Alive but non-infectious

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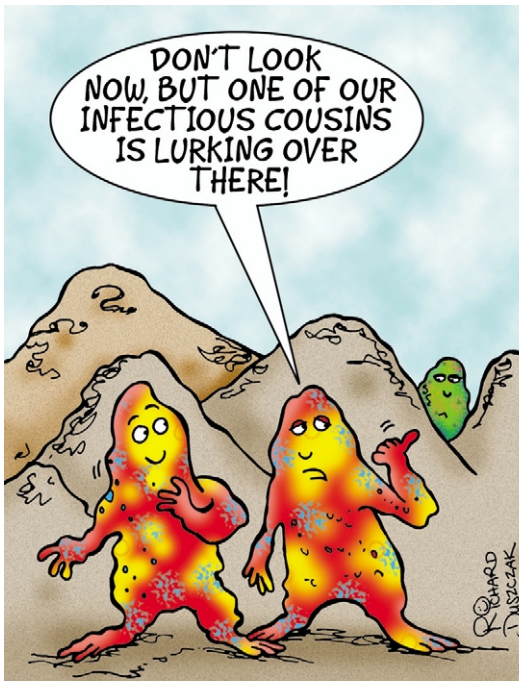
At last, there is concrete evidence that bacteria which exist in a viable but non-culturable (VBNC) state are not necessarily capable of infecting their "normal" hosts. Such organisms—which can be detected by molecular probes and by tests for physiological activity—may not therefore pose threats as hidden reservoirs of infectious disease. That at least is the reassuring conclusion that might be drawn from a report in *Microbiology* (2002; **148:** 2717) by Rebecca Smith and her colleagues at the universities of Leicester and Newcastle upon Tyne, UK.



Caution first. The researchers have not established that the results of their studies with *Salmonella enterica* serovar Typhimurium in mice are universally or even widely generalisable. Nevertheless, the work marks a major advance in the oft heated debate about the VBNC state (whose very existence has been challenged by some authorities). At the same time, the possibility that the findings do apply to other pathogens holds important practical implications for the battle against communicable disease.

The VBNC controversy really began just over 20 years ago when Debra Bashford and co-workers, working in Kent, UK, found *Vibrio cholerae* in streams and drainage ditches, including sites with negligible chance of sewage contamination (*Lancet* 1979; **1:** 436). It was an unsettling discovery, despite the fact that most of the isolates were non-toxigenic.

Shortly afterwards, Rita Colwell and co-workers reported the presence of *V cholerae* in Chesapeake Bay, Maryland, USA. When they incubated the organism in artificial sea water, they found that it remained viable but lost the capacity to form colonies on culture media (*Microbial Ecology* 1982; **8:** 313). The same applied to *Escherichia coli*.

Soon after these disclosures, *Salmonella enteritidis*, *Shigella sonnei*, and *Legionella pneumophila* joined a growing catalogue of organisms claimed to be capable of entering a state in which they failed to show up on nutrient agar yet took up substrates and betrayed their vital status in other ways. In short, the new discoveries appeared to threaten the centrepiece of medical (and environmental) microbiology—the use of laboratory media to recover and enumerate bacteria and to link them with, or absolve them from, pathological and other activities. Hence the spectre of pathogens lurking in the environment and creating dangers that could not be quantified or even confirmed.

Now, after two decades of uncertainty and acrimony, we have a key advance. Rebecca Smith and her colleagues used Carlos Hormaeche's standardised animal infection system (*Immunology* 1979; **37:** 311) to determine the pathogenic potential, if any, of non-culturable cells of *S enterica* serovar Typhimurium. The organisms they used were exponential-phase cells, rendered non-culturable through carbon and nitrogen stress. To achieve this, the researchers maintained the organisms in phosphate-buffered carbon and nitrogen-free medium in the presence of chloramphenicol.

The organisms were inoculated, either orally or intraperitoneally, into BALB/c mice in doses exceeding the LD_{50} values by three and a half and two orders of magnitude, respectively. But these challenges provided no evidence whatever of either infection or colonisation. This was true even when the inocula contained organisms defined as active but non-culturable. These were cells that, in the assay of Kogure et al (*Can J Microbiol* 1979; **25:** 415), failed to elongate in response to low nutrient levels and exposure to a quinolone antimicrobial.

Smith and her colleagues have worked with unusual rigour in searching for evidence of infection, paying careful attention to the statistical limits of their assays and giving the best possible opportunity for their cells to resuscitate and cause infection/disease. It is now up to others to apply similarly exacting criteria and precautions in studying other VBNC organisms.

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