ANNEX B

GENUS *VIBRIO* RELATIVE TO FOOD HYGIENE

A presentation made for FLEP by Bjørn Gondrosen , Norwegian Food Control Authority

Family: *Vibrionaceae*
Genus: *Vibrio*

General description

Gram-negative, facultative anaerobic curved or linear rods. Most pathogenic *Vibrio spp* are motile. With 35 species having been reported, recent literature indicate 12 species to be human pathogens – 8 of which are capable of causing foodborne infections.

Natural occurrence

Found in brackish and salt waters in temperate and tropical regions across the world, pathogenic *Vibrio spp* form part of the "normal" microflora; they increase in prevalence at algal blooms.
V. cholerae and *V. mimicus* occur in lakes, rivers, and with birds and herbivors (plant-eaters) in regions far in-land from coastal areas. At water-temperatures below 20°C, qualitative and quantitative indications of pathogenic *Vibrio spp* decline.
The bacteria are detectable at all depths, and in surface layers of seabed sediments. *Vibrio spp* are often found with mussels and shellfish. Humans incur illness by consuming Vibrio spp-infested water and foods, and from wound infections

Pathogenic Vibrio spp

Manual of Clinical Microbiology, (ASM 1999), says that 12 *Vibrio* species have been reported as being human pathogens, of which 8 are held to be of food hygiene relevance:

- *V. cholerae O1, O139, and O1/O139*
- *V. fluvialis*
- *V. furnisii*
- *V. hollisae*
- *V. mimicus*
- *V. parahaemolyticus*
- *V. vulnificus*
- *V. metschnikovii*

Causing almost exclusively wound infections – possibly with septicaemia – and eye infections, *V. alginolyticus* is, notably, nevertheless not listed.

The most important species

V. cholerae

From the environment onto food

From its early description back in 1883 until the early 1980s, *V. cholerae* was thought as having the human intestinal tract as its sole reservoir, and being unable to survive, for instance, in water for long. Thus, infection had to be due to recent fecal contamination of drinking water and foods. Information of a much later date on *V. cholerae's* capability for entering a rest-period during adverse conditions has altered this opinion. *V. cholerae* can apparently survive in marine waters – frequently associated with phyto or zoo plankton - for up to several months. At plankton blooms, during periods of favourable temperatures and

nutrition, the bacteria may multiply enormously. Still, fecally contaminated drinking water constitutes the cardinal source of infection with *V. cholerae*. By contact, such water may subsequently contaminate other foods. Infection also easily transfers among individuals following a fecal-oral route.

V. cholerae is divided into three serologically based main groups: O1, O139, and non-O1/O139. In its turn, sero-group O1 divides into two biotypes: classical, and El Tor. Typical cholera cases arise from the cholera toxin (CT) – a potent entero-toxin whose genes are lodged in bacterial chromosomes.

CT is produced by serogroups O1 and O139; there are CT-negative *V. cholerae* O1, too. CT can also be produced by some non-O1s, underpinning the significance of serological typing and testing for toxins of isolated strains from foods. The CT toxin is unstable at heat.
V. cholerae non-O1/O139 is capable of producing hemolysin and heat-stable entero-toxin, a group giving rise to mild forms of gastroenteritis. Most commonly found at infections occurring in the USA.

Infective dose - at bacteria counts in the range $10^6 - 10^{11}$. The high infective dose relates to the low pH of gastric acid. With individuals low-producing in gastric acid – or using antacids (acid neutralizers) – this dose will prove lower.

V. parahaemolyticus

Though primarily belonging to coastal and brackish waters whose temperatures will reach up to 30°C, *V. parahaemolyticus* has also been isolated from countries of colder climes. Illness has often been associated with consumption of marine products such as fish, crawfish (crustaceans), or mussels. In south-east Asia *V. parahaemolyticus* occasions a number of foodborne outbreaks. For example, in Japan the bacterium is found being causal in 40-70% of all foodborne infections. Infections develop into acute gastroenteritis, accompanied by unspecific symptoms such as vomiting and diarrhea.

Pathogenicity

The bacterium is invasive; does not develop entero-toxin.
Divided into serological groups; a number of strains. For convenience, partitioned into Kanagawa-positive and Kanagawa-negative. Since not all strains are toxic, toxicity tests are necessary. Pathogenicity is not exclusively associated with Kanagawa-positive strains; diarrhea can result from Kanagawa-negative strains. Some serotypes are more virulent than others. The 1998 outbreaks in the USA could be traced to serotype O3:K6 – a Kanagawa-negative. Eating one raw oyster was enough for contracting illness.

Infective dose – in the $10^2 - 10^6$ bacteria range, depending on serotype/virulence. Gastric acid has an inhibitory effect.

V. vulnificus

Pathogenicity

Several unknown mechanisms for pathogenicity are used and this species is considered extremely invasive. Like *V. parahaemolyticus* found mainly in coastal and brackish waters at water-temperatures reaching 30°C. Though reported as infesting fish harvested off Senegal, bivalves harvested off Rio de Janeiro, and in mussels and samples from surface waters off Japan, most studies on the occurrence of the bacterium have been conducted along US seaboards. The bacterium may cause severe illness in the form of septicaemia and wound infections. Individuals ridden by other primary diseases, such as liver dysfunctions, are particularly at risk – with death rates hovering above 60%.

Infective dose – is low, less than 10^2 bacteria.

V. alginolyticus

No firm indications tie it to foodborne illness; pathogenicity occurs at wound and eye infections.

Closing remarks

Vibrio spp. are generally held to form a somewhat mixed group, and comes in many serotypes harbouring various pathogenic properties. Gastric acid dampens bacterial activity with all groups. Some factual details of interest are listed below:

As *Vibrio spp* forms part of the normal microflora in marine areas, establishing the occurrence of these bacteria by seafood studies would be natural. For *V. cholerae*, serotypes O1/O139 are chiefly detected in areas of endemic fecal contamination, whereas non-O1/139 are most commonly found in other parts of the environment.
Of current interest is whether a risk assessment on seafood consumption should involve both species diagnosis and serotyping as well as consider the virulence factors (toxins) of the identified *Vibrio*.
Summing up current knowledge on Vibrio spp. pathogenicity, we get:
V.cholerae: though low-frequent, serogroups O1 and O139 pose the greatest hazards. Even though frequently detected, non-O1/O139 serogroups are considered a minor threat to public health. Relatively high infection doses are seen for all types.
V. parahaemolyticus: of serotypes of different virulence, its presence is commonly established.
V. vulnificus: pose serious hazards, but uncommon with foods.
V. alginolyticus is a likely candidate for the group "Other Vibrio species relevant to food hygiene".

Miscellaneous

Going mainly unreported is any food hygiene implications posed by the *Vibrio spp* capacity for becoming dormant at unfavourable conditions.
Though well-known that *V. parahaemolyticus* can develop in foods (above 5°C), for other species this capacity – greatly affecting food hygiene - seems to have been only superficially investigated.
Refrigeration does not ensure the elimination of *Vibrio spp.*
V. cholerae is more heat-resistant than, for example, *V. parahaemolyticus*; and cooking, unfortunately, cannot guarantee it not being present (high D-value).
Also creating problems for assessing *Vibrio* findings in foods is the fact that pathogenicity within each species varies widely. Several methods for identification of *Vibrio* species have been developed: Nordic Committee on Food Analysis (NMKL) have developed a method for Isolation from Foods – NMKL method No 156/1997.
Scientific Committee on Veterinary Public Health has been asked by the EU Commission to make a scientific evaluation on *Vibrio spp.*

19th of September 2001
Bjørn Gondrosen
Norwegian Food Control Authority

FLEP-bag-vibrio-document.doc