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# Alcohol and its influence on the survival of *Vibrio cholerae*

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Microbiologists in Inverness have become interested in the great cholera outbreak in Britain in 1831–32.[1] In 1832 the immoderate drinking of alcohol was considered a predisposing cause in the acquisition of cholera, as its incidence was much higher in the heavy drinker than among the general population.[2] In response to cholera's impending arrival in the capital of the Highlands, the newly formed Inverness Board of Health advised the population "to have great moderation in the use of fermented and spirituous liquors".[3] Was this advice correct?

More recently, in 1979, restrictions on alcohol also formed part of the public health advice during an outbreak of El Tor cholera in Tanzania, where locally made alcoholic beverages, which were made largely with untreated water, were banned for fear that these would act as vehicles of infection.[4]

The drinking water in Inverness, which was drawn from the River Ness during the first cholera epidemic in the late summer of 1832, would have been contaminated with the excreta of cholera victims. The spirits that would have been drunk in Inverness may have been diluted with this water before being sold.

There was no legal definition of proof spirit until 1879 and retailers in the 1830s diluted spirits with water for extra profit.[5] Thus, this study is designed to examine the effects of alcohol on *Vibrio cholerae* survival.

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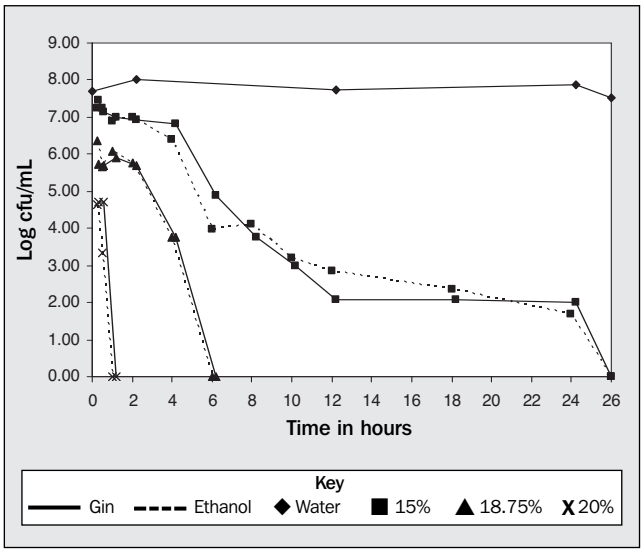


**Fig. 1.** Survival of *V. cholerae* in gin and ethanol.

The organism used was *V. cholerae* (NCTC 11348). Dry London Gin (37.5%, Tesco) was tested in parallel with equivalent concentrations of ethanol in a series of four experiments. A 16- to 18-hour culture of *V. cholerae* in alkaline peptone water was suspended in sterile de-ionised water (pH 7.0) to give a density of $10^7$ colony forming units (cfu)/mL.

Contaminated water (5 mL) was added to an equal volume of the gin and the ethanol to give a final alcohol concentration of 20%, 18.75% and 15% (5.34 mL of gin to 4.66 mL of contaminated water for 20% gin). The test solutions were incubated at 13°C, which was the average local ambient temperature when cholera was present in Inverness in 1832.[6]

A sample (1 mL) was removed and decimal dilutions were performed using 9 mL 0.1% peptone (Oxoid CM 0733) with NaCl (pH adjusted to 8.0) at 15 min, 30 min, 1 h, 2 h and at two-hourly intervals thereafter until an end point was reached. Aliquots of 200 µL were removed from each decimal dilution, plated on two thiosulphate citrate bile salt (TCBS) agar (Oxoid CM 0333) plates and incubated at 37°C for 18–24 h. The density of contaminated water was tested at the beginning and the end of the experiments.

If sparse or no growth was apparent at 24 h, the plates were re-incubated for a further 24 h and bacterial growth was assessed again at 48 h. Bacterial growth was measured as the number of yellow colonies seen with the naked eye, and the results of the duplicate plates were averaged and multiplied by the dilution factor. The lower limit of detection was 20 cfu/mL.

Figure 1 shows the survival of *V. cholerae* in 20%, 18.75% and 15% gin over 26 h. In 20% gin there was a 3 log reduction in the number of *V. cholerae* isolated at 15 min, with a total reduction in numbers after 1 h. The reduction was less dramatic in 18.75% gin, with no *V. cholerae* surviving after 6 h. In 15% gin, the numbers fell slowly to zero after 26 h. Identical survival times were found in all three ethanol dilutions tested (Fig. 1). In contrast, the number of *V. cholerae*

present in water showed no reduction over the same period.

The concentration of *V. cholerae* ($10^7$ cfu/mL) in the contaminated water used in this study has been shown to produce cholera diarrhoea in susceptible individuals.[7] Although studies on healthy individuals in the USA in the 1970s showed that *V. cholerae* at $10^{11}$ cfu/mL was necessary to produce cholera diarrhoea,[8] the concentration required can be as low as $10^4$ cfu/mL when hypochlorhydria is induced.[7] Contaminated water sources in cholera-endemic areas usually exhibit rather low concentrations of cholera vibrios; however, gastric hypochlorhydria may be present in such populations, which would facilitate the spread of the disease.[9]

The poor socio-economic conditions present in cholera-endemic areas today may be very similar to those found in early 19th-century Inverness,[10] and the concentration of *V. cholerae* in the contaminated water used in the experiments reported here was designed to reflect the quality available to such populations.

The image of the cholera victim in 1832 was lower-class, poor, filthy and drunken.[2] The low cost of gin, which meant that persons could be "Drunk for a penny; dead drunk for tuppence", depicted graphically in Hogarth's cartoon 'Gin Lane', made it an extremely popular drink in this section of society.[11] It was for this reason that gin was chosen as the "spirituous liquor" for the experiments.

There was no legal definition of proof spirit in 1832 and a glass of gin in one shop might contain 76% proof spirit, while it could be 50% in another shop.[5] The highest concentration of ethanol used in the present experiments was 40% ethanol ABV (alcohol by volume) before dilution with contaminated water. It is representative of some gins available and the authors believe that the higher concentrations that may have been available in 1832 would have had greater bactericidal effect. The concentration of the Dry London Gin purchased was 37.5% ABV, and 30% ABV was chosen as the lowest level for historical reasons.

When an equal volume of contaminated water was mixed with gin or ethanol to give a final concentration of 20% alcohol, there was a 3 log reduction in the number of *V. cholerae* isolated at 15 min and a total reduction in numbers after 1 h. Similar results were found with konyagi, a Tanzanian gin-like beverage with an alcohol content of 30%, which did not allow survival of *V. cholerae* beyond 1 h.[4]

A reduction of only 1.25% in ethanol concentration increased significantly the time necessary for the bactericidal effect to be apparent: it took 6 h for a similar effect in 18.75% gin and ethanol, while a further reduction to 15% extended the time to 26 h. Beer has been shown to reduce *V. cholerae* counts to zero within a day.[12] A similar effect was found with 10% tequila using salmonellas, shigellas and *Escherichia coli* at $10^5$–$10^6$ cfu/mL; however, no bactericidal effect was seen with 10% ethanol.[13] Lema *et al*.[4] demonstrated that *V. cholerae* can survive in 10% ethanol, confirming the authors' previous unpublished research.

Other studies have found that 12.5% ethanol had no bactericidal effect on enterobacteria; however, *Salmonella enteritidis* counts were reduced from $10^5$–$10^6$ cfu/mL to zero in 24 h when the pH was adjusted to that of wine (pH 3.5).[14] This increased bactericidal effect may be attributed to the combined effect of the ethanol and the low pH. In support of this, the authors found that the low pH of deionised water used in previous experiments reduced the number of viable *V. cholerae* in the contaminated water over time, and thus deionised water used in the experiments was adjusted to pH 7.0.

Several studies have found that wine has a substantially faster bactericidal effect than the equivalent concentration of ethanol (pH adjusted or unadjusted) and counts of enterobacteria were reduced to zero between 5 min and 4 h.[13,14] As long ago as the late 19th century in Paris, Dr Alois Pick demonstrated that neat and diluted wine killed *V. cholerae* within 15 min.[15] The antimicrobial agent in wine appears to be a polyphenol liberated during fermentation, which is active against bacteria at an acid pH.[13]

The results of the present study have shown no difference in survival time between the equivalent concentrations of gin and ethanol. It can be concluded, therefore, that the bactericidal effect seen is due solely to the ethanol present.

The results show that the ethanol in the gin tested can render a drink non-infectious. In addition, light to moderate drinking has a protective effect on the risk of developing an alcohol-related disease, compared to the heavy drinker or the abstainer.[16] In contrast, alcoholics are generally malnourished, have a reduced secretion of gastric acid and an elevated intestinal permeability to bacterial endotoxins,[17] making them more susceptible to cholera.[7]

The advice "to have great moderation in the use of spirituous liquors", given in Inverness during the cholera epidemic of 1832, at a time when the causative organism was yet to be identified, was correct, as modern laboratory techniques have been able to confirm.

*The authors would like to thank Robert Polson, Highland Health Sciences Library, and Linda Brady for their invaluable help and assistance. The authors are very grateful to Dr. Andrew Hay and Glen Widdows for their cooperation.*

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# Hepatitis B virus: a study of genotypes in an infected Saudi cohort

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Hepatitis B virus (HBV) is a small non-cytopathic virus with a circular DNA genome that contains four genes (*C*, *S*, *X* and *P*).[1] The *S* gene codes for the surface antigen (HBsAg). Hepatitis B virus is polymorphic and is classified into eight genotypes (A–H).[2–4] Hepatitis B virus genotypes differ by 8%,[5] while subgenotypes differ by at least 4%.[6] Genotypes and subgenotypes show a distinct geographical distribution,[7] with the former appearing to correlate with disease progression[8–10] and response to treatment.[11–13]

In Saudi Arabia, HBV infection is declining, and this is due mainly to the introduction of a successful vaccination programme. The authors have observed this decreasing rate of HBV infection in Saudi blood donors,[14] and the aim of the present study is discover which HBV genotypes are present in Saudi patients with acute HBV infection.

A total of 65 Saudi patients (mean age: 30.2 years + 15.3; 62 males, three females) with a positive HBeAg marker were recruited to the study. Extracted DNA was obtained using AmpliPrep sample processing. Hepatitis B virus DNA was extracted from plasma using the CAP instrument and TNAI kit, according to the manufacturer's instructions.

Genotyping was performed using INNO-LiPA HBV genotyping (Innogenetics, Ghent, Belgium). This is a line probe assay designed to identify all eight of the hepatitis B virus genotypes[15] by detecting type-specific sequences in the HBV polymerase gene domain (B to C). Table 1 shows the genotyping results obtained.

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**Table 1.** Hepatitis B virus genotype distribution in 65 infected Saudi patients.

| Genotype | Number | % |
|---|---|---|
| B | 3 | 4.6 |
| C | 1 | 1.5 |
| D | 61 | 93.9 |

Several studies have evaluated the molecular epidemiology and the relevance of HBV genotypes to clinical outcome. In the present study, it was found that HBV genotype D is the predominant genotype in Saudi Arabia. Other genotypes are present at much lower frequencies.

The results present here support those of Abdo *et al*.,[16] who published a study of the frequency of HBV genotypes in Saudi Arabia. They found genotype D to be the most common (81.4%), with a few instances of genotypes A, C and E, but not a single case of genotype B.

Saudi Arabia is home to foreign workers from different parts of the world. The largest communities comprise peoples mainly from Indonesia and the Philippines. The most prevalent HBV genotypes in these countries are genotypes B and C.

Hepatitis B virus genotypes have a geographical distribution.[7] Genotype D is found mainly in Mediterranean countries; in Albania, the Middle East, Turkey and Iran. Genotype B is found mainly in south-east Asia, Taiwan, Japan, Indonesia, China, Hong Kong, Vietnam and Thailand. Genotype C is found mainly in eastern Asia, Taiwan, Japan, Korea, China, Hong Kong, Thailand, Indonesia, Vietnam, the USA and Brazil.

It is now well established that HBV genotypes influence the severity of liver disease and its response to interferon and lamivudine. Genotype C appears to carry a higher risk for chronic disease and a lower response to treatment.[17]

In a prospective study, Thakur *et al*.[18] examined the prevalence and clinical significance of HBV genotypes A and D in Indian patients with chronic HBV infection. They found that genotype D is associated with more severe liver disease and that it may predict the occurrence of hepatocellular carcinoma (HCC) in young Indian patients.

Further studies are needed to investigate the effect of HBV genotypes on acute versus chronic infection, on response to treatment and on the risk of HCC in Saudi patients.

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