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The detection of *Cryptosporidium parvum* and *Escherichia coli* O157 in UK bivalve shellfish

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Abstract

Optimised immunomagnetic separation methods to detect *Cryptosporidium parvum* and *Escherichia coli* O157 in UK shellfish are described. Whole tissue homogenates gave the best recoveries for *C. parvum* oocysts compared with gill or haemolymph extracts. The sensitivity of recovery from spiked samples was comparable to that achieved when processing water and varied from 12–34% in mussels, 48–69.5% in oysters and 30–65% in scallops. Maximum recovery of *E. coli* O157 was achieved by enriching in buffered peptone water supplemented with vancomycin at 42 °C. Increasing enrichment temperatures from 37 to 42 °C gave a significant increase in target number recovery. Implementation of these methods into monitoring programmes and end-product testing will enable shellfish producers to better assess product safety.

Keywords: *C. parvum*; *E. coli* O157; Shellfish; Immunomagnetic separation

1. Introduction

Shellfish are a major food source worldwide; in 1997, the global catch of bivalve shellfish exceeded 7 million tonnes (Potasman et al., 2002). In the UK, 15,000 tonnes of mussels (*Mytilus edulis*) and 20,000 tonnes of scallops (*Pecten maximus*) were harvested from the wild in 2001 and during the same period a further 14,000 tonnes of mussels were farmed along with 1500 tonnes of Pacific oysters (*Crassostrea gigas*).

Bivalve molluscs are filter feeders and have the ability to concentrate and retain micro-organisms, some of which may be pathogenic to man. This is of particular concern if the shellfish is either eaten raw (as is often the case with oysters) or undercooked at

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the time of consumption. The current hygienic status and presence of specific micro-organisms in UK shellfish is limited to testing for *Escherichia coli* (indicator of faecal pathogens) and *Salmonella*, those being included in the Shellfish Classification Monitoring Programme. However, the presence of other pathogens is seldom investigated, which in part is due to the limited sensitivity and specificity of conventional detection methods.

Two pathogens whose incidence in human disease has increased significantly over the last decade are *Cryptosporidium parvum* (Guerrant, 1997) and *E. coli* O157 (Griffin and Tauxe, 1991). *C. parvum* is a protozoan parasite capable of causing disease in both neonatal ruminants and humans (Messner et al., 2001), and, like *E. coli* O157, is found in the gut during infection. Both are faecally transmitted, giving a common route of human food poisoning. *Cryptosporidium* is regarded as a waterborne pathogen because it is most often associated with surface and drinking waters and has been implicated in a number of outbreaks associated with contaminated drinking water, the largest being in Milwaukee where >400,000 cases of illness were reported and >100 deaths amongst HIV-infected individuals (MacKenzie et al., 1994). *E. coli* O157 is less common than *Cryptosporidium*, but the sequelae can be more severe—an outbreak in Central Scotland, arising from the consumption of contaminated meat, resulted in more than 500 falling ill, of whom 17 died (Cowden et al., 2001). The numbers of these pathogens shed by ruminants is sometimes underestimated. Strachan et al. (2001) reported concentrations of *E. coli* O157 in sheep faeces in excess of 10^6/g, which were linked to a human outbreak, while Omisakin et al. (2003) found *E. coli* O157 in cattle faeces at levels >10^5/g. Kemp et al. (1995) showed *C. parvum* can be shed at even higher concentrations where values reached 10^8 oocysts/g faeces in young calves. The potential exists therefore for both organisms to contaminate inshore shellfish by run off from agricultural practices or human waste disposal systems, although this latter problem may now be lessened with the implementation of EU legislation preventing direct disposal to sea. There are approximately 250 water treatment plants in England and Wales, and a further 50 in Scotland, considered 'at risk' from cryptosporidial challenge, indicating *C. parvum* oocysts are regularly detected in the source water feeding these plants.

Microbiological methods for the isolation and detection of *C. parvum* and *E. coli* O157 have improved recently with the introduction of immunomagnetic separation (IMS), a method of increasing popularity because it is easy to perform, relatively cost-effective and confers the required sensitivity and specificity. The approved method of testing water for *C. parvum* includes IMS and the same technique is frequently used for foods that require screening for *E. coli* O157. This study optimises IMS procedures specifically for the isolation of *C. parvum* and *E. coli* O157 from shellfish.

2. Materials and methods

Shellfish naturally contaminated with *C. parvum* and *E. coli* O157 were unavailable to test and therefore separate spiked samples were prepared by seeding seawater plastic tanks (Allibert type 21626, volume 650 l, with integral waterproof pump, flow valve, each stacked with six plastic mesh containers to hold the shellfish) containing mussels, oysters and scallops (from the retail market) separately with each pathogen. Tanks were filled with either filtered natural sea water (for *E. coli* O157) or Instant Ocean (Aquarium Systems, Sarrebourg, France) (for *C. parvum*).

2.1. Production of C. parvum oocysts

Propagation of *C. parvum* oocysts is confined to animal passage, there being no effective tissue culture systems for production of this obligate parasite. Male lambs (age 4–5 days) were each orally infected with 10^6 *C. parvum* oocysts, and their total faecal output collected over a period of 14–21 days using a harness and bag collection system. The *Cryptosporidium* isolate used was the Moredun cervine isolate, in laboratory use since 1986.

Oocyst numbers in the faeces were quantified by dilution counts, performed by diluting faeces 1:5 (w/v) with water, emulsifying and further diluting 1:10 with an aqueous solution of 0.2% malachite green/1% sodium dodecyl sulphate, then performing haemocytometer counts on the 1:50 diluted faeces. The malachite green stains the faecal debris but not the oocysts to aid oocyst