This can be a major problem in clinical microbiology, as significant pathogens can go undetected in non-selective culture media because the clinical specimen had been taken after the initiation of antibiotic therapy. In such cases, it can be difficult to isolate bacterial pathogens because they are non-culturable but may remain viable. In these cases, organisms are not thought to develop any elaborate resting stages, but may actively promote mechanisms of antibiotic resistance, such as point mutations (*gyr*A) and efflux pumps.

Thus, additional studies are required with larger numbers of isolates within these species to examine different aqueous environments, including tap water and fresh (river/lake) water, in order to fully elucidate the survival dynamics of these pathogens in such environments.

In conclusion, this short study demonstrates that *Pseudomonas aeruginosa* is capable of survival in sea water for prolonged periods of up to a year, whereas isolates of *B. multivorans* and *B. cenocepacia* could not be cultured after this period. Use of the bacterial viability assay indicates that, although non-culturable, cells of *B. multivorans* remained viable, as determined by the uptake of viability dyes, possibly indicating the presence of a viable but non-culturable (VNC) state for *B. multivorans*. □

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Enhancement of diaminobenzidine staining of chorioretinal specimens by cobaltous ions

Y. D'SOUZA[*], C. J. P. JONES[†] and R. BONSHEK[*,‡]
[*]Academic Unit, Manchester Royal Eye Hospital; [†]Maternal and Fetal Health Research Centre, University of Manchester, St Mary's Hospital; and [‡]National Specialist Ophthalmic Service, Manchester Royal Infirmary, Manchester, UK.

Chorioretinal specimens normally contain melanin, which is dark brown in colour. Diaminobenzidine (DAB) is a widely used substrate for immunohistochemistry and lectin histochemistry, as it is insoluble in alcohol and other organic

Correspondence to: Richard Bonshek
Academic Unit, Manchester Royal Eye Hospital, Oxford Road,
Manchester M13 9WH, UK
Email: richard.bonshek@manchester.ac.uk

solvents and is stable in storage. However, DAB staining results in a brown reaction product, which is difficult to differentiate from melanin. In the course of a study on the glycosylation of drusen deposits in the eye, in which melanin was a confusing factor, the authors have investigated a range of modifications to DAB staining using cobalt, copper and nickel salts,[1,2] in order to clearly differentiate the DAB final reaction product on drusen from endogenous melanin.

Table 1. Summary of the results of DAB staining with and without metal salts.

	Colour	Ease of differentiation from melanin	Background staining	Stability of end-product
DAB alone	Brown	No	No	Yes
DAB with $CoCl_2$	Purple/blue	Yes	No	Yes
DAB with $CuSO_4$	Grey/blue	Yes	No	No
DAB with $NiCl_2$	Brown/grey	No	No	Yes

Two eyes, both of which had been fixed in formaldehyde and embedded in paraffin wax, were used in this study. One had been enucleated surgically for chronic uveitis and the other for malignant melanoma. Drusen (accumulations of extracellular material) were an associated finding in both eyes on histopathological examination. Lectin histochemistry was carried out as described previously.[3]

To reveal the sites of lectin binding, the sections were pre-incubated for four minutes in 300 mL 0.05 mol/L Tris-buffered saline (TBS, pH 7.6) containing 0.05% (w/v) diaminobenzidine tetrahydrochloride dehydrate (Aldrich Chemical Co., Gillingham, UK) to which 2 mL of 1% (w/v) cobalt chloride, copper sulphate or nickel chloride had been added. Then, 0.015% (v/v) hydrogen peroxide (100 volumes [approx 30%]; BDH, Poole, UK) was added and incubation was allowed to continue for a further four minutes. The sections were rinsed in tap water, counterstained with 0.25% methyl green, dehydrated, cleared and mounted.

The sections were evaluated (Table 1) for intensification by metallic ions, the colour difference between melanin and enhancement of DAB stained by metallic ions, background staining, and the stability of the end-product (assessed on the basis of the stability of the binding pattern over five years after the specimens were originally stained).

The divalent metallic ions Co^{2+}, Cu^{2+} and Ni^{2+} modified the colour of the DAB final reaction product on the drusen to purple/blue, grey/blue and brown/grey, respectively (Fig. 1a–d). The purple/blue colour of $CoCl_2$ proved most easy to distinguish from the brown of melanin granules. There was no increase in background staining and assessment of sections over five years old showed that the product was stable over this period of time. The results presented here confirm previous studies which show that the colour obtained from $CoCl_2$ and DAB is quite stable and does not change during subsequent reactions or counterstaining.[1]

Therefore, the authors recommend the use of cobalt

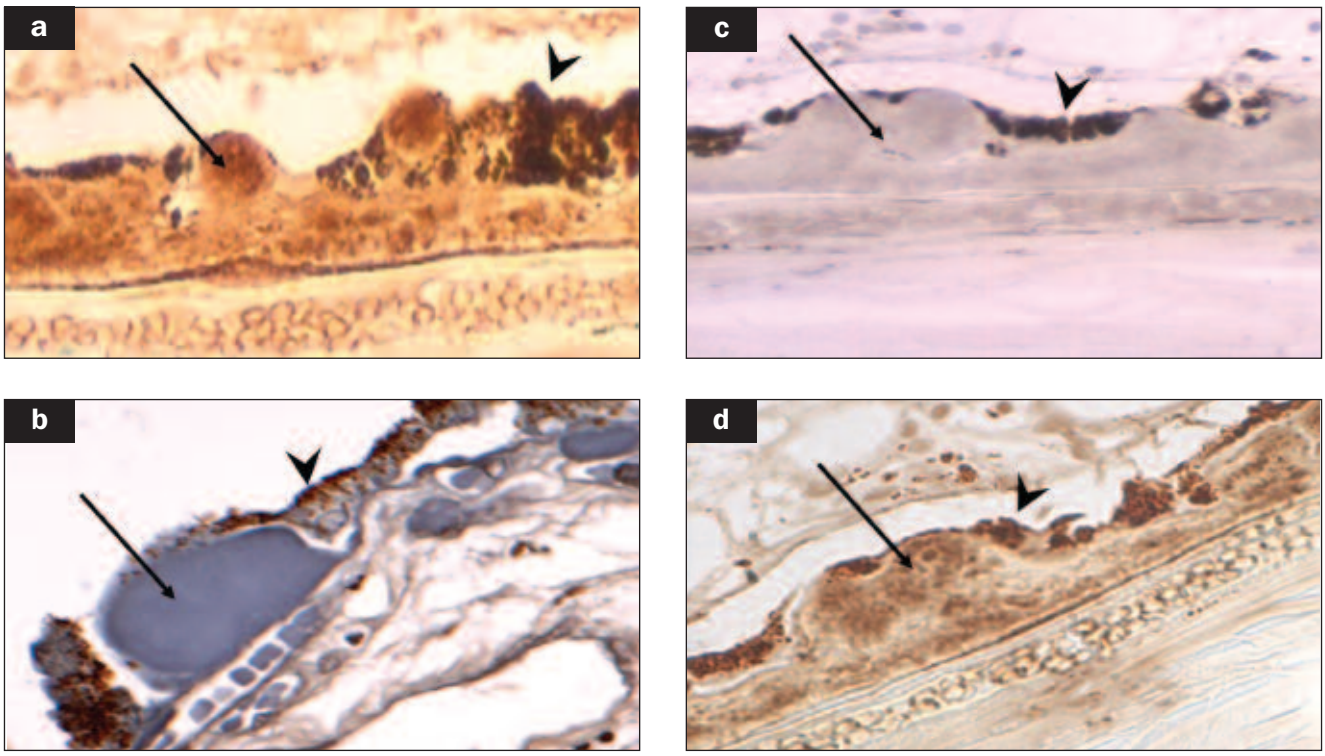

Fig. 1. Enhancement with metallic ions. Con A staining of drusen (arrows) in an eye enucleated following chronic uveitis. Arrow heads indicate retinal pigment epithelium containing melanin (original magnification x600). **a)** DAB stains drusen brown, making it difficult to differentiate from melanin; **b)** enhancement with cobalt chloride stains drusen purple/blue, making it easily differentiated from melanin; **c)** enhancement with copper sulphate stains drusen grey/blue, which contrasts well with melanin; and **d)** enhancement with nickel chloride stains drusen brown/grey, which makes it difficult to differentiate from melanin.

chloride enhancement of DAB for use in histochemical studies of chorioretinal specimens or other tissues where melanin might be an obstructive factor. □

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Molecular detection and identification of *Cryptosporidium* species in lettuce employing nested small-subunit rRNA PCR and direct automated sequencing

M. SHIGEMATSU[*,†], Y. NAGANO[*], B. C. MILLAR[*], F. KENNY[‡], C. J. LOWERY[§], L. XIAO[¥], J. R. RAO[#], V. NICHOLSON[‡], M. WATABE[*], N. HEANEY[*] O. SUNNOTEL[§], K. McCORRY[*], P. J. ROONEY[*], W. SNELLING[§], J. S. G. DOOLEY[§], J. S. ELBORN[§], M. MATSUDA[†] and J. E. MOORE[*]

[*]Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Belfast, Northern Ireland; [†]Laboratory of Molecular Biology, School of Environmental Health Sciences, Azabu University, Sagamihara, Japan; [‡]Sligo Public Health Laboratory, Sligo, Ireland; §School of Biomedical Science, University of Ulster, Coleraine, County Londonderry, Northern Ireland; [¥]Division of Parasitic Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia, USA; [#]Applied Plant Science Research Division, Agri-Food and Biosciences Institute, Newforge Lane, Belfast; and [§]Department of Respiratory Medicine, Queen's University, Level 8, Belfast City Hospital, Lisburn Road, Belfast, Northern Ireland

Human cryptosporidiosis emerged as an important gastrointestinal infection during the 1990s, due to the ingestion of mainly contaminated water and to a lesser extent foodstuffs containing the protozoan parasite *Cryptosporidium parvum*. A limited number of studies have examined the occurrence of the parasite on vegetables, including lettuce.

Fresh lettuces (n=50; iceberg and round) were obtained at retail sale in Northern Ireland during 2005 and were examined for the molecular presence of *Cryptosporidium* spp. by nested polymerase chain reaction (PCR) amplification of the small ribosomal RNA (rRNA) subunit. Just one lettuce was positive for this parasite, which was further confirmed by direct automated sequencing of the small rRNA subunit amplicon as either *C. parvum* or *C. hominis*, with 361/361 (100%) bases identified to either of these species.

Horticultural producers of lettuce should therefore place special emphasis on developing suitable and efficient Hazard Analysis and Critical Control Points (HACCP) strategies for the critical control of cryptosporidial oocysts, depending on the type of unit operation employed and the lettuce being processed.

Correspondence to: Dr. John E. Moore
Email: jemoore@niphl.dnet.co.uk

Fertilisation of horticultural crops, including lettuce, with manure from cattle and sheep containing viable oocysts of *Cryptosporidium* represents a significant public health risk to the contamination of these produce. Recently, Hutchinson *et al.*[1] demonstrated that persistence of *C. parvum* oocysts in farmyard wastes on a grass pasture ranged from eight to 31 days for a 1-log reduction in *C. parvum* levels and demonstrated that the protozoans were significantly more persistent than the bacteria. Oocyst recovery was more efficient from wastes with a lower dry matter content.

These workers concluded that horticultural crops might become contaminated with this parasite. Consequently, with a growing consumer trend away from intensively produced crops towards organic produce, this could increase the likelihood of contamination and the threat to public health.

In addition, horticultural produce may become contaminated by free-living wildlife, including wild birds such as gulls[2], as well as insects including flies[3] and dung beetles,[4] which have been shown previously to be carriers for the parasite, Furthermore, in certain countries, particularly the Far East, the practice of adding untreated human sewage (night soil) to fertilise horticultural crops is of considerable risk for the spread of *Cryptosporidium* to humans who ingest such produce.[5]

The main cause for concern in the horticultural industry is the risk of irrigation with untreated water of crops that do not require any thermal processing prior to consumption. As untreated surface waters are frequently contaminated, use of such supplies should not be used without adoption of control mechanisms to eliminate viable oocyts.

To date, there have been no reports on the contamination of lettuces consumed on the island of Ireland. Additionally, there have been limited reports on the use of molecular techniques, such as 18S rRNA PCR and sequencing, for the detection of this parasite from food produce. Consequently, the aim of this study is to employ molecular techniques to determine the occurrence and identification of the parasite on lettuces on retail sale in Ireland.

Fresh lettuces (n=50; Class I) were purchased from retail supermarkets in Northern Ireland during August to December 2005. These consisted of produce from Northern Ireland (iceberg lettuce [n= 8], round lettuce [n=12]) and from Spain (iceberg lettuce [n=30]). Produce type and country of origin were noted for all produce examined. Lettuces were transported to the laboratory for examination and were examined within four hours of purchase.

Lettuces were sliced with a sterile blade and a portion (25 g) was placed in a stomacher bag, to which 0.1% (w/v) peptone saline (139.5 mL; Oxoid CM0733, Basingstoke, UK) incorporating sodium laurylsulphate (0.22 g/1000 mL; Sigma, St. Louis, MI, USA) was added and pulsified (Microgen Bioproducts, Camberley, Surrey, UK) for 15 sec. After pulsifiying, the suspension was further agitated by stomaching for 120 sec (Stomacher 400 Circulator, Seward, Thetford, Norfolk, UK) on a high-speed setting in filter-lined stomacher bags (Seward).

The filtrate was decanted into sterile centrifuge tubes (50 mL, Falcon, Becton Dickinson, Oxford, UK) and centrifuged at 4000 x*g* for 10 min at room temperature (Labofuge 400,