There is much evidence suggesting that such a reservoir of bacterial pathogens multiply in intra- and extra-protozoal environments of animals, soil, water and key foods/catering. There is growing concern about the persistence of pathogens in sewage effluents disposed of on land now that green laws prohibit sea dumping. Soils contaminated with organic matter and sewage waste contain greatly increased numbers of protozoa such as *Acanthamoeba* polyphaga (5), as has a non-O157 strain (11). As protozoa are widely distributed in water, soils and effluents (14), they are likely to constitute an important environmental reservoir for transmission of *E. coli* O157 and other pathogens. Yet, the potential role of protozoa as reservoirs of human/animal pathogens has received little or no attention by the UK regulatory authorities (8).

The danger is magnified by the findings that phenotypic changes (3) brought about by intra-protozoal growth have been associated not only with enhanced environmental survival (11) but also with increased invasion and virulence (7), and greatly increased resistance to biocides (2) and antibiotics (4). Vero-cytotoxin-producing *E. coli* can also survive and replicate in bovine mammary cells (13).

The role of protozoa in the survival of *E. coli* O157 in the natural environment has not been studied, although the organism can survive in cattle manure slurries for at least several weeks (12). There is growing concern about the survival of pathogens in sewage effluents disposed of on land now that green laws prohibit sea dumping. Soils contaminated with organic matter and sewage waste contain greatly increased numbers of protozoa such as *Acanthamoeba* polyphaga (14). Thus, it is highly likely that *E. coli* O157 in soil and slurry will be preyed on by free-living protozoa that will be potential vectors for the spread of this pathogen. Intracellular growth not only enhances survival of pathogens after release from protozoa, but also the intracellular location is a per se mechanism to provide further protection (11). Thus, protozoal trophozoites could provide a protective niche for *E. coli* O157 (and other pathogens) against adverse conditions. This is especially so if the organism is able to survive in amoeba cysts as it has been shown for *Legionella pneumophila*, *Vibrio cholerae* and *Mycobacterium avium* (6, 18). Bacteria trapped within amoeba cysts could be blown through the air and enhance distribution. Grazing cattle will ingest protozoa in silage and grass, and if the ingested protozoa contain pathogens, this may be a significant route of transmission between and within cattle herds. Thus, the recommendation in paragraph 8.40 of the Joint Report (16) for a 3 week period between livestock and recreational usage of land could be a serious underestimate.

There is now much evidence to support the idea that the co-evolution of prokaryotic organisms is equipped some species of bacteria not only for environmental survival but also for invasion of, and survival in, higher-order eukaryotic animal cells/tissues (1, 6, 10, 17, 19). We suggest that this area of medical ecology requires further investigation to enhance our understanding of environmental pathogens such as *E. coli*, *Salmonella* spp. and *Mycobacterium* spp., where protozoa and other lower-order eukaryotes have a potential role in persistence.

Michael R. W. Brown, Anthony W. Smith, John Barker, Thomas J. Humphrey & Bernard Dixon

1Department of Pharmacy and Pharmacology, University of Bath, Bath, UK.
2Pharmaceutical Sciences Research Institute, Aston University, Birmingham, UK.
3Department of Clinical Veterinary Science, University of Bristol, Langford, UK.
4130 Cornwall Road, Ruislip Manor, Middlesex, UK.

Author for correspondence: Michael Brown.
Tel: +44 1225 323340. Fax: +44 1225 826114.
e-mail: prsmb@bath.ac.uk

GUIDELINES

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for the publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of *Microbiology* do not necessarily agree with the views expressed in *Microbiology Comment*.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.
Effect of diet on the shedding of *Escherichia coli* pathogens within protozoa during chlorination.


**In reply**

I welcome the comments of Brown et al. on the possible role of protozoa in the survival of *E. coli* O157, which raise an interesting hypothesis.

Nevertheless, the Task Force was tasked with making recommendations, in the light of currently available information, which were practical and proportionate. On this basis, the recommendation of a 3 week period between livestock withdrawal and the use of land for recreational purposes was a pragmatic approach to a difficult situation, based on experience gleaned during outbreak investigations. None of us live in a sterile environment. The guidance recognized that *E. coli* O157 may still be present in the environment after 3 weeks but at levels reduced by 2–3 log units.

If evidence subsequently emerges that *E. coli* O157 (and other pathogens) survive in greater numbers than currently believed because of protozoa and other environmental organisms, the 3 week withdrawal period may have to be increased. In the absence of such additional information, the 3 week period is a reasonable and practical approach to the enjoyment of our environment, whilst keeping risks within an acceptable level.

I support their call for further investigation into the area of medical ecology and the wider role of the environment in the survival of potential pathogens. Whereas there are currently a number of research projects into the survival and movement of *E. coli* O157 through soil and water, the role of protozoal activity has yet to be defined within an acceptable level.

W. J. Reilly, Chairman, *E. coli* Task Force
Scottish Centre for Infection and Environmental Health, Clifton House, Clifton Place, Glasgow, G3 7LN, UK.
Tel: +44 141 300 1123. Fax: +44 141 300 1170.
e-mail: Bill.Reilly@scieh.csa.scot.nhs.uk