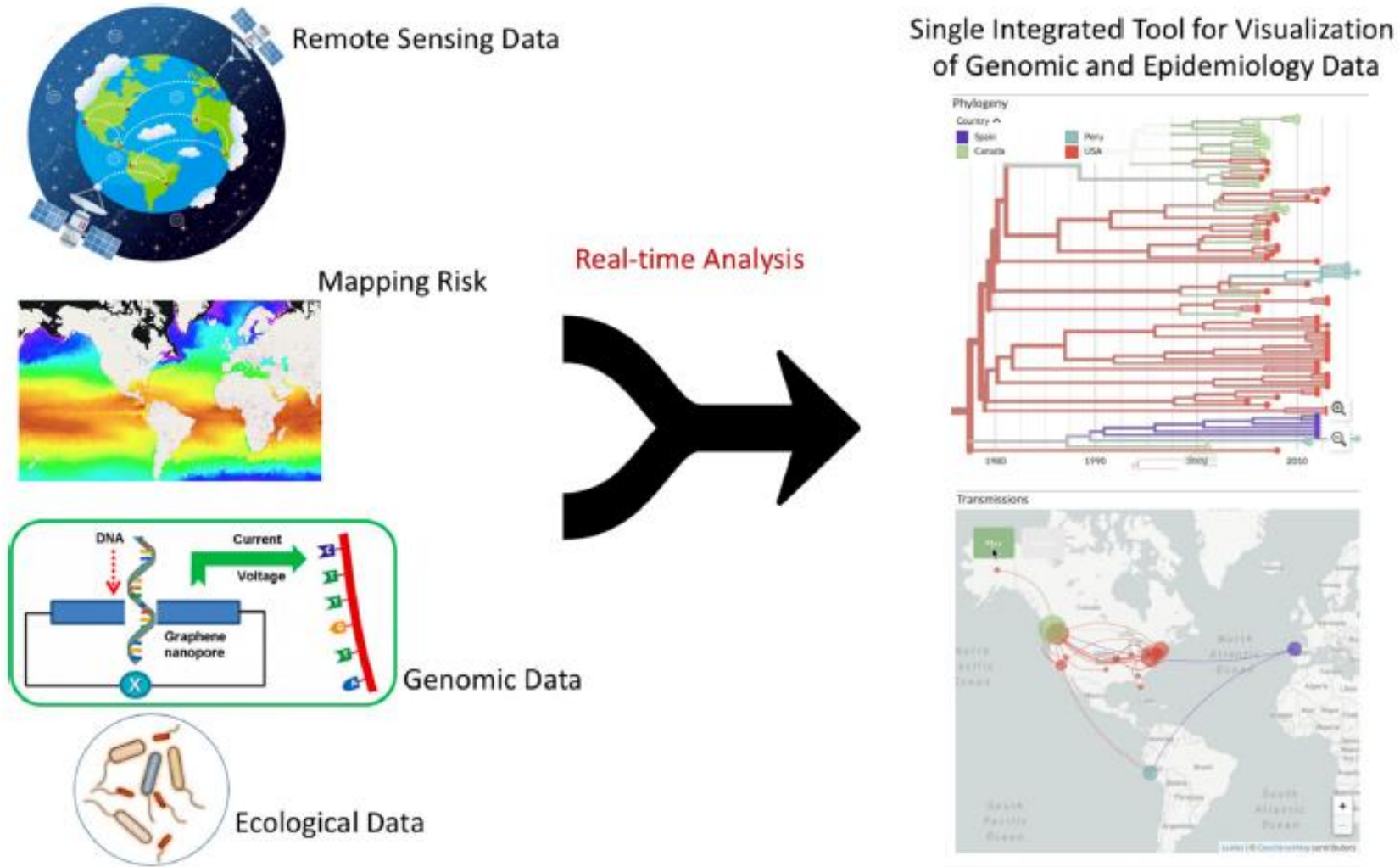
Summary

- Globally, norovirus, Hepatitis A and vibrios are the most important human pathogens associated with seafood.

- Dire need for improved systems of epidemiology, surveillance and reporting of shellfish-associated disease – again globally but also in the UK (under-reporting of infections is a very common issue).

- Increased international trade of shellfish, climate change, coupled to demographic and epidemiological changes may likely increase risks.

- Variety of different methods for tackling emerging diseases have been developed in the last decade, including risk prediction tools (e.g. remote sensing, genomics), as well as detection and quantification methods.

Many thanks for listening

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Acknowledgements