

Opinion on risk to health from fruit and vegetables and their products grown in areas where cholera (*Vibrio cholerae*) has reached epidemic levels (expressed on 4 June 1998)

Terms of reference

To advise the Commission on the risk to human health arising from the consumption of fruits and vegetables and their products originating in or consigned from areas where cholera has reached epidemic levels. The Committee is requested to indicate in its opinion the parameters for the survival of *Vibrio cholerae* in or on these foods, based upon an assessment of the available scientific data.

Background

The Commission has recently introduced safeguard measures through Commission Decision 98/116/EC Commission Decision of 4 February 1998 adopting special measures for the import of fruit and vegetables originating in or consigned from Uganda, Kenya, Tanzania, and Mozambique. Official journal No. L 31, 06/02/1998, p. 28-29) adopting special measures for the import of fruit and vegetables originating in or consigned from Uganda, Kenya, Tanzania, and Mozambique due to a cholera epidemic in these countries under the article 10 of the hygiene of foodstuffs directive 93/43/EEC (Council Directive 93/43/EEC of 14 June 1993 on the hygiene of foodstuffs. Official journal No. L 175, 19/07/1993, p.1-11).

Such measures have been used previously in Europe and in other parts of the world for public health protection.

History of cholera

V. cholerae causes cholera, an enteric disease characterized by watery diarrhoea caused by a potent enterotoxin.

A study of the history of cholera provides valuable information on the ecology and epidemiology of *V. cholerae*.

Cholera is known to have been present in the subcontinent of Asia since ancient time. It achieved global recognition when it spread to other areas of the world in 1817. This was the start of the first pandemic which reached Europe in 1822 and ended 3-4 years later only to be followed by the second pandemic in 1829, which appeared in North America in 1832. Increasing travel at that time was one of the important modes of transmission, as we have experienced in our time. The third Pandemic ought also to be mentioned since it hit Europe very hard. The year 1853 is known in Denmark as "the year of cholera". The fifth and sixth pandemics have been attributed to the classical biotype of *V. cholerae* O1, which dominated until the current, seventh pandemic, when *V. cholerae* O1 El Tor spread from Indonesia to largely replace the classical biogroup in endemic areas leaving only a small endemic focus in the Bengal area. At the end of the sixth Pandemic, around 1923, cholera retreated to its homeland in the deltas of the Ganges and Brahmaputra rivers. We are currently in the seventh Pandemic of cholera, with sporadic cases continuing to occur world-wide. The seventh Pandemic started in 1961 in the Indonesian region and in the subsequent period through 1970, it took a jump every year and finally reached south east Europe and Africa in 1970. It reached Napoli from Tunis the same year but has since declined. Importation of fresh fruit from the same area of Italy at that time however never resulted in outbreaks, because of the low viability of *V. Cholera* in most fresh fruit and vegetables.

Environmental contamination may play a role in spread of cholera though only for a limited period of time, unless there is repeated contamination by a human source.

Cholera is thought to remain endemic in the subcontinent of India and in parts of Asia and Africa, whereas sporadic cases are reported from North America and Australia.

In 1992, a non-O1 serotype of *V. cholerae*, designated O139 Bengal, was isolated from typical outbreaks of cholera in southern India and rapidly spread to other parts of India and Bangladesh (Albert et al., 1993). Outbreaks in Latin America in 1991-93 with *V. cholerae* O139 is assumed to have been introduced in the area by ballast water in a ship from India. In view of the severity of the disease and the potential for epidemic spread, the O139 serogroup has been considered equivalent to the O1 serogroup, and the emergence of the eighth pandemic has been heralded (Nair et al., 1995).

Taxonomy of *V. cholerae*

The genus *Vibrio* belongs to the family Vibrionaceae and it shares many similarities with the other pathogenic vibrios such as *V. vulnificus*, *V. parahaemolyticus*, and *V. alginolyticus*.

V. cholerae is classified into two serogroups on the basis of its O antigens. Those agglutinating in O antisera are termed serogroup O1 and those which fail to are referred to as *V. cholerae* non-O1.

V. cholerae serogroup O1 is further divided into two biotypes, Classic and El Tor, based upon certain physiological characteristics or bacteriophage sensitivity. The biotypes of serogroup O1 may then be further subdivided into three serovars, Ogawa, Inaba, and Hikojima.

Until recently, the non-O1 serogroup has been isolated only from sporadic cases or outbreaks of food-borne enteric disease. In 1992, a non-O1 serogroup of *V. cholerae*, subsequently designated O139 Bengal, was isolated from typical outbreaks of typical cholera in southern India as mentioned above.

Microbial ecology and epidemiology of *V. cholerae*

V. cholerae is thought to have its main habitat in the enteric system of humans, but observations from USA and Australia seem to indicate that *V. cholerae* could also belong to the autochthonous flora of brackish water, estuaries and coastal areas.

Generally the transmission of cholera is related to faecally polluted water. Routes of cholera transmission can be summarised as follows: via water; via foods contaminated by water; via direct person-to-person spread; and via direct contamination of food by food handlers excreting the organism, with or without the symptoms of illness (Roberts-D, 1992). Sea-food, as general vehicles of faecal bacteria, has been implicated as an important source of transmission, whereas other foods are only occasionally implicated, and then often as a result of unhygienic food preparation practices, involving direct contact with human carriers or shedders.

Growth and survival of *V. cholerae* in water

Until the last two decades man was believed to be the only reservoir of toxigenic *V. cholerae* O1 and the movement of man can be related to the spread of the current and past pandemics. The bacterium has been detected in surface waters in cholera endemic areas although this has been considered transient and the result of recent human faecal pollution.

In endemic and epidemic areas *V. cholerae* enters surface water systems, including those used as sources of potable water, from sewage, and from there contaminate the entire aquatic environment. The aquatic environment may form a natural habitat and the critical reservoir appears to be estuarine waters where the bacteria are part of the normal microflora. The incidence varies according to water temperature and may also be related to the greater availability of chitin which vibrios are able to utilise from marine crustaceans.

When the O1 El Tor was first described at the beginning of this century the organisms was isolated from surface waters and from the cadaver of a pilgrim who had died of causes other than cholerae. The body was found close to the

lighthouse of Cairo (hence the name El Tor) and there was no evidence of disease in the associated community.

Related to the debate on the existence of an aquatic reservoir of *V. cholerae* O1, is the ability of the organisms to enter into a dormant, viable but non-culturable (VBNC) state, as well known in *Campylobacter* (Colwell and Huq, 1994, Oliver, 1996, Oliver et al., 1995). Whether these forms have a role to play in the epidemiology of the disease remains to be further investigated. Such forms may also be formed in other stress situations of the bacteria on foods, vegetables and fruits, necessitating application of special detection methods.

It should be noted that a bacterial population without growth will normally die-off following an exponential model. This implies that a fixed fraction dies off at each time unit, usually expressed using D-values (= the time necessary to reduce a population by 90%). This also implies that presenting death (or survival) as fixed time statements, such as 'V. cholerae survives in water for 20 days', cannot be used as comparable scientific statements, unless the original concentration and the detection limit is also presented. Unfortunately, this information is often missing in the sparse literature relating to this subject.

A growth potential of *V. cholerae* in reconditioned wastewater containing sufficient nutrients to support bacterial growth has been shown at temperatures from 5 to 42 °C. However, these results were obtained in laboratory settings using waters filtered to eliminate the natural flora (Rajkowski et al., 1996). Likewise, other enteric pathogens, such as *E. coli* and *Salmonella spp.*, will generally die-off in water, but under conditions with a very high organic load, growth can be shown. A connection between contaminated cargo ship ballast and the occurrence of *V. cholerae* in sea-food in Alabama, USA, has been shown (McCarthy and Khambaty, 1994). The ships had taken on ballast water in cholera-infected countries. These results underline the potential of *Vibrio* transmission through faecally polluted, improperly treated water, and the dangers of using such waters for agricultural processing purposes.

Growth and survival of *V. cholerae* in foods

Cholera has long been known as a waterborne disease. Food-borne transmission is however increasingly recognised as important especially for the El Tor biovar. In endemic areas, water may be the primary vehicle of transmission. As the disease spreads within the community, secondary transmission via food and food handlers and person to person increases (Miller et al., 1985).

Of particular interest for EU is the ability of the bacteria to survive in or on fresh or processed foods imported from endemic areas. As the result of multiple sources of contamination, a wide variety of foods have been directly or indirectly implicated as a vehicle, the most common being fish, shellfish and crustaceans, but a wide variety of other foods have been implicated in the transmission of cholera, including soft drinks, fruit and vegetables, milk, seafood, locally brewed beer, egg and asparagus salad, potatoes as well as gruel (Hackney and Dishrag, 1988). Food may in some cases serve only as a passive vehicle of infection, while in other circumstances growth in foods is of major importance. It has been the traditional view that *V. cholerae* does not multiply in foods, but recent studies (Kolvin and Roberts, 1982) have indicated that it might grow well in a variety of cooked foods at 22, 30 and 37 °C (e.g. in cooked rice and pulses) and especially in high pH foods, such as cooked prawns, hard boiled eggs and cooked mussels.

It is interesting to notice that the El Tor type of *V. cholerae*, responsible for the recent seventh pandemic, is less virulent than the agent of the classical disease, but leads to a great many carriers who are barely sick so that carriage of *cholerae* may pass unnoticed. The less virulence of the El Tor cholerae bacteria is, from an epidemiological point of view, balanced by the fact that it is more robust than the classical cholerae bacteria. It grows at lower temperatures, down to 12 °C, and it survive for quite long periods in frozen seafood. *V. cholerae* poorly tolerates such environmental stresses as drying, exposure to sunlight, and competition with other organisms, but may remain viable for several days in food that is alkaline and moist, and eventually multiply provided that competing organisms do not overgrow it.

Heat resistance of *V. cholerae* expressed in decimal reduction values (D) in foods at aw 0.99 and pH = 6.5-7.0, has been reported to be 18 seconds at 72 °C (Mossel et al., 1995). This is slightly higher than the HTST pasteurization required for milk.

The range of parameters which control the growth of *Vibrio cholerae* in foods is shown in Table 1. Temperatures have a

significant effect on both the growth and survival of *V. cholerae*. The bacterium survives under refrigeration (< 10 °C) considerably longer than at ambient temperature. Survival is between 2-4 weeks on foods such as vegetables, meat, fish, milk, and cooked cereals when refrigerated, and may be longer when frozen (-20 °C). The bacteria grow in a variety of foods at 22 °C and 37 °C. The lag time in foods is less than 1 hour when the temperature is over 30 °C (ICMSF, 1995). In inoculated mussels and prawns it has been shown to increase from 10² - 10¹⁰ /g within 12 hours (Minz et al., 1994).

Table 1. Optimum conditions and growth limits for *Vibrio cholera* El Tor. (ICMSF, 1995)

Parameter	Optimum condition	Growth range
Temperature (°C)	37	10-43
pH	7.6	5.0-7.6
aw	0.984	0.970-0.988
NaCl (%)	0.5	0.1-4.0

Growth and survival of *V. cholerae* in fruits and vegetables

The contamination of vegetables and fruits with *V. cholerae* will presumably primarily originate from the use of sewage or manure as fertiliser on the growing crop. However, the use of faecally contaminated irrigation or process water or direct contamination resulting from improper production or sanitation procedures or facilities in the harvesting or production line can also represent contamination sources.

The *V. cholerae* reaching the vegetable or fruit will normally thereafter enter the die-off stage, corresponding to the situation explained under water survival. Again, specific die-off rates are very difficult to obtain from the scientific literature (see above). However, *V. cholerae* is thought to die off more rapidly than, for instance, *Salmonella spp.* or *E.coli* on soil or leaves. For *Salmonella* on fruit and vegetables survival conditions corresponding to D-values of hours to days have been suggested (Geldreich and Bordner, 1971). The 'total' survival period for *V. cholerae* at ambient temperature (30-32 °C.) has been reported to be 1-7 days for fresh vegetables and 1-3 days for fruits (WHO, 1997).

Contamination reaching fresh fruits and vegetables during the production stage will not be eliminated at a later stage, since these products will generally not receive further bacteriocidal treatment before consumption. An important special situation occurs with the production of seeds for sprouting since the production methods involves a stage where a multiplication of most bacteria present on the seeds is inevitable (Aabo & Baggesen, 1997). However, although a number of food-borne disease outbreaks related to sprouts have involved *Salmonella spp.* and EHEC, *V. cholerae* has not been implicated in such outbreaks, which is probably related to the inability of this bacterium to compete with the indigenous microflora present on the sprouts.

V. cholerae is able to survive for shorter periods on vegetables or fruits and depending on the original concentration of bacteria, the time since contamination, and the environmental conditions, *V. cholerae* can theoretically reach the consumer through imported foods.

Minimal infective dose of *V. cholerae*

In human volunteers, the infectious dose of *V. cholerae* O1 is very high. However, in cholera endemic areas with populations of poorer health status it has been estimated the infectious dose may be low (WHO, 1980). As with other pathogens this may have a significant effect on the infectious dose. Patients with gastric abnormalities are susceptible to low infectious doses and food may also provide protection for the bacterium during passage through the stomach (WHO, 1980).

Where larger infectious doses are required, growth of the organisms likely occurs prior to ingestion in the vehicle of infection, such as food.

Cholera outbreaks related to food in general

Most recent cases of cholera in the United States have been related to international travel, four US outbreaks have been linked to the consumption of *Vibrio cholerae* contaminated food imported from other countries. In two cases crab meat was implicated, one was associated with frozen coconut milk and one presumably with canned palm fruit (Bailey N. et al., 1995).

In an investigation into an outbreak of cholera from food served on an international aircraft out of Peru in 1992, Eberhardt-Phillips et al. (1996) performed a case-control study showing a strong association between the consumption of sea-food and disease. This outbreak occurred during the 1991-93 Latin American cholera epidemic.

In a case-control study, Koo et al. (1996) showed a significant association between cholera in Guatemala City and the consumption of street-vended foods, especially processed food, whereas disease was not associated with drinking municipal tap water. The *V. cholerae* strain related to this outbreak was multi-drug resistant and was not related to the 1991-93 Latin American cholera epidemic.

Cholera outbreaks related to fruits and vegetables

Fresh fruits and vegetables can become contaminated through contact with faecally contaminated soil (fertiliser) or water (irrigation, washing or injection to increase weight and turgor). The consumption of contaminated raw vegetables has been listed as a risk factor for the outbreak of cholera. In an outbreak in Israel in 1970, vegetables irrigated with inadequately treated wastewater were implicated, but not proven to be the source (Cohen, 1971). An outbreak in Chile was thought to have been caused by vegetables watered with sewage-contaminated river water (Anon., 1992). Consumption of vegetables and fruits from fields where raw sewage was used for irrigation was associated with a cholera outbreak in Peru (Swerdlow et al., 1992). However, these examples are few in comparison to the examples implicating other foods, such as sea-food (water contamination) or cooked grains and legumes (recontamination or improper heat treatment followed by multiplication caused by lack of cooling).

Food items are currently imported into regions where cholera is virtually absent, from many countries where cholera is endemic. However there are few documented outbreaks associated with imported foods (Taylor et al., 1993), and none of these involve raw vegetables or fruits. Cholera can certainly also occur as sporadic cases, but in regions, where cholera is virtually absent, even the occurrence of isolated cases of this disease would probably be recorded and looked into. Therefore, it would seem likely that the risk of disease from imported vegetables and fruits is extremely low.

Since the burden (concentration) of *V. cholerae* on vegetables and fruits grown with hazardous practices will increase in outbreak situations it is likely that the risk of *V. cholerae* reaching a consumer in an importing country will also increase. However the absence of reported cases of transmission of cholera in such situations still indicates that the overall risk is very low.

The reason why the final risk related to the importation of potentially contaminated fruits and vegetables is so low can not be explicitly determined from the available scientific data. The low pH especially in fruits is likely to play an important role together with competing organisms in the case of vegetables. Therefore, both the relatively poor survival potential on fruits and vegetables and the high infective dose, which has been suggested as more than one million *V. cholerae* for otherwise healthy individuals (Anon., 1997), are important factors. This links to the general notion that cholera outbreaks involving non-sea foods will often include a multiplication stage in the food.

Control of the transmission of *V. cholerae* by foods from endemic areas

Referring to the risk-based food safety management systems (such as Hazard Analysis and Critical Control Points, HACCP) the prevention of contamination is favoured over pathogen reduction treatment. One important point to

control food borne disease risk from fresh fruits and vegetables is therefore to avoid the use of untreated sewage sludge, animal slurry and irrigation or washing water of non-potable quality.

Since one cannot rely on end-product testing to ensure safety, there is a need to ensure the introduction of hygienic practices for primary production, harvesting and packaging of fresh fruits and vegetables.

Health protection measures have earlier been considered necessary for countries importing food from areas with cholera epidemics. Mossel et al. (1992) suggested an alternative to barring importation, including strict adherence to rigorous measures of longitudinally integrated microbiological safety assurance. The method was based upon the principles of the 'Wilson triad', including:

- a) minimising colonisation of raw material and increasing lethality of processing,
- b) preventing recontamination through aseptic packaging and treatment after packaging, and
- c) storing the products under conditions to prevent microbial growth.

The same authors suggest a strategy applicable to the acceptance of imported foods, based, among other things, on audit of the operations in the exporting country and testing of representative numbers of each consignment. However, since raw vegetables and fruits are generally not processed, the only 'Wilson' factor relevant here would be the prevention of original contamination. This will primarily mean the avoiding of the use of manure/sewage for fertiliser and faecally contaminated water for irrigation or processing and upkeeping workers sanitation measures during harvesting, processing and packaging.

Provided cross-contamination is prevented and correct processing methods are used for production of e.g. canned, irradiated, dried or frozen goods, few processed foods are hazardous. *V. cholerae* is destroyed by heating foods to a core temperature of 70 °C (Anon, 1991).

Conclusion

It is likely that microbial contaminants, including *V. cholerae*, will lose viability as a result of environmental stress and there is evidence that *V. cholerae* is more sensitive to such stress than either *Salmonella* or *E. coli*. The total survival period for *V. cholerae* at ambient temperature (30-32°C) has been reported to be 1-7 days for fresh vegetables and 1-3 days for fruits (WHO, 1997).

Since there are no recorded incidents linking cholera outbreaks or cases in non-cholera regions to the importation of raw fruits or vegetables, the risk of human illness from exposure to *V. cholerae* from imported fruits and vegetables is probably very low. There is no direct evidence that this risk changes significantly in situations where exporting countries move from an endemic to an epidemic situation.

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