Assessment of the health risk emanating from *Vibrio cholerae* strains, present in the large alkaline Central European Lake Neusiedler See

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Introduction

Vibrio cholerae is a human pathogen thriving in aquatic environments. *S*erogroups O1/O139 have been associated with epidemic cholera, while nonO1/nonO139 serogroups usually cause human disease other than classical cholera. *V.cholerae* nonO1/nonO139 were shown to be endemic to Lake Neusiedler See, a large alkaline Central European lake (5), and have been causing ear or wound infections, including one case of fatal septicaemia (4). The last patient was suffering from an underlying malignancy and received immunosuppressive treatments.

To assess the health risk emanating from *V. cholerae* strains for tourists performing recreational activities in the lake, a schematic model was developed, considering the concentration, ecological niches, diversity, and pathogenicity of the present strains, the input of "external" strains into the lake and the potential infection pathways to the patients (Fig. 1). Several investigations to fill the model with data were performed in the past eight years, enabling a first estimation of the risk.

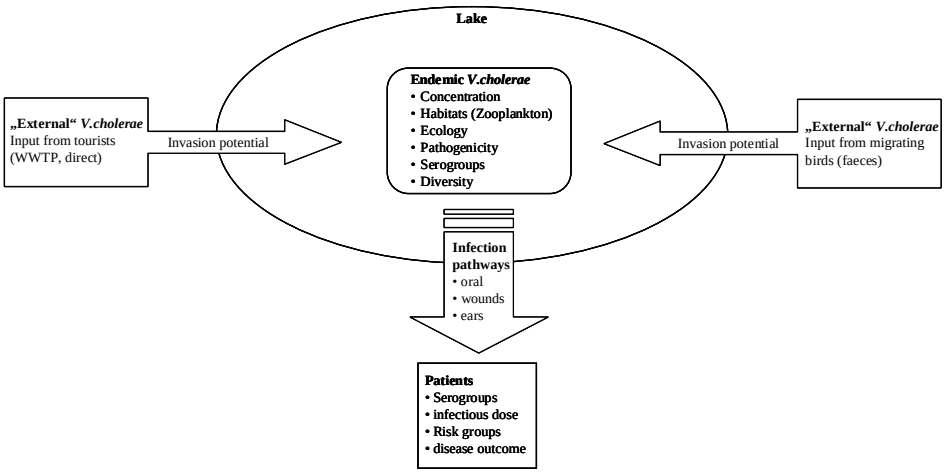

Figure 1: Schematic model of information necessary to assess the health risk from *V.cholerae* in the Lake Neusiedler See. WWTP: wastewater treatment plant

Materials and Methods

Study site – Lake Neusiedler See is a shallow, turbid, alkaline lake (Z_{max}: 1.8 m, pH: 8.5 – 9.1; salinity: 1 – 2 g L^{-1}) located in Eastern Austria. More than 50% of its 320 km^2 area are covered with reed, resulting in high concentrations of humic substances in the lake (7). The region is the most important bird sanctuary in Central Europe at the intersection point of East-West and South-North routes of migratory birds.

Sampling – Samples for all different kinds of specimens (water, zooplankton, faeces) and investigations (direct detection and isolation of *V.cholerae*, lab experiments) were taken from two to six representative sites in the open water area and in the reed belt. In addition, a series of environmental variables was determined.

V.cholerae abundance – *V.cholerae* were quantified by cultivation based methods, followed by biochemical tests and multiplex-PCR (2). Alternatively, *V.cholerae* were enumerated via a newly developed FISH protocol (6).

Laboratory experiments – For testing specific hypotheses (association of *V.cholerae* with different zooplankton species; influence of humic substances from the reed belt on growth of *V.cholerae*; invasion potential of *V. cholerae* O1 and O139 strains into the lake) batch culture lab experiments were performed.

Results and Discussion

Seasonal development, planktonic growth and association with zooplankton – A seasonal study over a period of 4 years showed that cultivable *V.cholerae* were significantly correlated with the presence of cladocerans but not with copepods. In lab cultures, a significant association of *V.cholerae* with the cladocerans could be found, while the presence of copepods had a negative influence on *V.cholerae* growth. Cladocerans significantly enhanced *V.cholerae* growth in the surrounding water and on their surfaces with concentrations up to 8×10^5 cells per individuum (6). Moreover, we could show that also in the absence of Cladocerans planktonic *V.cholerae* showed high growth rates, comparable to the natural bacterial population. Growth was significantly reduced in water from the reed belt due to inhibition by humic substances (5).

Potential pathogenicity of isolated V. cholerae strains – All of the strains isolated between 2002 and 2009 ($n > 600$) were identified as *V.cholerae* nonO1/nonO139. In no isolate the ctx and tcp gene were detected. However, virulence factors like hlyA, toxR, ompU and ompW were present in 88% to 100% of the strains.

Invasion potential of V.cholerae O1/O139 – *V.cholerae* strains O1 biotype ElTor and classical as well as O139 smooth and rugose were tested for their growth potential in lake water microcosms in the lab. The experiments showed that with the exception of *V.cholerae* O1 ElTor, all strains were able to multiply to high concentrations in the lake water. Growth was stronger in water from the reed belt as from the lake centre.

Quantitative data of V.cholerae concentrations – During a seasonal cycle from February to September *V.cholerae* concentrations in the lake water and on zooplankton were quantified via cultivation and FISH. Concentrations were rather constant over the period, as determined via FISH, while no *V.cholerae* could be detected via cultivation from February to April. During summer, both methods yielded similar results with 3×10^3 - 48×10^3 cells/CFU L^{-1}. Lower concentrations were observed in the reed belt than in the open lake.

Risk assessment concerning cholera – The fact that, up to now, no *V.cholerae* O1/O139 strains were isolated from the lake and that no isolate was positive for the ctx and tcp genes, indicates that the presence of *V.cholerae* O1/O139 in the lake and the acquisition of cholera is extremely unlikely. However, as our experiments showed an invasion of *V.cholerae* O1/O139 strains into the lake cannot be excluded for the future. Promoted by climate change (the average water temperature of the lake has increased by 1.5 °C during the past 30 years) and increased mobility of travellers, the probability of invasion will increase.

Risk assessment concerning gastrointestinal infections caused by V. cholerae nonO1/nonO139 – For this estimation it was assumed that the published infectious dose values for gastrointestinal infections caused by *V. cholerae* O1 and non-O1 strains are identical. This means that with concentrations of up to 50×10^3 *V. cholerae*

cells L^{-1} and a reported infectious dose of 10^5 - 10^8 CFU (3, 8), 2 to 2000 L of lake water have to be swallowed to cause infection. For hot-spots of *V. cholerae* on zooplankton with up to 8 x 10^5 cells per individuum (*Cladocerans!*) and a concentration of 100 cladocerans L^{-1}, swallowing of much less volume of water may be sufficient to cause infection.

Risk assessment concerning ear, wound and blood infections caused by V. cholerae nonO1/nonO139 – The infectious doses of *V.cholerae* for ear, wound and blood infections have not been investigated so far. The concentration to cause infections is, however, supposed to be much lower than for intestinal infections. As all patients had either an underlying immunosuppressive disease or a chronic local inflammatory disease, it can be assumed that few cells are sufficient to cause disease in health-impaired persons, while healthy persons are not affected.

Conclusions

In conclusion it can be stated that the risk of getting a severe disease caused by *V. cholerae* during recreational activities in the Lake Neusiedler See and other comparable environments, (like e.g. the Baltic Sea; 1), is extremely low for healthy persons. The risk of cholera can be excluded for the moment, as *V.cholerae* O1/O139 strains have not been detected in the lake so far. Immunosuppressed persons at high risk status, however, should exercise caution, especially in the case when having wounds. When infections are observed after a visit of the Neusiedler See or other comparable ecosystems, the possibility of an infection caused by *V. cholerae* should be taken into consideration. Furthermore, increased travel activities and temperature increase due to climate change may enhance the invasion of *V. cholerae* O1/O139 strains also into saline waters of the temperate climate zone increasing the risk in the future.

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