RISK PROFILE:
CRYPTOSPORIDIUM SPP.
IN
SHELLFISH

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by

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RISK PROFILE: *CRYPTOSPORIDIUM* SPP. IN SHELLFISH

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SUMMARY

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles include elements of a qualitative risk assessment, as well as providing information relevant to risk management. Risk profiling may result in a range of activities e.g. immediate risk management action, a decision to conduct a quantitative risk assessment, or a programme to gather more data. Risk Profiles also provide information for ranking of food safety issues. This risk assessment is focused on the food/hazard combination of *Cryptosporidium* spp. in shellfish.

There are two pathogenic species of the *Cryptosporidium* parasite that infect humans; *C. parvum* and *C. hominis*. *C. parvum* can also infect bovines. When excreted by the host, the environmental form of *Cryptosporidium* is the oocyst, which contains four sporozoites, and is a very resilient organism that can withstand many environmental stresses including chlorination.

Shellfish are defined for this Risk Profile as bivalve filter-feeding shellfish, such as oysters, mussels, clams, cockles, pipis and scallops. Where relevant, this Risk Profile discusses shellfish in terms of two distinct categories; commercially farmed shellfish and feral shellfish that are accessible to recreational collectors.

Bivalve filter feeding molluscs have been frequently found contaminated with oocysts overseas. But with low sensitivities of detection methods, the numbers reported may be an underestimation. It should be noted that there is a potential for false-positives to be reported such as the mistaken identification of other similar sized organisms. There are also issues surrounding the viability of oocysts, for example empty oocysts may be enumerated by some detection methods but would be non-viable.

Although in many cases shellfish are consumed cooked, there are occasions where shellfish such as oysters are consumed raw or lightly cooked. Some cooking methods may not eliminate risk; a study has shown that steaming of mussels was insufficient to destroy all oocysts present.

A quantitative risk assessment on *Cryptosporidium* in oysters from Chesapeake Bay, USA, published in 2007, concluded that the actual number of viable oocysts consumed is significantly less than would be estimated by previous exposure assessments, may often be too low to cause cryptosporidiosis in healthy individuals.

The lack of reported human cryptosporidiosis associated with shellfish must be a key consideration when interpreting the literature on the subject of *Cryptosporidium* oocysts in shellfish. Despite reports of shellfish contamination, the ineffectiveness of depuration, survival of the oocyst in seawater and many shellfish being consumed raw or lightly cooked, the expected disease burden in the human population is either not being detected or not occurring in the first instance. Feral shellfish gathered from areas impacted by human sewage or livestock runoff may be contaminated with oocysts but the available data is insufficient to quantify this risk. Commercially grown shellfish are gathered from waters where sanitary surveys are usually conducted to detect risks from human and livestock sewage runoff.

The data overseas on oocyst prevalence must be treated with caution because of the inherent difficulties with oocyst detection methodologies and differences in samples e.g. homogenates, gills, gastro-intestinal tract, haemolymph etc. In terms of oocyst contamination in sewage influents and effluents, the concentration of oocysts in New Zealand appears to be similar to the overseas data. It should be noted that seawater salinity values and temperatures differ globally. For example, much of the data is published centred on the Chesapeake Bay on the east coast of the USA where the salinity baseline value is between 9 to 15 ppt and temperatures range between 12 – 16°C. New Zealand salinity values are between 22 – 33 ppt and temperatures between 10-26°C, it is unknown what effect these different environmental conditions may have on *Cryptosporidium* uptake in New Zealand shellfish.

Datagaps at present would make a quantitative risk assessment difficult to construct.

Data gaps

- There is very little evidence to support the use of faecal coliforms to indicate the presence of *Cryptosporidium* spp. This may be an area for further research;
- Immunity status of the population;
- Prevalence and quantitative data on *C. parvum* or *C. hominis* oocysts in shellfish, particularly in feral shellfish areas impacted by sewage and agricultural runoff;
- Prevalence and quantitative data on *C. parvum* or *C. hominis* oocysts in New Zealand marine waters, particularly in feral shellfish areas impacted by sewage and agricultural runoff;
- *Cryptosporidium* oocyst carriage rates in waterfowl and sea-gulls in New Zealand; and
- Effect of the different salinities and temperatures in New Zealand seawaters on the uptake of *Cryptosporidium* oocysts and retention by bivalve filtering feeding shellfish. In addition, the viability and infectivity of oocysts in the New Zealand marine environment.

1 INTRODUCTION

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. The place of a risk profile in the risk management process is described in "Food Administration in New Zealand: A Risk Management Framework for Food Safety" (Ministry of Health/Ministry of Agriculture and Forestry, 2000). Figure 1 outlines the risk management process.

Figure 1: Risk Management Framework

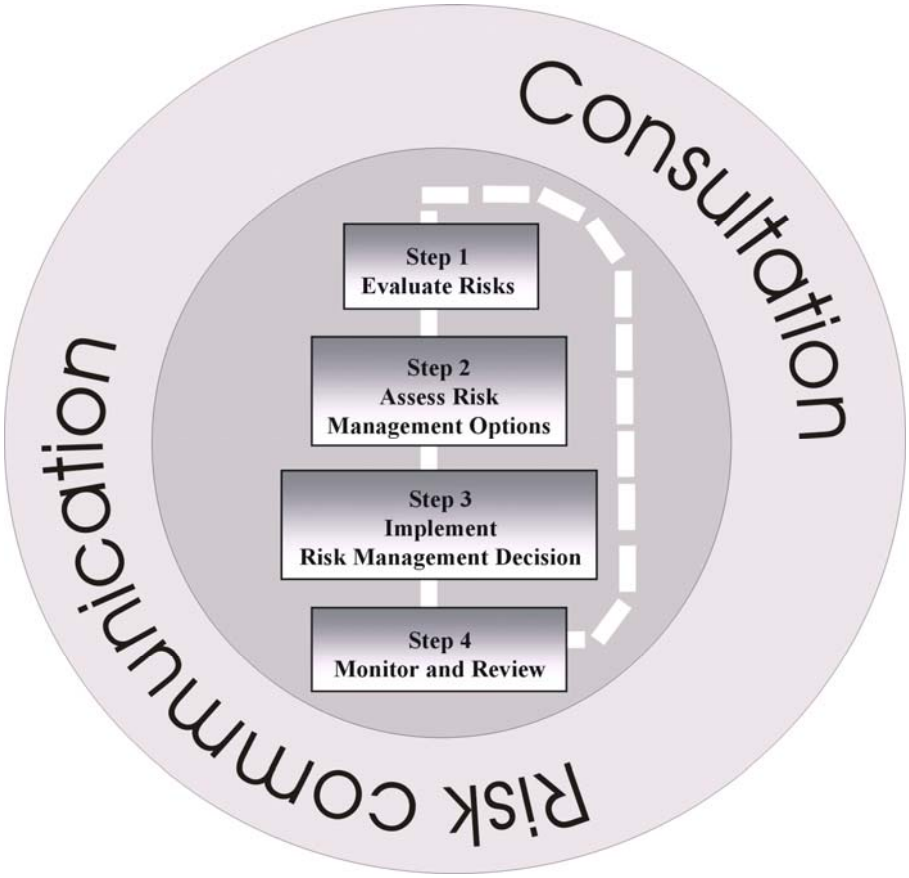


Figure reproduced from "Food Administration in New Zealand. A risk management framework for food safety" (Ministry of Health/Ministry of Agriculture and Forestry, 2000).

In more detail, the four step process is:

1. Risk evaluation

- identification of the food safety issue
- **establishment of a risk profile**
- ranking of the food safety issue for risk management
- establishment of risk assessment policy
- commissioning of a risk assessment
- consideration of the results of risk assessment

2. Risk management option assessment

- identification of available risk management options
- selection of preferred risk management option
- final risk management decision

3. Implementation of the risk management decision

4. Monitoring and review.

The Risk Profile informs the overall process, and provides an input into ranking the food safety issue for risk management. Risk Profiles include elements of a qualitative risk assessment. However, in most cases a full exposure estimate will not be possible, due to data gaps, particularly regarding the level of hazard in individual foods. Consequently the risk characterisation part of a risk assessment will usually rely on surveillance data. The Risk Profiles also provide information relevant to risk management. Based on a Risk Profile, decisions are made regarding whether to conduct a quantitative risk assessment, or take action, in the form of gathering more data, or immediate risk management activity.

This Risk Profile concerns the parasites *Cryptosporidium parvum* and *Cryptosporidium hominis* in shellfish. Bivalve shellfish are filter feeders that can process large volumes of seawater, from which they extract and concentrate particulate material, including parasites. The origin of the parasites in water is from human and animal sewage.

The sections in this Risk Profile are organised as much as possible as they would be for a conventional qualitative risk assessment, as defined by Codex (1999).

Hazard identification, including:

- A description of the organism
- A description of the food group

Hazard characterisation, including:

- A description of the adverse health effects caused by the organism.
- Dose-response information for the organism in humans, where available.

Exposure assessment, including:

- Data on the consumption of the food group by New Zealanders.
- Data on the occurrence of the hazard in the New Zealand food supply.
- Qualitative estimate of exposure to the organism (if possible).
- Overseas data relevant to dietary exposure to the organism.

Risk characterisation:

- Information on the number of cases of adverse health effects resulting from exposure to the organism with particular reference to the food (based on surveillance data).
- Qualitative estimate of risk, including categorisation of the level of risk associated with the organism in the food.

Risk management information:

- A description of the food industry sector, and relevant food safety controls.
- Information about risk management options.

Conclusions and recommendations for further action

2 HAZARD IDENTIFICATION: THE ORGANISM

The information contained in this Risk Profile is current to the date of publication. Please be aware that new information on the subject may have arisen since the document was finalised.

The following information (unless otherwise stated) is adapted from a data sheet http://www.nzfsa.govt.nz/science/data-sheets/cryptosporidium-parvum.pdf prepared by ESR under a contract for the Ministry of Health (and now kept on the NZFSA website). The data sheet is intended for use by Regional Public Health Units.

2.1 *Cryptosporidium* Species

2.1.1 The organism

All species of the genus *Cryptosporidium* are obligate intracellular parasites. They infect fish, amphibians, reptiles, birds and mammals. There are sixteen recognised species at present, ten have been isolated from immunocompromised humans. They are; *C. baileyi, C. canis, C. felis, C. hominis, C. meleagridis, C. muris, C. parvum, C. andersoni*, *C. suis* and *C. cervine*. Only *C. parvum* and *C. hominis* infect humans and only *C. hominis* uses humans as the primary host, although isolated reports indicate that *C. hominis* has infected the dugong, a marine mammal (Morgan *et al.*, 2000) and a sheep (Ryan *et al.*, 2005). *C. parvum* was once thought to be a single species infecting 155 mammalian hosts (based on oocyst morphology). Now *C. parvum* has been subdivided into two species; *C. hominis* and *C. parvum*, following observations of genetic distinction (Awad-El-Kariem, 1999). The renaming occurred in 2002 (Morgan-Ryan *et al.*, 2002). *C. hominis* oocysts transmit from human to human (anthroponotically) while *C. parvum* transmits animal to animal or animal to human (zoonotically) (Awad-El Kariem, 1999).

It should be noted that the renaming of *C. parvum* creates some difficulties when reviewing the scientific literature before 2002, because the genotype for *C. parvum* is not always stated. This means that *C. parvum* may be stated as the species, when, in fact, it was presumably *C. hominis.* For the purposes of this risk profile, *C. parvum* and *C. hominis* will be used where stated.

C. parvum and *C. hominis* are the most widespread and frequently reported zoonotic species of *Cryptosporidium* in humans, and are the subject of this Risk Profile.

In 1907, Ernest Tyzzer first described the life stages of the protozoan and the oocysts in the faeces of his laboratory mice. In 1910 he proposed the name *Cryptosporidium muris* after the association with his laboratory mice and in 1912 named another new species *C. parvum* with stages only developing in the mouse small intestine, the oocysts were smaller than *C. muris.* Upton and Current (1985) have demonstrated using morphologic and biologic evidence that *C. muris* is a species distinct from *C. parvum*. It took until 1976 for *C. parvum* to be reported as a human pathogen when two descriptions of cryptosporidiosis in immune compromised humans were published (Nime *et al*., 1976; Meisel *et al.,* 1976). In 1982, 21 AIDS cases in six cities (Boston, Los Angeles, Newark, New York, Philadelphia and San Francisco) were reported to have cryptosporidiosis (Goldfarb *et al*., 1982) and scientific interest in the parasite began.

The recognised strains of *C. parvum* include Iowa, Moredun, Texas A&M University (TAMU), Ungar *Cryptosporidium parvum* (UCP), Maine and Glasgow. The names referring to the laboratories from where they were first isolated and can be purchased. There are different infectious dose values for the different strains.

2.1.1.1 Lifecycle

The oocyst is a spore-like body of four crescent-shaped sporozoites surrounded by a tough protective wall. Once an oocyst is ingested by a suitable host, the four motile sporozoites are released from the cyst, invade and parasitise the gastrointestinal tract. The time from ingestion of infective oocysts to excretion (life cycle completion) can be as short as 3 to 5 days but can take up to 2 weeks (Fayer, 2004). Most oocysts have already sporulated and become infective by the time they are excreted by the infected host (O'Donoghue, 1995).

Around 80% of the oocysts remain in the host's gut and autoinfect the host. These oocysts have thin walls. Approximately 20% develop with thick, bi-layered walls and are environmentally resilient. They are released from the gut wall into the faeces to infect new hosts (O'Donoghue, 1995). As long as the bi-layered wall remains intact, oocysts can survive months in cool, moist conditions (Laberge *et al.,* 1996).

2.1.2 Growth and survival

An important distinction to make is that viability and infectivity are two different states. Viability concerns whether the organism is still alive or not whereas infectivity measures the organism's ability to infect a host. Where possible these distinctions will be made in the text.

In terms of growth, as a protozoan parasite, the organism can not grow outside of a mammalian host therefore growth is not an issue in shellfish or while in the environment although it will be a factor when infecting humans.

Temperature: Fayer (1994) demonstrated that oocysts could survive and remain infective when heated in water up to a temperature of 67.5°C for one minute.

Freezing at any temperature will render a portion of the oocysts non-infectious, but may not completely eliminate infectivity.

Fayer and Nerad (1996) found that oocysts in frozen water could retain both their viability and infectivity, they also found that the oocysts could survive longer at higher freezing temperatures. For example mice inoculated with oocysts frozen at –10°C (for 8, 24 and 168 hours (7 days)), -15°C (for 8 and 24 hours) and –20°C (for 1, 3 and 5 hours) were later found to be infected. This work was later confirmed by Fayer *et al.,* (1998a) where oocysts retained their infectivity when frozen at –10°C for 7 days and at the higher temperature of –5°C, oocysts were still infectious after 8 weeks.

No detectable parasites were found in the mice that received oocysts frozen to –20°C for 24 hours and 168 hours. Other time/temperature combinations that resulted in no infectivity were –15°C for 168 hours and –70°C for 1, 8 and 24 hours. Oocysts frozen for up to 7 days at –10°C, -15°C and –20°C were indistinguishable from unfrozen controls, whereas more cracked cell walls were observed in the –70°C samples.

Water activity: The survival of oocysts in river and drinking water at ambient temperature has been reported at 176 days (25 weeks) with 1 – 11% survival (Robertson *et al.,* 1992).

In de-ionised water at 0, 5, 10, 15 and 20°C, oocysts were still found to be infective after 24 weeks (Fayer *et al.,* 1998a)

A study on the survival of oocysts in river water (Medema *et al.,* 1997) concluded that the ability of the oocyst to survive several months was instrumental in its environmental transmission, although several factors are probably influential such as temperature, presence of predators and exo-enzymes.

An important finding was that in untreated river water, the inactivation rates of *E. coli* and faecal enterococci were approximately ten times faster than the die-off rates of the oocysts. This has implications for the microbiological testing of shellfish growing waters and using faecal coliform testing. The authors concluded that the rapid die-off of *E. coli* and enterococci made them problematic as indicators of oocyst presence in water.

2.1.3 Inactivation, Critical Control Points and hurdles

Note that in microbiological terms "D" refers to a 90% (or decimal or 1 log cycle) reduction in the number of organisms.

Reviews of the effect of various physical and environmental stresses on oocysts have been published (Robertson *et al.,* 1992; Enemark *et al.,* 2003).

Currently physical removal by filtration is one option to prevent oocyst contamination in drinking water supplies (Ministry of Health, 2005). The oocyst size is 4-5μm in diameter so the use of 1μm pore size filtration systems has generally been found effective, but such systems are only useful in low volume water through-puts and complete removal can not be guaranteed. Wound fibre filters are nominal pore size whereas laser drilled filters are of absolute dimensions.

Inactivation methods have been extensively reviewed (Millar *et al*., 2002; Erickson and Ortega YR, 2006); these include pressure, dessication, extreme temperatures, prolonged storage, sedimentation, UV light, filtration, high energy radiation, ultrasound, electric field photo-oxidation, disinfectants, ozone and monochloramine and gaseous disinfection.

Temperature: In water and milk, the pasteurisation process (71.7°C for 15, 10 and 5 seconds was sufficient to inactivate >99.99% of oocysts, i.e. no mice pups inoculated with oocysts treated to these temperatures became infected (Harp *et al.,* 1996). Fayer (1994) has also carried out heat trials and found that the infectivity of oocysts was lost when heated in water to 72.4°C for 1 minute or 64.2°C for 2 minutes (in a 5 minute heating cycle).

Steam cooking of mussels was insufficient to destroy oocysts completely (Gomez-Couso *et al.,* 2006a). The internal starting temperature of the mussels was approximately 20°C rising to approximately 60-65°C after 5 minutes.

Rapid freezing is more effective at inactivating oocysts compared to a slower rate. Snap freezing resulted in 100% inactivation, while slow freezing at –22°C for 21 hours resulted in 67% inactivation. After 152 hours (6.3 days), 90% were dead. Even after one month, a small proportion of slowly frozen oocysts were still alive (98.2% dead) (Robertson *et al*., 1992).

pH: A pH of 4.0 results in some loss of oocyst viability. Alkaline conditions provided by pH conditions alone have minimal effects although work with ammonia has proved promising in reducing viability of oocysts (Jenkins *et al.,* 1998).

Water activity: Oocysts are sensitive to drying; only 3% of oocysts were still viable after 2 hours of ambient air-drying, after 4 hours, 100% were non-viable (Robertson *et al*., 1992).

The unit of ocean salinity used throughout this Risk Profile is the physical quantity "ppt". This is a measurement of the kilogrammes of salt per kilogramme of water in parts per thousand.

There are mixed results regarding the effect of salinity on the degradation of the oocysts. More details of these studies are given in Table 3.

Oocysts lost infectivity when stored at water activity (a_w) of 0.85 for 24 hours at 28°C, or 1 week at 7°C. At a water activity of 0.95, infectivity was lost after 1 and 2 weeks at 28°C or 7°C respectively.

Radiation: The oocysts are sensitive to ultra-violet rays due to optical and thermal processes, with strong synergistic effects noted at temperatures exceeding 45°C. It is suggested that the oocyst wall permeability is increased by warmer water temperatures, facilitating the transport of hydrogen peroxide and superoxide ions into the oocyst, thereby achieving a greater biocidal effect (Méndez-Hermida *et al*., 2005).

Study of the effect of ultra-violet radiation on five strains of *C. parvum* (Iowa, Moredun, Texas A&M University, Maine and Glasgow) has shown that all five were highly susceptible, achieving a 4-$\log_{10}$ inactivation even at low doses (at 10mJ/cm^2) (Clancy *et al.,* 2004).

UV exposure is divided into three components; UV-A (315 – 400 nm), UV-B (280 – 315) and UV-C (less than 280 nm). The wavelength emitted by treatment equipment is 254 nanometers or UV-C radiation. In nature, most UV-C is shielded from Earth by atmospheric ozone but the shorter wavelength UV-B does reach ground level mainly due to the thinning atmospheric ozone layer. In terms of New Zealand's environmental UV range, because of various factors such as lower ozone, closer Sun-Earth separation and atmospheric clarity, New Zealand experiences more UV radiation than the northern hemisphere during the summer months. Batch solar disinfection (SODIS) to inactivate oocysts in drinking water has been used successfully in Southern Spain and has been advocated as an appropriate technology for point of use water disinfection (Méndez-Hermida *et al*., 2005; McGuigan *et al*., 2006; Mendez-Hermida *et al.,* 2007). No similar research using environmental UV radiation has been carried out in New Zealand.

Ultra-violet treatment is specifically mentioned in the Drinking Water Standards for New Zealand (Ministry of Health, 2005).

Other forms of radiation studied include electron beam irradiation and microwave energy on *C. parvum* oocysts in oysters (Collins *et al*., 2005a), a product frequently consumed raw. Oysters were artificially inoculated and after treatments, oyster tissues were fed to mouse pups. Significant reductions in infectivity were observed for in-shell and shucked oysters treated with electron beam irradiation (doses 1.0, 1.5 and 2 kGys) as opposed to the untreated controls. The 2 kGy dose completely inactivated all *C. parvum* infectivity with the additional benefit of not causing any colour changes to the oyster meat. Microwave energy was less successful, at 2100 watts (frequency 915MHz), exposure times were 1, 2 and 3 seconds at 43.2°C, 54.0°C and 62.5°C respectively. A non-significant reduction in infectivity was found and after 2 and 3 seconds of exposure, changes in texture and colour of the oyster meat occurred.

Disinfectants: O'Donoghue (1995) has reviewed disinfection methods. Only five disinfectants out of 35 tested have been found effective with short exposure times. These are listed below, with corresponding concentration percentages and exposure times. Steam heat, and fumigation (with formaldehyde or ammonia) were also found to be effective.

- Ammonia: 5% for 2 hours, 50% for 30 minutes
- Hydrogen peroxide (10 vol): 3% for 30 minutes
- Formalin: 10% for 2 hours
- Exspor (Chlorine dioxide based): "working dilution" for 30 minutes
- Oo-cide (ammonia): 5% for 5 minutes

Castro-Hermida *et al*., (2006) evaluated two commercial disinfectants on viability and infectivity. The products contained formaldehyde and hydrogen peroxide respectively. A decrease in oocyst viability was associated with an increase in exposure time for each of the concentrations used. The intensity of infection (in mice) was significantly lower ($P < 0.05$) than in control litters.

Chlorine dioxide is a more effective disinfectant than free chlorine. Korich *et al.,* (1990) reported that 1.3 ppm chlorine dioxide gave approximately 90% inactivation after 1 hour exposure.

Bacterial spores are more sensitive to chlorine dioxide than *C. parvum* oocysts and are therefore not useful as direct indicators of inactivation for this protozoa (Chauret *et al.,* 2001).
In a study carried out by Castro-Hermida *et al.* (2006), two commercial disinfectants developed originally for food processing and farm industries contained formaldehyde and hydrogen peroxide respectively. The first chemical, "agri'germ 1000" contained 13.16% formaldehyde, 13.37% glutaraldehyde and 3.21% chlorure didecyl dimethyl ammonium. The second chemical "agroxyde II" contained 5% peracetic acid, 20-30% hydrogen peroxide and 5-10% acetic acid. The authors exposed *C. parvum* oocysts to both disinfectants at different concentrations (mixed with distilled water) at room temperature under continuous agitation conditions. Agri'germ 1000 was diluted to 3.03% (vol/vol), 15.2% (vol/vol) and 30.4% (vol/vol) while Agroxyde II was diluted to 1% (vol/vol), 5% (vol/vol) and 10% (vol/vol). Exposure times were 10, 20, 30, 60 or 120 minutes. The oocysts were then tested for viability by fluorescence microscopy and infectivity (using mouse bioassay). Results showed that oocyst viability declined with increasing concentrations and exposure times. After 120

minutes, the decline was approximately between 25 – 43%. In the control assays of distilled water, the percentage of viable oocysts after 120 minutes remained at 97%. *In vitro* effects of both disinfectants were not significantly different after exposure times of 60 and 120 minutes. The results from the mouse bioassay tests showed that with increasing Agri'germ 1000 concentration, the number of infected mice declined, the infection rates ranged from 36% to 21.7% respectively. The figures for Agroxyde II showed a similar decline with increasing concentration (20% to 10.7%).

In the study by Korich *et al*. (1990), ozone was shown to have the greatest biocidal effect on the oocysts when compared to chlorine dioxide, chlorine and monochloramine. Exposure to 1 ppm of ozone at 25°C inactivated at least 90% **(**based on doses of 600, 6000 and 60,000 oocysts**)** when exposed for 3 minutes, between 90% – 99% at 5 minutes and between 99 – 99.9% when exposed for 10 minutes. The rate of decrease was highest for the first minute of exposure and declined over the remaining time. Ozone and chlorine dioxide more effectively inactivated oocysts than chlorine or monochloramine. Exposure to 1.3 ppm of chlorine dioxide yielded 90% inactivation after one hour, while 80 ppm of chlorine and 80 ppm of monochloramine required approximately 90 minutes for 90% inactivation.

Other chemicals that the oocyst shows resistance to are; phenol, ethanol, isopropanol and lysol.

The drinking water standards for New Zealand (Ministry of Health, 2005) specifically give contact time values for ozone disinfection using *Cryptosporidium* as the baseline organism. The contact times depend upon water temperature, the ozone concentrations are in the range of 0.2 – 5.0 mg/l.

Pressure: The effect of high pressure on *C. parvum* oocysts recovered from oysters has been studied (Collins *et al.,* 2005b). The oysters were artificially exposed to 2 x 10^7 oocysts in seawater conditions and then shucked. High hydrostatic pressure (HP) was then used. Oocyst infectivity was assessed by inoculating oyster tissue into neonatal mice. Pressures of 305, 370, 400, 480, 550 Mpa were all effective in reducing infectivity. The maximum decrease in infected mouse pups was achieved at a pressure of 550 Mpa at 180 seconds holding time (93.3%). Processing times from 120 seconds to 360 seconds at this pressure caused a small increase in whiteness in the oyster meat.

2.1.4 Sources

Human: Person-to-person secondary transmission can occur.

Animal: 155 species of mammal have been identified as hosts for *C. parvum* and *C. parvum*-like organisms (Fayer, 2004). The disease in humans is known to increase during lambing and calving in some parts of the world (Goldsmid *et al*., 2003; Learmonth *et al*., 2003). Preweaned ruminants appear especially vulnerable to infection (Fayer, 2004). Nydam *et al.,* (2001) enumerated the total number of oocysts shed by 478 naturally infected calves. Shedding increased from age 4 days, peaking at day 12 and then declining. A calf infected at six days old was shown to shed 3.89×10^{10} oocysts by the time it was 12 days old.

Studies on flies have shown them to be potentially involved in transmission, although no epidemiological evidence has been documented. Mechanical transmission is possible as the

oocysts pass without alteration through the fly gastro-intestinal track without losing infectivity (Graczyk *et al.,* 2004). Exposure of the adult fly to bovine diarrhoea containing oocysts later resulted in oocysts being deposited in fly faeces (Graczyk *et al.,* 1999a).

Food: Oocysts have been detected in raw milk and meat, raw fruits and vegetables. Vehicles identified from incidents of human infection include salad, raw milk, sausage and frozen tripe. Yoghurt made from contaminated milk can harbour the oocyst but there was no survival in ice cream because of the freezing effect. Although the oocysts have been detected in food, direct evidence linking food to cases of cryptosporidiosis is scarce. Some reasons put forward for this paradox are the low numbers of oocysts in food samples associated with outbreaks, lack of an equivalent to the bacterial enrichment culture to enhance oocyst recovery, poor sensitivity tests and under-reporting of diarrheal illness (Laberge *et al*., 1996; Enemark *et al.,* 2003).

Environment: The oocyst is the only stage found outside the host and it is particularly resilient. Under cool, moist conditions, the oocyst can remain infective for many months, especially in water temperatures that are low (below 5°C) but above freezing (Fayer, 2004). Faecally polluted water is a potential reservoir of oocysts, and temperature appears to be an important factor, as temperatures increase above 5°C, survival time decreases. Robertson *et al.* (1992) found that oocysts submerged in semi-solid cow faeces for 176 days declined in viability to reach 66% inactivated (stored outside in bucket – as close as possible to 4°C) while in human faeces after 178 days at 4°C, 78% of oocysts were dead.

Transmission Routes: Infected people and animals pass up to 10 billion *C. parvum* oocysts over the course of the infection (Fayer, 2004). There are three main routes for transmission, from person-to-person, directly from animals and from faecally contaminated water or food. There has been a single report of airborne transmission to a human in a veterinary situation involving a severely infected calf (Højlyng *et al*., 1987).

Marine waters may be contaminated directly from human sewage and/or animal faeces or indirectly (via runoff into rivers). Electrostatic forces between oocysts and soil particles are insufficent for oocysts to attach to soil (Dai and Boll, 2003). Direct ingestion of contaminated seawater or consumption of contaminated raw shellfish can result in oocyst ingestion.

Micro-crustacean and waterborne amoebae have recently been reported as possible transmission routes into shellfish. Mendez-Hermida *et al.* (2006) demonstrated that *Artemia franciscana,* a microcrustacean commonly used in the live diet of shellfish during larviculture, can ingest oocysts. Gomez-Couso *et al.* (2006b) demonstrated that the amoeba *Acanthamoeba* was a predator of oocysts, a maximum of six oocysts per *Acanthamoeba* were detected. The authors concluded that free living amoebae may act as carriers of oocysts and play an important role in transmission to shellfish. Using protozoa and rotifers to predate *Cryptosporidium* has been studied as a possible removal mechanism (Stott *et al*., 2003). All organisms investigated ingested oocysts although variations were observed with predation activity and rates of ingestion related to predator species and prey density. Ciliated protozoa demonstrated the greatest predation activity. However the fate of ingested oocysts within protozoa is unknown as is the effect on the oocysts viability. Dispersal is thought possible by faecal pellets or excreted boluses. This area of research demonstrates beneficial applications

but also the potential for environmental reservoirs and vectors, it is an area identified for further study overseas.

2.2 *C. parvum* and *C. hominis* Isolation and Typing

Two isolation and enumeration techniques have been published for oocysts in shellfish. Homogenisation is used generally as a first step in order to release oocysts from the matrix.

The first method is Immunofluorescent assay (IFA) that enumerates the number of oocysts under microscopic examination. The second method is Polymerase Chain Reaction- (PCR-Restricted Fragment Length Polymorphism (RFLP) (18rRNA gene) and is used for detection as well as genotyping.

Differences in results between the two methods are attributed to method sensitivity and the presence of empty (excysted) oocysts. These may be detected under the microscope but not recognised as empty, while they will not be detected using PCR. Molecular methods have been shown to detect as few as 10 oocysts per 10 ml environmental filtrate sample) and also have the ability to differentiate species and thus identify pathogenic species to man (Lowery *et al*., 2001).

Four methods to determine oocyst viability and infectivity have been identified, these are reviewed by Millar *et al.,* (2002). They are animal infectivity, in vitro excystation, exclusion/inclusion of vital fluorogenic dyes and reverse transcriptase- PCR.

For quantitative risk assessment purposes, it is generally recognised that data should include consideration of the viability and infectivity of oocysts. The mouse is the most common animal model used to assess infectivity of *C. parvum* oocysts, although the infectivity may be different for humans. The use of mice and their shortcomings are discussed under risk assessment in section 6.2.4.

Miller *et al.* (2006) have recently evaluated methods for the detection of oocysts in mussels. Methods used were real-time Taqman PCR, conventional PCR and Direct Immunofluorescent antibody assays (DFA) with and without Immunomagnetic Separation Methods (IMS). The object was to identify the best method for parasite detection in mussel haemolymph, gill washings and digestive glands. They concluded that the most sensitive method for detection of *C. parvum* in oocyst-exposed mussels was IMS concentration with DFA detection, using this method, 80% of individual and 100% of pooled digestive gland samples were found positive. Taqman was comparable to conventional PCR, and allowed for automated testing, high throughputs and semi-quantitative results. One interesting finding was that none of the tank exposed gill wash samples tested positive by any of the methods used, suggesting that more oocysts are retained in digestive glands than in gill washings and haemolymph after mussels have filtered oocysts from their surroundings.

The 50% infectious dose (ID50) for mice ranges from 60 – 1000 oocysts depending on the age of the mice (review of 4 papers – Millar *et al*. (2002)).

The use of dyes is the most commonly used method for assessing viability, although it is recognised that this does over-estimate viability (Campbell *et al.,* 1992; Duffy and Moriarty, 2003).

A recent successful detection of *C. parvum* from shellfish using optimised IMS has been reported (MacRae *et al*., 2005). Whole tissue homogenates allowed better recovery rates than gill or haemolymph extracts. The sensitivities of recovery were 12 – 34% in mussels, 48-69.5% in oysters and 30-65% in scallops. Other authors have reported less satisfactory results using this method (Schets *et al*., 2007).

A review of the common methods used for the detection of *Cryptosporidium* spp. has been compiled (Sunnotel *et al*., 2006).

A cautionary note has been made regarding the use of acid-fast staining (AFS) techniques (Graczyk *et al.,* 1998b) because there is a potential for false-positive *Cryptosporidium* oocyst reporting. This is due to the standard procedure of excising and vortexing oyster gills that may free *Haplosporidium nelsoni* or *H. costale* spores, these spores display similar staining patterns with irregular black granules and have minor size differences.

3 HAZARD IDENTIFICATION: THE FOOD

3.1 Relevant Characteristics of the Food: Filter Feeding Shellfish

New Zealand has a coastal marine environment that can be categorised into three temperature related bioregions; northern, central and southern. Northern coastal waters are warm and salty with sub-tropical currents to the north and west. In the southern regions, the waters are cold, less saline and are influenced by sub-antarctic currents (Thomson, 2000). Shellfish diversity and distribution are influenced by these factors.

Shellfish are typically 70-80% water, 10-20% protein with small amounts of fat and carbohydrate. They can contain high concentrations of metallic elements through bioaccumulation.

Bivalve molluscs feed and respire by inducing a current of water to flow over a series of complex gill structures which capture suspended particles, passing them towards the mouth where they may be ingested or rejected as "pseudo–faeces". The gills have a pore size similar to oocysts (Gómez-Couso *et al*., 2003).

Molluscan shellfish contain haemolymph, a circulatory fluid which includes water, amino acids, sugars, salts, and white cells like those of blood (haemocytes). In molluscs it also circulates oxygen and carbon dioxide. Tests for *Cryptosporidium* oocyst sometimes involve examining the haemolymph.

During a study of Asian clams by Graczyk *et al*., (1998), it was observed that when oocysts were deposited in faeces, the oocysts were always surrounded by the faeces and occurred in clusters. No free oocysts were observed.

Shellfish filtration rates vary widely depending on a large number of factors such as particle size in the water column, condition and size of the bivalve etc. Due partly to the incorrect methods used and partly to different experimental conditions, the results that have been published are difficult to interpret and compare. Riisgård (2001) has reviewed the literature in this field and began by clearly defining and explaining the differences between the filtration rate and the clearance rate. The author has collated a table of filtration rates as a function of size and occasionally shell length under each of five experimental methods used. The rates reported vary from 2.5 to 11.6 l h^{-1} by weight and 0.0012 to 0.027 l h^{-1} by shell length.

3.2 The Food Supply in New Zealand: Shellfish

"Shellfish" are defined for this Risk Profile as bivalve filter-feeding shellfish. This definition includes dredge and pacific oysters, mussels, clams, tuatuas, scallops, cockles and pipis. The definition does not include spat (juvenile shellfish taken for purposes of on-growing). Both commercial and "feral" shellfish are covered. Feral is defined as shellfish harvested by private individuals from areas not considered commercial growing waters.

The following information on shellfish derives from a review of wild foods in New Zealand (Turner *et al.,* 2005) unless otherwise stated.

Mussels

The green-lipped mussel (*Perna canaliculus*) is the most well known species due its commercial popularity (Greenshell™). It is widely distributed in New Zealand, living on rocky shores between low waters to 60 metres and exposed open shores. The blue mussel (*Mytilus* species.) live in the same habitats as green lipped mussels although they prefer colder waters in the South Island. Other common species are the little black mussel (*Xenostrobus pulex*) found in dense beds at the mid tide level on exposed rocky shores, the ribbed mussel (*Aulacomya atra maoriana*) found in South Island rocky shores and the large horse mussel (*Atrina zelandica*) found below low tide to 45m from estuaries to open coast.

Oysters

Pacific oysters (*Crassostrea gigas*) can be found in inter-tidal zones of harbours and estuaries. They are a commercial species. The rock oyster (*Saccostrea glomerata*) inhabits similar zones although it prefers warmer North Island waters. Bluff, dredge and flat oysters are common names for *Ostrea chilensis*, found in inter-tidal zones and depths down to 50 metres around New Zealand. Harvest conditions for oysters in New Zealand have been recorded at 22 – 33 ppt (mostly around 30 ppt) salinity and 10-26°C (McCoubrey, 2000).

Pipis

Paphies australis lives in sheltered harbour and estuarine waters, just beneath sand from mid-tide to 5 metre depths.

Tuatua and toheroa

Tuatua (*Paphies subtriangulata* and *Paphies donacina*) and the larger toheroa (*Paphies ventricosa*) are common bivalves mostly found on open sandy beaches, just under the sand.

Cockles

The common cockle (*Austrovenus stutchburyi*) is the most abundant and widely distributed bivalve shellfish in New Zealand waters. Habitats include harbours, estuaries and sheltered beaches, just beneath the sediments in the inter-tidal zone.

Scallops

The habitat of *Pecten novaezelandiae* is sand or mud of open waters and harbours, living in low tide to depths of 50 metres.

3.2.1 Economic considerations

The Seafood Industry Council was created in 1996 to represent all sectors of the fishing industry. A number of more specialist industry organisations exist, these can be accessed from the Seafood Industry Council website;http://www.seafood.co.nz under the industry organisations link.

With exports at 315,000 tonnes, worth approximately $NZ 1.25 billion in 2007, the seafood sector ranks fifth in the New Zealand exporting sector and represents 2% of the total global

seafood trade. Australia, the European Union, Hong Kong, USA, Japan and China are the largest markets (Seafood Industry Council: www.seafood.co.nz/factfile).

New Zealand's Exclusive Economic Zone (EEZ) is the fourth largest coastal fishing zone in the world at 2.2 million square miles (Seafood Industry Council, http://www.seafood.co.nz/business/ accessed 12.04.06). The zone extends 200 nautical miles out from the coastline. Approximately 130 species of fish are commercially caught, of which 43 species are commercially significant. Predominantly these are the deepwater species such as hoki, hake, ling, orange roughy, oreo dories, squid, and silver warehou as well as spiny red rock lobster, paua (abalone), greenshell mussels, and snapper. A fisheries quota management system was implemented in 1986 to control the marine catches in order to sustain yields (Ministry of Fisheries, 1999). Lengthening shelf life by improved storage techniques is an important area of development for New Zealand due to the distance of markets when exporting.

In 2007, NZ Greenshell mussels were the largest component of the shellfish export market at 35.5 tonnes worth $NZ 174 million. Mussel exports to the USA account for approximately 30% of total mussel exports (SeaFIC, websites accessed 27.02.2007: http://www.seafood.co.nz/business/ecotrade/exportsum.asp and http://www.seafood.co.nz/doclibrary/exportstats/reporta1.pdf). Other relevant shellfish exports in 2007 (fresh or frozen) were: oysters 2.2 tonnes ($16.5 million); scallops 83 tonnes ($1.5 million) and "other" shellfish (excluding squid) 1.2 tonnes ($8.2 million).

Little information is available on the amount of shellfish which enter the domestic market because the seafood sector is predominantly aimed at the export market. The domestic market for fish is about $NZ120 million p.a., figures for shellfish could not be located.

The Food and Agriculture Organization of the United Nations (FAO) consolidates information on production and consumption of commodities through their FAOSTS databases (http://faostat.fao.org/). Table 1 summarises information from this source for the production of shellfish species in New Zealand for the most recent available year (2004).

Table 1: Production of bivalve shellfish in New Zealand - 2004

Shellfish type	Total Production (tonnes)*	Production from	
		Capture (tonnes)	Aquaculture (tonnes)
Clams, cockles, arkshells	1,787	1,787	
Mussels	86,353	1,353	85,000
Oysters	2,488	465	2,023
Scallops	1,981	1,981	

* Production figures are 'greenweight', i.e. shell plus flesh
From http://faostat.fao.org

New Zealand shellfish production is becoming increasingly dominated by aquaculture, as opposed to capture of 'natural' resources. Mussels are farmed mainly in three regions; Coromandel, Marlborough/Tasman Bay and Stewart Island (http://nzmic.co.nz). Marlborough/Tasman Bay is the largest production area (approximately 78% of total harvest in 2005), followed by Coromandel (18% in 2005) and Stewart Island (3% in 2005).

Information from the Food Balance Sheet module of the FAOSTAT database indicates that approximately 92% of New Zealand's shellfish production is exported

3.2.2 Imported food

New Zealand imports significant quantities of shellfish and shellfish meat. Data obtained from Statistics New Zealand show that in the year to September 2005, New Zealand imported approximately 170 tonnes of oysters (95% from Korea), 560 tonnes of scallops (97% from China) and small quantities of mussels and clams. From the import statistics it is uncertain what proportion of this material was cooked, although the majority of it was frozen.

It is difficult to determine what proportion of shellfish consumption by New Zealanders these amounts represent, as there are no details on whether the weights include shells or just flesh.

Molluscs (cooked and raw) including clams, cockles, mussels, oysters and scallops are prescribed foods under the New Zealand (Mandatory) Food Standard 1997. This means that imports of these products can be required to demonstrate compliance with conditions related to the presence of heavy metals, marine biotoxins and pathogenic bacteria. Details of specific import requirements are given in on the Auckland District Health Board Central Clearing House website (http://www.akhealth.co.nz/akphp/Services/ImportedFood/imported_foods.htm).

3.3 *C. parvum* and *C. hominis* in Shellfish

3.3.1 Contamination

Shellfish are filter feeders, filtering water in order to obtain particles of food. Experiments have shown filter-feeders concentrate oocysts. In an experiment conducted by (Fayer *et al.*, 1998b) 24 oysters were used as follows; six were controls, and six oysters each were exposed to 10, 100 or 1000 oocysts in each of three aquaria. The gill washings from the oysters were examined 72 hours later. Three (50%), six (100%) and six (100%) respectively contained oocysts, as shown in Table 2. This experiment also demonstrated that when a single 200-µl aliquot of haemolymph or gill washing is examined and low numbers of oocysts are present, the test method may underestimate the true value. In this case, if duplicate tests had not been performed, oyster numbers 2 and 3 (10 oocyst seeding) and numbers 5 and 6 (100 oocyst seeding) would have returned negative results. In the same paper but a different experiment, it was found that the number of oocysts in the gill washings did not always correspond with the number found in the haemocytes from the same oyster.

Table 2: Number of oocysts detected (IFA) in each of two 200 µl aliquots of gill washings from 24 oysters – various oocyst exposure

	Number of oocysts detected			
Oyster no.	**Controls**	**Seeded with 10**	**Seeded with 100**	**Seeded with 1000**
1	0,0	1,1	8,5	46,26
2	0,0	2,0	6,3	28,22
3	0,0	2,0	3,1	17,15
4	0,0	0,0	2,1	11,7
5	0,0	0,0	1,0	8,6
6	0,0	0,0	1,0	2,2

In a three year study of Chesapeake Bay oysters, Fayer *et al*. (2002) found that oocyst contamination was greatest after significant rainfall events (agricultural runoff).

Cryptosporidium spp. have been transmitted by recreational swimming (Smith, 1992). In Honolulu, Hawaii, recreational bathing waters near to seawater sewage outlets were found contaminated compared to a control bathing area not influenced by sewage. A major problem encountered in this study was the presence of algae that interfered with recognition of oocysts (Johnson *et al.* 1995).

The low rate of sedimentation (0.5 µm/s) exhibited by the freely-suspended oocysts means that they can remain in suspension for long periods, possibly due to their relatively small size. (Rose 1997 cited in Gómez-Couso *et al.*, 2003). This theory is used to explain the results in the Gómez-Couso *et al.* (2003) survey in that higher levels of contamination were found on shellfish cultured on floating rafts (mussels and oysters were 38.2% positive) as opposed to species harvested in the sediments (clams and cockles 26.9% positive). However, these differences could be due to a number of factors such as the difference in uptake between different species of filter feeders.

The attachment of oocysts to particles is dependent on the matrix and surface charge of the material they are attaching to. Studies have shown that while the electrostatic forces between oocysts and soil particles are insufficient for oocysts to attach to soil (Dai and Boll, 2003), they are sufficient for oocysts to readily attach to biological particles in effluent (up to 75% after 24 hours) (Medema *et al.,* 1998). With the increased particle size, the velocity of sedimentation is increased. Oocysts readily attach to biological particles in effluent (up to 75% after 24 hours) (Medema *et al.,* 1998). With the increased particle size, the velocity of sedimentation is increased.

This provides an alternative explanation for the higher prevalence of contamination in floating raft shellfish; if the water is relatively clean with little biological particulate content, there would be greater retention of oocysts in suspension.

An important factor influencing *C. parvum* (and *C. hominis*) contamination of filter-feeding shellfish is that the shellfish are commonly grown in inshore environments, and their microflora reflects terrestrial influences (ICMSF, 1998). It is these terrestrial influences, particularly the effect of bovine waste runoff that may be having a significant effect on contamination. Fayer (2004) describes the situation in the USA where it is estimated that 1.2

billion tonnes of bovine manure was produced in 1997, nearly all preweaned calves infected and excreting oocysts and so cattle are believed to be a major source for water-borne *Cryptosporidium* oocysts.

3.3.2 Oocyst viability and infectivity

It should be noted in the following information that the infectivity of oocysts are tested predominantly on animal models such as mouse pups and chicks, this does not necessarily reflect infectivity in humans.

3.3.2.1 In shellfish

Conflicting evidence has been reported for the behaviour of oocysts in shellfish.

There have been no reports of oocyst contaminated shellfish associated with a human case of cryptosporidiosis, see Table 24. Nevertheless, *Cryptosporidium* oocysts that are infective to humans (*C. parvum* and *C. hominis*) have been found in shellfish, and with many types of shellfish consumed raw or lightly cooked, one might have expected a notified case of cryptosporidiosis as a result. A quantitative risk assessment for Chesapeake Bay in the US recently published by Graczyk *et al.,* (2007) presents potential explanations for this, and is discussed in more detail in section 6.2.4.

This paradox has been examined by Lee and Lee (2003). They posed the question " why is there an absence of reported raw-oyster-related human infections caused by coccidial parasites, while there are so many reported cases of illness caused by bacterial and viral agents attributable to the consumption of raw oysters?" Using raw oysters as a test, they studied the passage of coccidial parasites for 48 hours through the shellfish and the viability and later infectivity of the parasite. The authors worked with *Eimeria acervulina* (non-pathogenic to humans) as a safety precaution. The surrogate is a close relative to *Cryptosporidium.* The infectivity of the oocysts were tested in chicks.

Over three experiments, artificial seawater (at 20.5°C and 18 ppt salinity) [note New Zealand seawater has higher salinity values] was inoculated with 500,000 oocysts (12 oocysts per ml) and 24 to 33 oysters added to each tank. Three living oysters were removed from the tank after 6, 12, 24, 36, 48, 72 and 96 hours. It does not appear that the uptake of oocysts by the shellfish was determined. Shells were washed for 2 minutes then shucked. Gill and stomach contents from the three oysters were pooled and macerated, the macerate was gavage fed to each of five chicks. A control was also set up. In one of the experiments, to determine faecal accumulation of oocysts, 9 oysters were transferred after 24 hours to a clean tank of seawater (with no oocysts). After a further 48 hours, all oyster faeces were collected from the tank and fed to 5 chicks.

The viability of oocysts inoculated into seawater but without passing through oysters was also tested as a control.

Results showed that the water containing the oysters contained viable cysts for at least 24 hours. Oocysts in the inoculated water that had not passed through oysters remained viable for at least 48 hours. The oysters concentrated the oocysts from seawater within 6 hours of exposure, and in the same time-frame the oocysts retained infectivity (9/10 chicks excreted

oocysts). Oocyst infectivity decreased with time, after 24 hours, 11/15 chicks were infected while after 48 hours only 2 chicks out of 10 were excreting oocysts. Oysters contained no infective oocysts after 72 and 96 hours. The oyster faeces from the second clean tank all contained viable and infective oocysts with 5/5 chicks infected.

The authors concluded that coccidian parasites occur primarily in the alimentary tract and not elsewhere in the oyster. No replication takes place because *Cryptosporidium* can only grow in a mammalian host. The study concluded that almost complete depuration of the oyster occurred within 48 hours in contradiction to the findings of Gomez-Couso *et al.,* (2003) (see section 3.4, oocysts were still present after 5 days of depuration). However, the eliminated oocysts were concentrated in the oyster faeces where they remained infective after 48 hours. The authors suggest that oocysts become part of the sediment in saline water rather than remaining infective in the oyster itself. The authors concluded that it is possible for human coccidial infection to result from the consumption of raw oysters, but the oocysts pass the digestive tract of oysters in one day. Therefore unless contaminated waters (with oyster faeces) are ingested or oysters are harvested and consumed within a day of contamination, they are unlikely to be the cause of coccidial infections in humans.

There are conflicting reports to the Lee and Lee (2003) observations above. In Tamburrini and Pozio (1999), a study was conducted to determine if oocysts were retained in mussel tissue and how long they retained infectivity. Forty mussels (*Mytilus galloprovincialis*) filtered more than 4 x 10^8 oocysts from artificial seawater [salinity values not given] in a 24 hour period and oocysts were detected in gill washings up to 3 days post-inoculum (in haemolymph 7 days post-inoculum). The intestinal tract contained oocysts up to 14 days post-inoculum. Oocysts collected from the intestinal tract were used at 7 days and 14 days to inoculate mice pups, and were found to be infective.

Freire-Santos *et al*. (2002) used an experimentally contaminated saltwater aquarium to contaminate oysters and clams. There was a decrease of 15 – 25% in viability during the first 4 days, but oocysts were still isolated from the shellfish and found to be infective (mouse bioassay) after 30 days.

A longer term histological study tracking *C. parvum* oocysts through clams (*Tapes decussates*) has been recently published (Gómez-Couso *et al.,* 2005). Clam spats were contaminated with oocysts and over a period of 240 hours (10 days), five specimens were removed and killed every 24 hours. Oocysts were identified by the IFA method and were detected in siphons, gills, stomach, digestive diverticula and intestine. An observation that agrees with the Lee and Lee (2003) study was that oocysts in the intestine were free and mixed with other intestinal contents suggesting that release of the oocysts with the faeces was possible. The parasite was never observed to be internalised by the mollusc cells.

The gonads and gills are the largest components of the mussels eaten by humans, the remainder of the edible viscera consists of the stomach, digestive gland, intestine, kidney, heart and the foot structure. In a French study recently published (Li *et al*., 2006), the flesh was the most contaminated part followed by gills and then inner shell washings, with the numbers of oocysts in flesh homogenates higher than in flesh washings. The opposite was true with gill samples suggesting that oocysts gain deeper penetration of flesh than gills. Unfortunately the flesh is not anatomically defined in the text of the paper.

3.3.2.2 In seawater

Table 3 collates the information on viability and infectivity of oocysts in seawater (and one freshwater included for comparison). The results are mixed and difficult to extrapolate to the New Zealand marine environment due to the various test parameters involved. The nearest set of experiments were by Fayer *et al.* (1998) and Nasser *et al.* (2003) using 30 ppt, the results are in contradiction. New Zealand's saline conditions are between 22 and 33ppt (mostly 30 ppt) and temperatures are between 10 to 26°C depending on the latitude.

Table 3: Viability (and infectivity) of oocysts in seawater (and freshwater for comparison)

Type of water	Salinity values	Time period	Temp. of water	Oocyst Viable and infectious? - viability	Reference
Seawater Ayrshire coast, Scotland	Not given	5 weeks	4°C (in the dark)	Viable*	Robinson *et al.,* 1992
Artificial seawater	10 ppt 20 ppt 30 ppt	12 weeks	10°C 10°C 10°C	Viable and infectious Viability 4 weeks Viability 2 weeks	Fayer *et al.,* 1998b
Seawater	~ 30 ppt	30 days	30°C 15°C	No decrease in viability observed	Nasser *et al.,* 2003
Freshwater	NA	12 weeks	10°C	Viable and infectious (mice bioassay)	Fayer *et al.,* 1998b

* oocysts from intestinal tract used at 7 and 14 days to inoculate mice pups, oocysts were infective.
Not applicable

3.4 Depuration and Relaying

The difference between depuration and relaying is that depuration refers to land-based purification plants while relaying refers to purification in naturally clean waters. Depuration or relaying is required where there is a potential or actual risk of contamination and aims to reduce the number of pathogenic organisms accumulated by shellfish grown in moderately polluted waters.

Unfortunately depuration conditions such as water temperature, salinity, water volume per oyster, whether water flowed-through or was recirculated etc are not specified in the following papers making comparison analysis difficult. A depuration process of 72 hours has been suggested to remove all *C. parvum* (and *C. hominis*) oocysts from mussels (Gómez-Bautista *et al.,* 2000). In contrast, a survey by Gómez-Couso *et al.,* (2003) found oocysts in 42.6% of samples analyzed immediately before depuration, those that had received 5 days of depuration treatment still had 33.3% remaining contaminated and even after two weeks, some samples remained contaminated. The authors cite their own previous work (Gomez-Couso *et*

al., 2001– article in Portuguese) showing that oocysts released via mollusc faeces into the environment were "taken up by other individuals of the same species or of different co-existing species, thereby spreading the contamination".
The results of Lee and Lee (2003) (in section 3.3.2 above) suggest that almost complete depuration occurred in 48 hours. The depuration conditions reported in this study were a 40 litre saltwater tank at 20.5 (±0.5°C), with salinity at 18 ppt.

Freire-Santos *et al*. (2000) found oocysts in samples that had been depurated for greater than 72 hours, and even in samples treated for 300 hours, again depuration conditions were not cited. The authors also demonstrated a correlation between residual faecal coliform levels and oocysts. For the faecal coliform ranges $\leq$ 20 MPN and 20-300 MPN, the correlation with oocyst presence was approximately 40% and for >300 MPN faecal coliforms, the correlation was 100%.

In New Zealand, commercially harvested product for export to the United States must come from an area that complies with the USA National Shellfish Sanitation Programme, which means that because of that clean environment, depuration or relaying is not required.

3.5 Monitoring of Feral Shellfish Gathering

In the 1999/2000 year a survey of public health service providers and regional councils regarding the microbiological safety of feral shellfish gathering was conducted by ESR (Thomson, 2000). The report does not mention *Cryptosporidium* although information on monitoring of the microbiological quality of shellfish and marine waters was discussed. Some form of routine surveillance of feral shellfish for bacterial microbiological quality was performed by three District Health Boards (DHB) – Gisborne, Southland and Crown Public Health (Timaru – 1 site)- based on faecal coliforms, while routine monitoring of marine waters is conducted by all twelve regional councils, it is mostly for recreational bathing reasons and may not be indicative of feral shellfish growing regions. It should be noted that the quality requirements for swimming purposes are less stringent than shellfish gathering. As already mentioned, faecal coliforms have a die-off rate ten times faster than oocysts (Medema *et al.,* 1997) so the information gathered by this surveillance may not be indicative of *Cryptosporidium* contamination.

A greater diversity of feral shellfish is collected in Northland, the East coast (Northland to Hawkes Bay) and in Marlborough/Tasman area. The report commented that there was a lack of routine monitoring of feral shellfish in most parts of New Zealand and that safety could not be ensured based on data from faecal coliform testing.

During 1996, a survey on feral shellfish collection revealed that scallops, mussels and pipis were the most targeted (reported in Turner *et al.,* 2005). Data on the number of trips to collect which species of shellfish and the number collected was collated. More recently a survey from telephone interviews and diary records in 1999/2000 estimated the harvest of each species along with the most common method of collection. The results can be found in Table 4. Dredge oysters are not harvested in the Foveaux strait between 31 August and mid-November to allow for spawning.

Table 4: Estimated feral harvest of shellfish in New Zealand for 1999/2000, along with most common harvest method

Species	Harvest (numbers of shellfish)	Common method of harvest
Cockle	7,102,000[a]	Hand digging at low tide
Green-lipped mussel	1,989,000	Low tide, snorkelling, dredging
Blue mussel	254,000	Low tide collection
Rock oyster	313,000	Low tide collection
Pacific oyster	48,000	Low tide collection
Dredge oyster	39,000[b]	Dredging, diving
Pipi	10,793,000	Low tide collection
Scallop	5,097,000	Dredging, diving
Tuatua	2,061,000	Low tide collection (also toheroa)

Source: Turner *et al.,* 2005

[a] Year 2000: Whangarei 2,400,000: Otago Peninsula 1,476,000: Nelson/Marlborough 499,000

[b] The estimate for the Foveaux Strait is 430,000 oysters: In 1998, 143,949: 1999, 177,360: 2000, 223,332: 2001, 259,243.

4 HAZARD CHARACTERISATION: ADVERSE HEALTH EFFECTS

4.1 Symptoms

Incubation: 3 to 11 days, can be as long as 28 days.

Symptoms: In immunocompetent cases; watery non-bloody diarrhoea, abdominal pain, nausea, anorexia, fever and weight-loss. The patent period (the time over which oocysts are excreted) can last one to several weeks and oocysts may be detected only intermittently.

In the immunocompromised chronic persistent diarrhoea, severe abdominal pain and weight loss occur, and infection can last for several months. Severity of symptoms may correlate with intensity of oocyst shedding (Fayer and Ungar, 1986). Parasites are generally restricted to the lower small intestine.

The diarrhoea is characteristically profuse and watery, with up to 71 stools per day and up to 17 litres/day reported (Fayer and Ungar, 1986). The diarrhoea is more copious in immuno-compromised cases.

The period of illness can range from self-limiting (2-4 days) to severe and even fatal for some at risk groups. The illness has also been known to affect the respiratory system.

Excretion of oocysts varies between individuals (Fayer, 2004). Infectious oocysts are generally excreted for 6 to 9 days (range 1-15 days) and up to two months or longer after symptoms have ended. More than 10^8 - 10^{10} oocysts in total can be excreted daily in human faeces (and neonatal calves) (ESR datasheet, 2001; Fayer *et al.,* 2004). Intermittent negative samples also complicate the picture. Carrier status is therefore difficult to predict based on cessation of symptoms (Millar *et al*., 2002). Asymptomatic adult carriers may comprise 0.4% of the population, while the figure for children (immunocompetent) may be 6.4% (Millar *et al*., 2002).

Immunity can be obtained following an infection that will confer a degree of protection against subsequent re-infections.

Condition: Cryptosporidiosis

Toxins: Toxins are not produced

At Risk Groups: The oocysts can infect everyone. Children under 5 years and immuno-compromised groups are particularly at risk because of the severity of the disease experienced.

Long Term Effects: There is currently no therapy that will completely remove *Cryptosporidium* from an infected human. Fatality rates in immuno-compromised cases is between 50 – 60%. Cases are often seen in those with AIDS, even in those who have immunity. For example, a study by Cozon *et al*. (1994) has shown that even where AIDS cases have a high level of immunity (*Cryptosporidium* specific IgA), they can become re-infected with chronic cryptosporidiosis, suggesting that antibodies to the parasite are not effective on their own.

Invasion of the respiratory system can result in death.

Treatment: No effective treatment. Disease is self-limiting although infection in immuno-compromised cases can be fatal. There are no vaccines available for prevention in either animals or humans (Fayer and Ungar, 1986).

4.2 Types Causing Disease

Of the sixteen named species of *Cryptosporidium* that are infectious for non-human vertebrates, ten are also reported to have been isolated from immunocompromised humans; *C. baileyi, C. canis, C. felis, C. hominis, C. meleagrdis, C. muris* and *C. parvu, C. andersoni*, *C. suis* and *C. cervine.*

In England, a genetic analysis of faecal samples from 2414 cryptosporidiosis cases (Leoni *et al.,* 2006), found *C. parvum* in 56.1%. The study was conducted over the period 1985 to 2000. *C. hominis* was found in 41.7% of cases and a mixture of *C. parvum* and *C. hominis* in 0.9%. The following isolates were found in the remaining cases; *C meleagridis* (0.9%), *C. felis* (0.2%), *C. andersoni* (0.1%), *C. canis* (0.04%), *C. suis* (0.04%) and *C. cervine* (0.04%).

In a larger study, 13,112 faecal samples were examined from human cases in England and Wales since 1989. *C. hominis* was identified from 50.29% of samples, while *C. parvum* was identified in 45.6% of samples, and a mixture of the two in 0.5% of samples. The remaining isolates were *C meleagridis* (0.8%), *C. felis* (0.2%), *C. canis* (0.02%), *C. suis* (0.01%) and *C. cervine* (0.05%), skunk genotype (0.01%), CZB141 genotype (0.01%) and novel/undetermined (2.6%) (Nichols *et al*., 2006).

4.3 Dose Response

Table 5 shows the infectious dose value for a human volunteer set using three different isolates. The results show that the attack rates differed between isolates and were significantly different ($p = 0.045$). The authors concluded that *C. parvum* isolates differ in their infectivity. This paper was written before the species was subdivided in 2002.

Table 5: The ID50 value for healthy human volunteers – three different isolates

Isolate	ID_{50} value (no. of oocysts)	Attack rates (%)	Diarrhoea duration (h)
Iowa	87	52	64.2
UCP	1042	59	81.6
TAMU	9	86	94.5

Source: Okhuysen *et al*., 1999

In Texas, between March 1993 and January 1994, a challenge study on 26 healthy volunteers (HIV negative and absence of *C. parvum* serum antibodies) was carried out using the Iowa isolate (Dann *et al*., 2000). The inoculum was grown in a calf and the doses administered ranged from 30 to 10^6. The dose was grouped as follows; low dose 30-100 oocysts (12 volunteers); moderate dose 300-500 (9 volunteers); and high dose $\geq$ 1000 oocysts (5 volunteers). The volunteers were monitored for symptoms such as diarrhoea and presence of

oocysts in faeces as well as immunoglobulin (fIgA) response levels. Monitoring took place for up to 60 days with household contacts monitored for person-to-person spread. After oocyst challenge, there was a significant increase overall in total and specific fIgA levels, including those in the 30-100 oocyst dose group. Highest responses were associated with moderate-to-high challenge doses and/or oocyst shedding. The earliest detectable positive stool was seen at day 2 post-challenge with most subjects experiencing an initial response after 7 days. One individual became positive after 30 days with just one positive stool. Day to day variations in antibody secretions were noted with the largest response closely linked to the number of parasites in the challenge dose and to the establishment of infection.

After one year, 19 of the volunteers were rechallenged with 500 oocysts (Okhuysen *et al*., 1998). Fewer subjects shed oocysts following re-exposure (3/19; 16%) than after the first exposure (12/19; 63%), $P< 0.005$. The mean number of unformed stools passed was also lower after re-exposure; 8.62 v 11.25; $P<0.05$ that confirms that a degree of immunity is obtained following an infection.

Dupont *et al*. (1995) also carried out dose response studies on a group of healthy adults. Twenty nine healthy volunteers without previous evidence of cryptosporidiosis were given a single dose of 30 to 1 million *C. parvum* oocysts obtained from a calf. The human subjects were monitored for 8 weeks. Household contacts were also monitored. Infection results showed that 14/16 (88%) volunteers who received an intended dose of 300 oocysts or more became infected. At the lower dose of 30 oocysts, 1/5 (20%) became infected. Where doses of 1000-plus oocysts were given, all 7/7 volunteers became infected (100%). After the challenge dose, 18 subjects excreted oocysts, of which 11 had enteric symptoms and 7 had clinical cryptosporidiosis (diarrhoea and one other enteric symptom). The median infective dose (by linear regression) was 132 oocysts. All cases recovered and no secondary cases were reported amongst household contacts.

After one year, 17 of the 29 volunteers were rechallenged with *C. parvum* Iowa isolates (Chappell *et al*., 1999). These 17 had pre-existing anti-*C. parvum* IgG antibodies. A single dose of 500-50,000 oocysts was used and an ID_{50} value calculated. Thirteen of the 17 (76.5%) volunteers had presumed clinical infection, From these 13, diarrhoea was documented in 10, faecal oocyst shedding was reported for 7 and two were asymptomatic but were shedding oocysts and were included in the 13 infected. The remaining 4 of the 17 volunteers were both asymptomatic and negative for faecal oocysts. Calculated from previous studies, an ID_{50} of 83 oocysts (including those with presumed infection) was determined for antibody-negative volunteers (Chappell *et al*., 1999). From this study, the ID_{50} was 1880 oocysts, a 23-fold increase on the previous estimate. When calculation for oocyst shedders only were conducted (excluding those with presumed infection), the ID_{50} was 132 oocysts previously and 7,638 in the current study, a 57-fold increase. Another observation was that fewer oocysts were excreted by the group with pre-existing antibodies.

Using *C. hominis* (TU502) oocysts, a further experimental challenge of 21 healthy adults has been reported using a dose of between 10-500 oocysts. Sixteen (76.2%) had evidence of infection, which allowed an ID_{50} calculation estimated at 10-83 oocysts (using clinical and microbiological definitions of infection respectively). These figures are similar to those for *C. parvum*- induced illness. Diarrhoea occurred in 40% of the volunteers receiving 10 oocysts, increasing stepwise to 75% in those receiving 500 oocysts. A serum IgG response

was observed in those receiving doses of more than 30 oocysts – the greatest responses were seen in those with diarrhoea and oocyst shedding (Chappell *et al*., 2006).

5 EXPOSURE ASSESSMENT

5.1 The Hazard in the New Zealand Food Supply: *C. parvum* and *C. hominis* in Shellfish

5.1.1 *C. parvum* and *C. hominis* in shellfish

There have been no published New Zealand surveys on the prevalence of *C. parvum* or *C. hominis* oocysts in shellfish, either feral or commercial.

5.1.2 *C. parvum* and *C. hominis* in the New Zealand aquatic environment

There have been several published reports on the prevalence of the organism in New Zealand's aquatic environment, but no papers published on the prevalence of *Cryptosporidium* in marine waters.

5.1.2.1 Natural waters

The relevance of including fresh water information here is due to the run off of agricultural and human waste into freshwater systems that ultimately lead to the marine environment.

A nationwide survey on the distribution of *Giardia* and *Cryptosporidium* in natural freshwater systems has been published (Ionas *et al.,* 1998). Natural water was sampled at 100 km intervals (56 x 100km squares in a grid were drawn over a map of New Zealand to determine testing sites). The results show that *Cryptosporidium* species are widely distributed across the country, with oocysts detected in 13.0% of waters. The squares with the highest prevalence were also those used for intensive stock farming with numbers of up to 100 oocysts /100 litres of water being found (grid numbers 53 around Dunedin; number 18 around Gisborne and number 22 around Napier were highest). The modified APHA method was used. This detected approximately 8 – 15% of oocysts in spiked water, and the authors concluded that a more sensitive technique was needed to isolate, purify and concentrate oocysts. They claim that they have developed a new technique that concentrates oocysts 8-fold. This paper does not state in what season of the year the sampling took place, considering the spring-time association with calves and lambs excreting oocysts, the time of sampling could be a factor.

A study termed the "bad bug survey" was carried out across New Zealand from December 1998 to February 2000 (Ministry of Health/MfE, 2002). Twenty-five freshwater sites were chosen based on the selection of 5 different categories of environmental impact, i.e. birds, dairy farming, forestry/undeveloped, municipal and sheep/pastoral. In total, 725 samples were collected for *Cryptosporidium* oocyst analysis using the IMS method. In addition, 145 samples from water supplies in these regions were also tested. Table 6 shows the results, overall 33/725 samples were positive (5%) with freshwater sites impacted by birds having the highest rate of contamination at 9%.

Table 6: Freshwater survey across New Zealand, categorised by 5 environmental factors

Group	Number positive	Total number	% positive
All	33	725	5
Birds	10	116	9
Dairy	7	145	5
Forestry/undeveloped	10	203	5
Municipal	3	87	3
Sheep	3	174	2
Water Supplies	5	145	3

The low percentage of positive samples was a surprise to the authors, and they suggested a cyclical pattern over the year may be a reason.

Oocysts in storm waters following periods of heavy rainfall have also been studied (Ionas *et al*., 2004). Two sites were chosen; Turitea stream that is a tributary of the Manawatu River at Palmerston North. The stream is dammed and provides a catchment for the city's drinking water. Secondly the Mangaone stream that flows through farmland joining the Manawatu River in the southwest of the city. One hundred litre samples were filtered, IMS and IFA performed and the samples were collected from May 2003 to March 2004. No genotyping of isolates was undertaken. The fortnightly collections at the Turitea stream found either low numbers or no oocysts present, except surprisingly on the 3rd June 2003 when high numbers of oocysts were detected (at this time the Manawatu river level was less than one metre in depth). The authors suggested that the source of this contamination was the city's water treatment plant, where filters are back washed regularly.

In the case of the Mangaone stream, low numbers were present when the river level was below 2 metres depth but elevated numbers of oocysts were present when the river level was above 2 metres.

5.1.2.2 Wastewater treatment – influents and effluents

The following section summarises the information on wastewater treatment plants in New Zealand, it is included here because information on the influents gives some indication of the level of infection in the community and the effluents indicate the number of oocysts released into receiving waters that will ultimately end in the marine environment.

There are a number of reports that suggest that *Cryptosporidium* spp. are more prevalent in wastewater treatment plants that treat animal wastes and those serving rural communities. Work carried out by a Cross-Departmental Research Project, reported by Waste Solutions Ltd (2005) gives numerical information on the concentration of oocysts in influents and effluents. Sampling was carried out between 3rd December 2003 and 26th May 2004. Ten treatment works across New Zealand were chosen for the survey representing "small urban" and "large urban" communities. Three samples at each treatment works were taken, and the results are shown in Table 7. Green Island and Bells Island treatment works contained *Cryptosporidium* oocysts in all its samples, this may reflect the presence of abattoir wastes that it treats. The treatment performed by some of the plants did appear to reduce the protozoan concentration. Note, in the original report, the number of oocysts were expressed

as MPN/100ml, this has been clarified as oocysts per 10 litre grab samples with the laboratory that undertook the testing and the reviewer of the report.

Table 7: Number of *Cryptosporidium* oocysts in influent and effluent across ten wastewater treatment plants in New Zealand

Oocysts/10L	Influent 1	Influent 2	Influent 3	Effluent 1	Effluent 2	Effluent 3
Bells Island[2]	18	3	7	6	<1	3
Christchurch[2]	<1	1	<1	<1	7	<1
Foxton Beach[1]	3	3	<1	3	2	<1
Green Island[1]	8	14	11	5	3	1
Kaikohe[1]	<1	1	<1	<1	3	<1
Karori[2]	<1	<1	<1	<1	<1	<1
Mangere[2]	<1	<1	<1	<1	3	<1
Moa Point[2]	5	<1	<1	2	16	<1
Rotorua[2]	15	21	11	22	12	41
Waikouaiti[1]	<1	<1	10	<1	<1	56

[1] Small urban
[2] Large urban

The influent, effluent and percentage difference at each treatment works is shown in Table 8. Due to the limited data set, caution should be taken in making interpretations of these results. It is interesting to note that in some cases, the concentration of oocysts in effluent can be higher than the influent. Although not addressed in the paper, these results could be due to experimental error, the limit of detection being lower in the more particulate influent than the effluent and direct deposition from wildfowl and vermin at the treatment works.

Table 8: Averages of oocyst numbers and percentage difference between influents and effluents from ten wastewater treatment plants across New Zealand

Oocysts/10L	Influent Average	Effluent Average	Difference	Percentage
Bells Island[2]	9.3	3.0	6.3	68
Christchurch[2]	0.4	2.4	-2.0	-500
Foxton Beach[1]	2.0	1.7	0.3	16
Green Island[1]	11.0	3.0	8.0	73
Kaikohe[1]	0.4	1.1	-0.7	-167
Karori[2]	0.1	0.1	0.0	0
Mangere[2]	0.1	1.1	-1.0	-967
Moa Point[2]	1.7	6.0	-4.3	-248
Rotorua[2]	15.7	25.0	-9.3	-60
Waikouaiti[1]	3.4	18.7	-15.3	-451

The authors concluded that the occurrence of *Cryptosporidium* at most plants was relatively low.

This may be due to the time of sampling, which was predominantly during summer and autumn. Reported numbers of cryptosporidiosis are generally lower during the summer

months and the autumn peak of cryptosporidiosis due to *C. hominis* has been in decline for several years in New Zealand (Moriarty and Learmonth, 2007). There is a noticeably greater incidence of oocysts where plants are treating animal effluents such as abattoirs (Green Island and Bells Island).

Christchurch and Mangere have been the subjects of studies during the winter months of 2002 and 1999 respectively, which is useful for a general comparison over time. Note that volumes are expressed differently.

A study into the effectiveness of Christchurch wastewater treatment in removing pathogens at the primary (screening and sedimentation), secondary (trickling filters, aerobic contact and clarification) and tertiary treatment (oxidation ponds) stages has been presented at conference (Leonard *et al.,* 2003). Samples were collected between July and September 2002 and so offer a "snapshot" of winter conditions. Indicator organisms were compared to the behaviour of *Cryptosporidium* among other pathogens. A 2 $\log_{10}$ reduction in concentration was calculated for *Cryptosporidium spp.* The results show an overall reduction as the effluent is treated although caution must be applied to the interpretation due to the limited data set. In screened raw sewage, the median number of oocysts per litre were 10 with a range of <5 to 20, n=4. In secondary effluent, the median was 1 (range 0.02-2, n=4). Tertiary effluent median 0.1, (range <0.01-0.12). The limited study demonstrated that each pathogen responded differently to different stages of wastewater treatment and that traditional indicators did not necessarily reflect the reductions in protozoa. The paper concludes that direct measurement of pathogens was necessary to assess public health risk rather than reliance on traditional bacterial indicators.

The Mangere wastewater treatment plant study was conducted by Watercare Services Limited, (2002). The plant serves a population of approximately 1 million people. The results are shown in Table 9. The species was most probably *C. hominis* although the study was conducted before the differentiation in *C. parvum* was agreed. The variation in the concentration of ooocysts in raw sewage (over the nine samples) at the 50th percentile was 480 oocysts per litre and at the 99 percentile, 4,950 oocysts/l. These concentrations were considered generally consistent with reported concentrations of oocysts in raw sewage overseas, see Table 14.

Table 9: Number of oocysts/litre in three different stages at Mangere Wastewater treatment – during winter 1999.

Date (year 1999)	Raw Sewage	Biological Nutrient Reduction Effluent	Filtered effluent
	***Cryptosporidium* spp. (oocysts/l)**		
2 June	333	71	9
8 June	150	36	20
10 June	983	421	29
16 June	500	111	<1
17 June	625	<28	8
21 June	100	30	5
28 June	<125	13	<0.2
30 June	167	100	1.3
1 July	343	<167	2

5.1.2.3 Abattoir waste effluents

The possibility of oocysts being discharged into natural waterways from meat works effluent plants has been examined (Ionas *et al*., 2004; Ionas *et al.,* 2005). Effluents from four processing plants; two in North Island and two in South Island were examined over a two and a half year period (November 2002 – April 2005). Fortnightly effluent samples of 100 litres were collected just prior to river discharge. The samples were filtered and an IFA method used for detection. Recovery rates averaged 16% for *Cryptosporidium* due to high levels of suspended solids interfering with the immunomagnetic capture of oocysts. Therefore multiplying the result by a common factor of 6.25 estimates the true total. The results are shown in Table 10 and clearly illustrate the potential that these discharges hold for contaminating waterways.

Table 10: Concentration of oocysts in meat work effluent from four plants in New Zealand

Site	**1 (North I.)**	**2 (South I.)**	**3 (South I.)**	**4 (North I.)**
Meat processed	Beef only	Beef/sheep	Beef/sheep	Beef only
Treatment type	Ponds	Physical/ chemical	UV	Ponds
Discharge to:	River	River	River	Wetlands
Cryptosporidium oocyst maximum/ 100 litres	1,764	1918	12,713	83
Normalised result (x6.25)	11,025	11,988	79,456	519

5.1.3 *C. parvum* and *C. hominis* in the New Zealand terrestrial environment

Information is included here on terrestrial environment because of the potential for agricultural runoff of oocysts to ultimately end up in the marine environment.

In 2005, the number of cattle (dairy and beef) was 9.1 million, at a daily excretion rate of 25.25kg wet weight each, this equates to a yearly faecal output of 83,987,000 tonnes across New Zealand. Around a quarter of cattle in New Zealand are located in the Waikato (Moriarty and Sinton, ESR, personal communication, January 2007).

The most recently published work on *Cryptosporidium* in bovine faeces involves complex genetic analysis and was conducted by Massey University (French *et al*., 2006). The survey included four dairy farms in Otago during the winter and early spring months of August and September 2005. The survey was timed to cover the early spring calving season. Twenty-seven calf faecal specimens were collected and during the same period, 44 human faecal specimens from *C. parvum* infected cases in Otago were also analysed. A known positive specimen for *C. hominis* was included in the study for comparative reasons. PCR detection methods were used on all specimens. The allelic profiles for each specimen were determined and a population structure was then compared to the UK dataset. The *C. parvum* genotypes in New Zealand were found to be remarkably similar to each other and are genetically very distinct from the UK isolates.

Work has been carried out to determine the carriage rates for *C. parvum* and *C. hominis* (then called genotypes bovine and human) in cattle faeces in the Waikato region (Learmonth *et al*., 2003). Over spring (August – October) and early autumn (January – March) periods, faecal samples were collected from dairy cattle and humans in the region. The results are shown in Table 11. This study found approximately 10% of calves in the region were excreting *C. parvum,* mostly in the spring period. Note the shift in human cases caused by bovine genotypes (*C. parvum*) in the spring and human genotypes (*C. hominis*) in the autumn;

Table 11: Prevalence of *Cryptosporidium* species in faecal samples

	No. of faecal samples	**No. positive**	**% positive**	**Spring (no. and genotype)**	**Autumn (no. and genotype)**
Cows	354	2	0.6	2 "bovine"	
Calves	304	33	10.8	29 "bovine"	4 "bovine"
Humans (cases)	66	66	100	43 "bovine"	1 bovine 22 human
Total	724	101	13.9		

In a recent study of *C. parvum* oocysts in newborn calves involving 10 dairy farms in the Manawatu (Grinberg *et al.,* 2005), faecal samples from 156 calves were tested microscopically. The results showed that 33/156 (21.2%) calves were infected. The samples were enumerated and from four of the farms, calves were excreting more than 10^6 oocysts/g.

Ammonia occurs in natural environments as a product of urea hydrolysis and of the microbial degradation of proteins and other nitrogen containing compounds. Significant amounts of ammonia may be present in decomposing manure. The effect of increasing free ammonia concentrations and pH on the viability of *C. parvum* oocysts has been studied (Jenkins *et al.,* 1998). Ammonia from a commercial solution was administered in concentrations ranging from 0.007 to 0.148 M (a relevant range associated with manure slurries). Exposure times ranged from 10 minutes to 24 hours at a constant 24°C. The lowest concentration of ammonia significantly decreased viability after 24 hours, while increasing the concentration increased the inactivation rate although at the highest concentration a small fraction of viable oocysts remained. This effect appeared to be due to ammonia itself, as experiments with pH levels corresponding to those associated with the ammonia concentrations showed minimal effects of the alkaline pH. The authors concluded the environmentally relevant free ammonia concentrations may significantly reduce viability of oocysts. The concentration of ammonia was suggested to affect the permeability of the oocyst wall and sporozoite membranes leading to inactivation. Free ammonia does exist in manure albeit for a short time, several studies indicate that generally nitrogen in cattle manure is lost as gaseous ammonia to the environment.

Research has also been conducted on ammonia retention in manure (Ruxton, 1995; Whitehead and Raistrick, 1993). Ruxton carried out mathematical modelling on ammonia volatisation in slurry stores and applied this to viability of *Cryptosporidium* oocysts. The model predicts the change in ammonia distribution with depth, using a critical value of 0.1% ammonia solution for 48 hours (at 37°C and 22°C) that appears to destroy viability of the oocyst (this assumes that the same effect is achievable at lower temperatures). The results predicted that oocysts are unlikely to remain viable in slurry except in the top few

centimetres. The authors suggested that farmers occasionally stir slurry in order to reduce oocyst survival further.

In a study conducted by Chilvers *et al.* (1998), the prevalence of *Cryptosporidium* in wild animals on farmland was assessed. The catchment area was the south-east region of the North Island, animals were collected between May 1991 and January 1992. Oocysts were detected in the following animals (percentage positive);

> 39 possums (12.8%), 8 ship rats (37.5%), 17 house mice (11.8%), 61 house sparrows (8.2%), 14 thrushes (21.4%) and 1 of 2 starlings (50%).

It does not appear that any surveys of waterfowl or seagulls have been conducted. In relation to shellfish, carriage rates in these animals may be of interest.

5.2 Food Consumption: Shellfish

The Food and Agriculture Organization of the United Nations (FAO) consolidates information on the disposition of food commodities by country in their FAOSTAT database (http://faostat.fao.org/site/354/default.aspx). The most recent New Zealand statistics are for 2004. New Zealand's total production of marine molluscs was reported as approximately 80,000 tonnes of which approximately 92% was exported. Mean human consumption was 5.7 g/person/day.

The 1997 New Zealand National Nutrition Survey (1997 NNS; Russell *et al*., 1999) collected information on dietary consumption by New Zealanders aged 15 years and older. A FSANZ assessment of the data from the 1997 NNS, using a series of standard recipes to determine quantities of commodities in compound foods, estimated the proportion of respondents consuming molluscs to be 3.1%, with proportions consuming mussels, oysters and scallops as 1.9, 0.6, and 0.3% respectively (ANZFA, 2001). Mean consumption levels for molluscs, for all respondents, were slightly lower than the FAO estimates at 2.7 g/day. Mean daily consumption of mussels, oysters and scallops was 1.3, 0.6 and 0.2 g/day respectively.

The 2002 New Zealand Children's National Nutrition Survey (02CNS; MoH, 2003) collected equivalent information for New Zealand children aged 5-15 years. According to this survey, children are less likely to consume shellfish than adults with 0.3%, 0.06% and 0% of respondents reporting consumption of mussels, oysters and scallops respectively. This equates to mean daily intakes for all children of 0.14, 0.03 and 0g/day respectively for mussels, oysters and scallops.

USA statistics suggest that raw oysters are consumed on average once every six weeks (2.4% of the population consume on any given day (FDA, 2001)).

The FSANZ assessment of the 1997 NNS data reported a median amount eaten by consumers of 38.4, 45.9, 54.4 g/day respectively for mussels, oysters and scallops. As multiple shellfish meals are unlikely within a single day, these figures are likely to represent median serving sizes for shellfish meals.

The USFDA reported a mean value for a single serving of raw oysters of 110 g (FDA, 2001). Data from the 1997 NNS for oysters eaten as oysters rather than as a component of a recipe

gives a mean serving size for New Zealanders of 132 g, although this mean is highly skewed by one consumption event of 600 g of oysters. The median serving size for oysters was 90 g.

Analysis of the data from the 1997 NNS suggests that Maori respondents, on average, consume larger amounts of shellfish (average daily consumption of 160 g as compared to 113 g for non-Maori), although the data available do not suggest that they eat shellfish more frequently.

There is some evidence to suggest that certain ethnic groups in New Zealand (Maori, Pacific Islanders, Asians) are over-represented in non-commercial harvesting of shellfish (see studies reviewed in Hay *et al.,* (2000). It has been estimated that 14% are Maori, 2% Pacific People and 84% other ethnic groups are involved in non-commercial harvesting (Wilson, 1996). When this is put into terms of the 2006 census count (albeit some 10 years after the Wilson report was published), 14 % of the total New Zealand population reported themselves as Maori and 7% as Pacific Islanders (Statistics New Zealand website accessed 12.04.2007).

A survey of feral cockle collectors by Kearney (1999) was conducted in Whangateau Harbour (north of Auckland). 54% were Maori (collecting 55% of total cockle weight), followed by 26% New Zealand Europeans (19% weight). 15% were Asian (9% weight) and 4% were Pacific people (9% weight).

The harvesting of seafood by Maori, particularly in the far north, is an important cultural and dietary component. A survey in this region found that 11% of households reported collecting seafood more than once a week, 31% collected seafood at least weekly, and 52% reported collecting seafood at least fortnightly (n=499) (reported in Hay *et al.,* 2000).

5.3 Qualitative Estimate of Exposure

Internationally, there have been no documented cases of cryptosporidiosis associated from eating raw shellfish (Graczyk and Schwab, 2000; Millar *et al*., 2002).

There are data on consumption of this food type by New Zealanders but none on contamination levels. Consequently a qualitative estimate of exposure is not possible at the current time.

5.3.1 Number of servings and serving sizes

About three percent of the New Zealand population consume shellfish on any particular day.

Of the servings of shellfish identified in the 1997 National Nutrition Survey, 57% identified the product as being cooked, 20% raw, 19% marinated, 3% canned and 2% smoked. It is unclear from the data whether marinated is cooked or uncooked.

Serving sizes for shellfish are similar to those for medium-sized fruit such as figs or kiwifruit, mammalian offals or vegetables such as brussel sprouts, pumpkin or spinach. These foods form a 'medium' serving size group below the dietary staples such as meats and common fruits and vegetables, but above meal ingredients such as herbs, fats and oils, seeds and nuts.

5.3.2 Frequency of contamination

With very little published information on the frequency of *Cryptosporidium* contamination in New Zealand shellfish, predicted frequency of contamination is difficult to quantify.

A survey of 8000 oysters by Mike Hine of NIWA (cited in McCoubrey, 2000) used electron microscopic techniques on sections of oysters to look generically for disease. One or two sections 5 to 7 microns thick were examined per animal. The presence of *Cryptosporidium* oocysts was looked for, but no positive samples reported. In retrospect, the method may not have been suitable for oocyst detection because the accepted mainstream method of detection of oocysts in shellfish are molecular or immuno-fluorescent microscropy (Tecklok Wong, personal communication, ESR, February, 2007 (after consultation with Mike Hine - 21.04.2006).

The 8000 oysters originated from throughout New Zealand; Pacific oysters (*Crassostrea gigas*) from Northland and Mahurangi and dredge oysters (*Ostreidae* and *Tiostrea chilensis*) from Fouveaux Strait and Golden Bay.

5.3.3 Predicted contamination level at retail

With no published information from New Zealand, contamination at retail is difficult to predict. *C. parvum* and *C. hominis* cannot replicate in food, the parasite needs a living mammalian host to reproduce.

5.3.4 Heat treatment

Heat treatment is commonly applied to many of the filter feeding shellfish. However there are occasions, especially in the case of oysters, where the flesh will be consumed raw or lightly cooked.

Studies on the susceptibilities of the oocyst to heat suggest that most cooking temperatures are sufficient to destroy oocysts (Laberge *et al.,* 1996). However, steam cooking of mussels appears to be insufficient to destroy all oocysts present (Gomez-Couso *et al.,* 2006a). Contaminated mussels spiked with *C. parvum* were heated until they opened. The internal starting temperature of the mussels was approximately 20°C rising to approximately 60-65°C after 5 minutes. Gills and gastrointestinal tracts were immediately dissected and processed, and 100µl aliquots of concentrate were inoculated into neonatal mice. Out of 26 mice inoculated 13 were infected.

A common method for cooking mussels is exposure to boiling water for approximately 1.5 minutes until the shell opens. It remains unclear whether these parameters are adequate to destroy oocysts within mussels (Chalmers *et al.,* 1997).

Various cooking times were studied in three microwave ovens (various wattages and sizes) to determine the effect of oocyst inactivation. The mouse bioassay was used to determine viability of the oocysts (Ortega and Liao, 2006). Oocysts were completely inactivated by a minimum of 20 seconds at 100% cooking power in two of the three microwave ovens used (1100 W and 650 W) because a temperature greater than 80°C was achieved. Due to the variability of temperatures inside the various microwave ovens, a time parameter can not be

relied upon however for inactivation and a temperature of at least 80°C throughout needs to be achieved. *C. parvum* oocysts were found to be more sensitive to microwave heating than *Cyclospora* oocysts, which took up to 45 seconds of cooking time to be destroyed.

5.3.5 Exposure summary

Raw molluscan shellfish are consumed by approximately 3% of the New Zealand population on any given day. This is consistent with findings from normal 'benchmark' countries such as Australia and the US. Without any information on degree of contamination, it is not possible to ascertain the exposure to *Cryptosporidium* from either commercial or feral shellfish.

The percentages of the total consumption of shellfish is 44% mussels, 24% oysters, 13% scallops, 3% pipis and 2% tuatua, with the remainder made up with abalone (Russell *et al.,* 1999).

Exposure may be higher for ethnic groups such as Maori, Pacific and Asian Peoples, based on their over-representation amongst those harvesting feral shellfish compared to the ethnic composition of New Zealand (Drey and Hartill, 1993).

5.4 *C. parvum* and *C. hominis* in Shellfish Overseas

5.4.1 Presence of *C. parvum* and *C. hominis* in shellfish overseas

Data on the prevalence of *C. parvum* and *C. hominis* in shellfish overseas have been summarised in Table 12. Due to the importance of the method used (molecular and microscopy methods), and the sample type (homogenates/washings; gills, gastro-intestinal (GI), haemolymph) etc., these factors are also presented where known.

Table 12: Summary of *C. parvum* and *C. hominis* prevalence in overseas shellfish

Country	Shellfish	Comment on method / species	No. positive/ No. sampled (%)	No. Oocysts per kg	Reference
	Mussels				
France (Normandy)	Mussels (*Mytilus edulis*) (close to river outfalls)	*C. parvum*	1889/1889 (100)	8-148	Li *et al*., 2006
Northern Ireland (Belfast Lough)	Mussels (*Mytilus edulis*)	*C. hominis* PCR and IFA methods Samples collected between July-August 1999	None positive using IFA With PCR, 2/16 mussels positive (12.5%)	NS	Lowery *et al.*, 2001
Ireland (Sligo)	Mussels (*Mytilus edulis*)	IFA - Seashore 3 sites May – August 1996	Only one site positive, oocysts	NS	Chalmers *et al.*, 1997

Country	Shellfish	Comment on method / species	No. positive/ No. sampled (%)	No. Oocysts per kg	Reference
		(10 mussels per site to give 100ml whole tissue homogenate) *C. parvum*	detected 3 times (June). Site sampled 13 times		
Spain*	Mussels (*Mytilus galloprovincialis*)	***C. parvum* IFA, mouse bioassay and PCR	2/9 sites Site 1; 17 mussels 14 mussels Site 2; 5 mussels	 1.0×10^5 1.5×10^4 1.5×10^4	Gomez-Bautista *et al*., 2000
Spain (Galician coast)	Mussels (*Mytilus galloprovincialis*)	*Cryptosporidium* spp. Pool of 12 molluscs GI and gill homogenates - IFA	6/15 (40)		Freire-Santos *et al.*, 2000
Spain	Mussels *(Mytilus galloprovincialis)*	*C. parvum*	54/184 (29.3) (42/184 using IFA 26/184 using PCR. In 14 samples oocysts were detected using both techniques	25-275 oocysts per 6-8 mussel sample	Gómez-Couso *et al.*, 2006c
Spain (Galician coast)	Mussels *(Mytilus galloprovincialis*	Pooled gills and GI tracts from 6-8 mussels IFA PCR *C. parvum*	69/222 pools (31.1) IFA 54/222 PCR 28/222 Overlap in 13 samples	NS	Gómez-Couso *et al.*, 2006d
Canada	Zebra mussels (*Dreissena ploymorpha*)	*C. hominis*		220,000	Graczyk *et al*., 2001
USA (California coast)	*Mytilus californianus, M. galloprovincialis* (haemolymph samples)	*Cryptosporidium* species including novel species (PCR)	19/156 pools (12)	NS	Miller *et al.*, 2005
	Clams				

Country	Shellfish	Comment on method / species	No. positive/ No. sampled (%)	No. Oocysts per kg	Reference
Spain (Galician coast)	*Dosinia exoleta, Venerupis pullastra, Venerupis rhomboideus, Venus verrocosa*	*Cryptosporidium* spp. Pool of 12 molluscs GI and gill homogenates - IFA	1/1 3/6 0/1 1/1 Total: 5/9 (55.5)		Freire-Santos *et al.*, 2000
Italy	*Ruditapes philippinarum*	IFA method Pool of 12 molluscs GI and gill homogenates	5/8 clams (62.5)	NS	Freire-Santos *et al.*, 2000
Italy	*Chamelea gallina*	IFA method on haemolymph and tissues *C. parvum* 960 clams pooled into 32 samples	23/32 pools (71.9%) – PCR on the 23 positives revealed 2 pools positive for *C. parvum* (not clear what other 21 pools were)	NS	Giangaspero *et al.*, 2005
Italy *** (Northern Adriatic Lagoons)	*Ruditapes philippinarum*	From three clam farms, 60 clams collected per month and pooled i.e. 60 per pool PCR for *C. hominis* and *C. parvum*	2160 clams into 36 pools. 7 pools (19.4%) positive by PCR 1 pool *C. hominis,* 6 pools *C. parvum*	NS	Molini *et al.*, 2007
	Oysters				
USA (Chesapeake Bay)	*Crassostrea virginica*	*Cryptosporidium* spp.	Range Spring 5/30 (16.7) to 18/30 (60)	NS	Fayer *et al*., 1998b

Country	Shellfish	Comment on method / species	No. positive/ No. sampled (%)	No. Oocysts per kg	Reference
			Summer 2/30 (6.7) to 26/30 (86.7)		
USA (Chesapeake Bay)	*Crassostrea virginica*	*C. parvum*, *C. hominis* and *C. baileyi* Gill washings by IFA	312/1590 (19.6)	NS	Fayer *et al.*, 2002
USA Two methods used; IFA and PCR	NS 13 Atlantic coast states and one site in Canada.	*C. parvum*, *C. hominis* and *C. meleagridis* Gill washings from 25 shellfish at each site	34/925(3.7) by IFA method By PCR method 65/185 (35.2) (each pool containing 5 samples)	NS	Fayer *et al.*, 2003
Spain (Galician coast)	*Ostrea edulis*	*Cryptosporidium* spp. Pool of 12 molluscs GI and gill homogenates - IFA	4/6		Freire-Santos *et al.*, 2000
UK	*Ostrea edulis*	IFA method. Pool of 12 molluscs GI and gill homogenates	1/1		Freire-Santos *et al.*, 2000
The Netherlands	Portuguese oysters	*C. parvum*	6/133 (13)	NS	Schets *et al.*, 2003
The Netherlands	*Crassostrea gigas* - commercial -non-commercial - total	*Cryptosporidium*, species not stated	5/133 (8) 4/46 (9) 9/179 (5)	NS	Schets *et al.*, 2007
	Cockles				
Spain	*Cerastoderma edule*	**Recovered after first 48 hours of collection only. Most recovered after 24 hours.	6 cockles	3 x 10^4 oocysts in sample	Gomez-Baustista *et al.*, 2000

Country	Shellfish	Comment on method / species	No. positive/ No. sampled (%)	No. Oocysts per kg	Reference
	Combination				
Spain****	Molluscan shellfish (mussels, oysters, clams, cockles)	*C. parvum*	69/203 (34) (Spain) 14/ 38 (36.8%)(EU)	NS	Gómez-Couso *et al.*, 2003
Spain Two methods used; IFA and PCR	18 clam 22 mussel 9 oyster Samples originated from Spain, UK, Italy and Ireland	*C. parvum* and *C. hominis*	56% IFA 44% PCR	NS	Gómez-Couso *et al.*, 2004

Source: adapted from Millar *et al*., 2002 with additional information added.
NS Not stated
* Oocysts recovered from water filtered during first 24 hours in aquaria but not in tissue homogenate

** Recovered only from areas near river mouths where river banks supported a high density of grazing ruminants

*** Positive months were February, March, June, August, September and October.

**** In the Spanish samples, 49.3% of the oocysts were viable. In the imported EU samples (30 from Italy, 5 from UK, 2 from Ireland and 1 from Portugal), 71.4% were viable oocysts. Each sample consisted of pooled gills and gastrointestinal tracts from seven molluscs. Technique used was IFA.

The following notes expand on the table above and are generally in the order that the study appears in the table.

Many of the sampling surveys in the USA were conducted in the Chesapeake Bay, on the East coast. This Bay is known for its high level of agricultural pollution and eutrophication. It should be noted that Chesapeake water parameters are different to New Zealand. The salinity of Chesapeake is 9-15ppt, with temperatures in the range 12-16°C. New Zealand water salinity is 22-33 ppt, with a temperature range between 10-26°C. It is unknown what effect these different environmental conditions may have on *Cryptosporidium* uptake in shellfish (McCoubrey, 2000). In addition, the effect on viability and infectivity of oocysts in New Zealand waters is unknown.

In the French study (Li *et al*., 2006), mussels were collected from three sites over the four seasons. Highest oocyst numbers in the mussels were recorded in January (midwinter) while lowest counts were recorded in October (Autumn). This correlated with heavy rainfall in January and low rainfall in October, with the conclusion that heavy rains appear to increase oocyst distribution into the sea probably due to agricultural runoff. However, infectivity of the oocysts was very low. Only one mouse infected with 100 oocysts (mussels from one site in January) showed developmental signs of cryptosporidiosis in the small intestine.

The risk to humans was considered low because mussels were usually well cooked in France before being eaten. In addition, the mean oocyst numbers were low compared to other

studies and it was suggested that the numbers of mussels that would need to be consumed to result in infection would probably exceed a one-meal serving.

The three-year Californian coast study using mussels as filter-feeding sentinels (Miller *et al.,* 2005) tested the hypothesis that oocysts would be detected more often in mussels collected from sites near livestock runoff and human sewage outlets rather than from sites distant from these two sources. The results did not support this hypothesis. Three sites each were chosen (3 near livestock runoff, 3 near sewage outlets and 3 controls). Sentinel mussels were outplanted at coastal study sites and harvested after at least a month. In year 1, batches were tested once during the wet and dry season. In years 2 and 3, this increased to quarterly testing. Mussels were transported chilled to the laboratory within 2 days, with 30 mussels per batch individually tested. Over the 3 years, 156 batches of mussels were collected totaling 4680 mussels. *Cryptosporidium* DNA was identified in 19/156 batches (12%). Within positive batches, 1 to 4 mussels tested positive with occasionally more than one genotype detected. While oocysts were detected in both wet and dry seasons, those batches collected near high freshwater outflows and those batches collected within a week of precipitation were the most likely to contain oocysts.

In the Schets *et al.,* (2007) Netherlands study, all isolations originated from the intestines (none from concentrated gill washings). Methodological shortcomings were highlighted as a possible reason for the low numbers of positives. The microscope slides were found to be difficult to read due to the thick layer of homogenised oyster tissue and it was thought this layer could mask oocysts present. The authors believed that 50% of oysters contaminated with 500 oocysts or less may have gone undetected because of this problem with digestive tract homogenates as opposed to the relativity easier gill washing examinations. IMS has been successfully reported by others for the isolation of *Cryptosporidium* from oyster tissues (MacRae *et al.,* 2005) but IMS did not perform well in the Netherlands study.

In the Fayer *et al.* (1998b) study, the oocysts were found in gill washings and lymph sites. Oysters positive for gill washings were not always positive in lymph sites and vice versa. No trend was observed for positive oysters affected by wastewater outfalls and animal agricultural runoffs.

The Fayer *et al.* (2002) study collected a total of 53 collections, from eight trips over a period of 3 years from seven sites in the Chesapeake Bay, USA and its tributaries. In the 53 collections, oocysts were detected in 81% of the 53 collections, the greatest percentage of positive oysters with oocysts coincided with times of greatest weekly and monthly rainfall with corresponding streamflows in to the Bay. The water temperatures at these times were also the lowest. *C. parvum*, *C. hominis* and *C. baileyi* were identified by PCR and gene sequencing in 28% of the 53 collections. Infectivity of the oocysts were further confirmed in 37.5% of 40 of the collections, where *C. parvum* infections were initiated in mice.

In Fayer *et al.* (2003), none of the positive samples were later found infectious using a mouse bioassay. Collectively *Cryptosporidium* spp. including *C. meleagridis* were found in 64.9% of commercial shellfish sites along the Atlantic coast (either by microscopy or molecular methods). Shellfish were examined during a period of exceptionally low rainfall, the authors therefore propose that the data are an underestimation of contaminated shellfish during normal and heavy precipition.

Further classification of collection waters according to EU directive 91/492 of the Gómez-Couso *et al*. (2006c) study were used as the basis for testing mussels grown in North West Spain. A total of 184 samples of mussels were tested, and overall, 54 (29.3%) were contaminated (each sample consisting of the pooled gills and gastrointestinal tracts of 6 to 8 mussels). Note that two methods; PCR and IFA were used and disparate results found. Only 14 of the 54 mussel samples were found positive by *both* methods. The mussels were collected from three categories of water;

Category A = less than 230 *E. coli* /100g
Category B = between 230-4,600 *E. coli* /100g, and
Category C =more than 4,600 *E. coli* /100g

The results were; Category A waters - 35/135 samples positive for oocysts (25.9%), 15/40 (37.5%) for Category B waters and 4/9 (44%) for Category C waters positive (44%), although with such a small sample size for the latter groups, these last figures are not particularly useful. All oocysts in the mussels were identified as *C. parvum* and were assumed to have originated from cattle via runoff.

In the Gómez-Couso *et al*. (2004) study, two mussel samples were harvested in New Zealand and obtained at retail sale in London. Unfortunately the paper does not clearly state the results from the New Zealand mussels, but combines results with UK origin mussels. A summary of the results can be found in Table 13. The authors concluded that the IFA and PCR methods were equivalent in ability to detect the oocysts (as mentioned in the Gómez-Couso *et al*. (2006c) study above) and that advantages and disadvantages were apparent with both methods. From the 26 PCR positive samples, 22 were *C. parvum*, 1 was *C. hominis* and the remaining 3 were a mixture of *C. parvum* and *C. hominis*-like organisms. Unfortunately it is not clear which samples came from which shellfish species or their origins although the majority will have originated from the Spanish study. As 22 were *C. parvum*, this ties in with the fact that the samples in Spain were collected between November and March. The authors concluded that if seasonal spring peaks previously noted in England applied to Europe, the peak time for excretion of oocysts by calves and lambs would be the Spring period that falls within this time-frame.

Table 13: Comparision of IFA and PCR methods

Shellfish species	Origin of samples	No. of samples	Method used			
			IFA No. positive	% positive	PCR No. positive	% positive
Clam	Spain	18	10	56	9	50
Mussel	Spain	22	12	55	8	36
Oyster	Spain	9	6	67	5	56
Mussel	NW England New Zealand	18 2	NT		3	15
Cockle	NW England	18	NT		1	6

NT Not tested.
Source Gómez-Couso *et al*., (2004)

5.4.2 Presence of *C. parvum* and *C. hominis* in seawater/runoff waters and sewage overseas

Table 14 summarises the results of studies into the presence of *Cryptosporidium* in seawater, run-off waters and sewage overseas.

Table 14: Presence of *C. parvum* and *C. hominis* in seawater/runoff waters and human sewage overseas

Country	Type of sample	Species of *Cryptosporidium*	No. positive/ No. sampled (%)	Reference
Rivers				
England	River waters	*Cryptosporidium,* spp.	691 mean concentration 0.38 oocysts / litre	Unpublished data cited by Lowery *et al*., 2001
Ireland (Sligo)	River waters in agricultural area (Owenboy River), collected May - August	*C. parvum*	Of 9 sampling sites; 3 sampling sites were positive over the course of the study - 5/24 samples were positive	Chalmers *et al.,* 1997
River and sewage effluent				
Northern Ireland (Belfast Lough)	River x 5 Sewage effluent (final treated) x 5	*C. parvum* and *C. hominis* IMS-IFA method PCR-IMS method	Using IFA, no positives detected. With PCR, 1 sewage (*C. hominis*), 1 river (*C. parvum*) positive 2/10 samples (20%)	Lowery *et al.,* 1997
Sewage				
Canada	Montreal	*Cryptosporidium*	6 oocsts/l	Cited in

Country	Type of sample	Species of *Cryptosporidium*	No. positive/ No. sampled (%)	Reference
		spp.	(range 1 to 560 oocysts)	Watercare Services Limited, 2002
England	7 wastewater treatment plants	*Cryptosporidium* spp.	Concentration 10 to 170 oocysts/L	Cited in Watercare Services Limited, 2002
Japan	Raw sewage	*Cryptosporidium,* spp.	10% positive (8-50 oocysts/litre)	Cited in Watercare Services Limited, 2002
The Netherlands	Sewage treatment effluent November December	*Cryptosporidium,* spp.	 7.9/litre (95% dead) 4.8/litre (90% dead)	Schets *et al.,* 2007
Norway	Sewage influent into 40 treatment works	*Cryptosporidium,* spp.	32/40 (80% of treatment works positive)	Robertson *et al.,* 2006
USA	Arizona, USA (2 year study) Milwaukee	*Cryptosporidium,* spp. *Cryptosporidium,* spp.	521 oocysts/litre Recovered sporadically, 13% samples positive	Cited in Watercare Services Limited, 2002

In the Netherlands study (Schets *et al*., 2007), numerous water samples were also taken from two sewage treatment effluents through to seawater samples. The authors found declining concentrations as expected. The effluents contained up to 7.9 and 8.8 oocysts per litre (95% - 93% dead respectively). The pumping engine and ditch sites showed 0.9 and 1 oocyst /litre respectively (76% to 82% dead), while a surface water canal (0.04 /litre – all alive) and seawater harvesting site (<0.04) contained very few oocysts.

The Norwegian study also estimated the risk of clinical infection in the population. The highest estimates were up to 5 per 100,000 per annum for two populations in eastern Norway. Removal efficiency at two treatment works with secondary treatment was approximately 50%. Minimal treatment resulted in negligible removal of oocysts. Information was also gathered about the sewage treatment works such as the size of the population served, daily volumes, inputs from animal sources such as abattoirs, type of treatments and end discharge destinations such as sea, river or lake. With the sewage influent into 80% of treatment works positive for oocysts, it was concluded that cryptosporidiosis infections were widespread

throughout Norway. However, because the disease is not notifable in this country (unless it is associated with AIDS), there is no way to assess the estimates against actual reported disease.

Experiments conducted to assess the effectiveness of sewage treatment found a 2-log reduction in the prevalence of *Cryptosporidium* oocysts (determined by indirect fluorescent antibody staining IFA) (Mayer and Palmer, 1996).

In terms of the international microbiological quality requirements for shellfish gathering waters, the following Table 15 has been reproduced here from WaterCare Services Limited (2002) report;

Table 15: International microbiological quality requirements for shellfish gathering waters

Country	Total coliforms	Faecal coliforms
United States (Environmental Protection Authority)	-	14[1] (43)[2]
California, USA	70[1] (230)[2]	-
Venzuela	70[1] (230)[2]	14[1] (43)[2]
Peru	1,000[3]	200[3]
Mexico	70[4] (230)[2]	-
Japan	70	-
Puerto Rico	70 (230)[3]	-

[1] Logarithmic average for a period of 30 days of at least 5 samples, expressed MPN/100ml.
[2] 90 percentile value
[3] 80 percentile value
[4] monthly average

5.4.3 Presence of *C. parvum* spp. in bovine faeces overseas

Prevalence rates for *Cryptosporidium* spp. in bovine faeces in studies overseas vary between 1.1% to 79% depending on the age of the animal and whether the animal is symptomatic. In general, worldwide studies have found a very high prevalence of *C. parvum* in calves aged between 4 and 30 days old, with excretion decreasing over time. For example, 29 dairy calves were examined weekly over 20 weeks on an Ohio farm. *C. parvum* was detected at 1 to 5 weeks at the following rates; 93.3, 95.5, 71.4, 26.3 and 30% respectively. From 6 to 20 weeks, 0,1 or 2 calves were found positive each week (Xiao and Herd, 1994). Several studies demonstrate that as cattle age, *C. parvum* is less likely to be excreted. Table 16 summarises some of the overseas studies.

Table 16: Prevalence of *Cryptosporidium spp.*in bovine faeces overseas

Country	Animal	No. of samples	Percentage positive	Species#	Reference
Canada	Beef cattle	669	18.4	*C. parvum*	McAllister *et al.,* 2005
Denmark	Cattle* Year 2001 2002	2509 2825	11.4 10.9		Anonymous 2002; 2003;

Country	Animal	No. of samples	Percentage positive	Species#	Reference
	2003 2004 2005	NS NS NS	16.0 19.9 21.6		2004; 2005
Germany	Calves (up to 6 months)	4060	21.5	NS	Joachim *et al*., 2003
Ireland	Cattle at slaughter <30 months	288	7.3	45.5% *C. parvum* 54.5% *C. andersoni*	Moriarty *et al*., 2005
Japan	Beef cattle	512	4.7	*C. muris*	Kaneta and Nakai (1998)
Spain	Predominantly young diarrhoeic animals	NS	6.4	*C. parvum*	Villacorta *et al.,* 1991
USA	Adult beef cattle (California)	NS	1.1	*C. parvum*	Hoar *et al.,* 2001
USA	Pre-weaned calves (2-60 days) Post-weaned calves (3-11 months)	503 468	50.3 19.7	85% *C. parvum* 1% *C. parvum*	Santín *et al*., 2004
USA (Eastern)	Pre-weaned calves Post-weaned calves 1-2 yr heifers (dairy)	393 447 571	41 26.2 11.9	 0.7% *C. parvum*	Fayer *et al.,* 2006

NS; Not stated

*Unclear whether calves were included in the testing

Note that due to taxonomic changes over recent years, the species stated in the paper is reported here, accuracy is therefore uncertain in this respect

Detection of *Cryptosporidium* species in six of eleven farm waters destined for bovine consumption has also been reported (Da Fonseca *et al.,* 2000). This preliminary study was carried out in Portugal.

6 RISK CHARACTERISATION

6.1 Adverse Health Effects in New Zealand

6.1.1 Incidence

Cryptosporidiosis became notifiable on 1 June 1996. A total of 889 cases of cryptosporidiosis were notified in 2005 (ESR, 2006). The infection appeared to peak in 2001 although rates have been over 20 per 100,000 since 1998, except in 2004. Table 17 summarises the number of cases and rate per 100,000 since cryptosporidiosis became notifiable in New Zealand. Figure 2 illustrates the total number of notified cases from 1996 to 2005 while Figure 3 illustrates the peaks and troughs in the number of notifications each month since 1999. There is a distinct seasonal pattern with the largest number of notifications in October of each year.

Table 17: Number of reported cases and rates of cryptosporidiosis from June 1996 to 2005 in New Zealand

Year	Number of cases of cryptosporidiosis	Rate per 100,000*	Reference
1996**	110	6	Galloway *et al*., 1998
1997	357	9.9	Galloway *et al*., 1998
1998	866	23.9	Perks *et al*., 1999
1999	977	27.0	Kieft *et al.*, 2000
2000	775	21.4	Lopez *et al.*, 2001
2001	1208	32.3	Sneyd *et al*., 2002
2002	974	26.1	Sneyd and Baker, 2003
2003	818	21.9	ESR, 2004a
2004	612	16.4	ESR, 2005a
2005	889	23.8	ESR, 2006

* The New Zealand population increases by up to an estimated 2% per annum (http://www.stats.govt.nz/analytical-reports/dem-trends-05/default.htm). The cryptosporidiosis rates are calculated using the most recent census data (e.g. 2001 census for rates from 2001 to 2005). An annual rate increase of more than 2% therefore represents an increase in reported notification rate.

** June to December

There is uncertainty about the ratio between the total number of cryptosporidiosis cases in the community compared to those notified. In a study in England and Wales by Adak *et al.*, (2002), an estimated 1 case in every 7.4 cases was reported. The foodborne percentage was estimated at 5.6%. However this ratio may also be a substantial underestimation due to not all cases being stool positive (especially those with partial immunity) (Chappell *et al.*, 1999, - study discussed in section 4.3). On the available evidence, Nichols *et al*., (2006) estimate around 15 cases in the community for every case diagnosed. This suggests that the total rate of cryptosporidiosis in New Zealand using the most recent data for the year 2005 is approximately 360 per 100,000.

Screening all submitted diarrhoeal samples from a community for cryptosporidiosis oocysts was carried around 20 years ago in Auckland and Taranaki.

Between 19th December and 4th March 1985, faecal samples from children with significant diarrhoea were screened at the Princess Mary Hospital, Auckland. In total, 36 patients were screened for certain pathogens including cryptosporidiosis. Ocysts were present in eight children (22%). Duration of diarrhoea ranged from 10 days to several months, age ranges were five months to five years (Te Waita and Lennon, 1985).

In Taranaki, a study was conducted to determine the incidence of *Cryptosporidium* associated diarrhoeal disease in immunocompetent individuals. A total of 1669 diarrhoeal specimens from 1273 patients were submitted for oocyst examination over a 12 month period. Of these, 73 samples (4.4%) from 54 patients contained oocysts. This makes a total of 54/1273 patients positive as oocyst shedders (4.2%). In terms of age, those aged 1 to 15 years had the highest incidence at 7.2% and in terms of gender, 61% were males. Of the 54 cases, 40 (74%) were diagnosed in the Spring and early to mid Summer months. All cases were self-limiting. The authors concluded that direct contact with farm animals was the probable source of infection and recommended that clinical laboratories routinely screen faecal samples in diarrhoeal samples (Carter and Anzimlt, 1986).

The annual notification rates vary throughout the country from 7 per 100,00 notified in Waitemata through to the highest rate recorded in South Canterbury of 85.3 per 100,000. Rates over 30 per 100,000 notified to District Health Boards in descending order are as follows; Lakes (62.5 per 100,000), West Coast (49.6 per 100,000), Waikato (44.4 per 100,000), Wairarapa (36.6 per 100,000), Capitol and Coast (35.4 per 100,000), Southland (32.9 per 100,000) and Hawkes Bay (32.7 per 100,000). Auckland had a notified rate of 9.8 per 100,000. South Canterbury had the highest regional rates for the three years prior to 2005 (ESR, 2006).

The age distribution of cases is predominantly in the under ten years age group. Highest notifications were in the 1-4 group (144.3 per 100,000; 312 cases) followed by those less than 1 year (56.7 per 100,000; 31) and 5 – 9 years (46.5 per 100,000; 133) (ESR, 2006). These figures are adjusted for the New Zealand population age distribution based on the 2001 census.

The reported rates of cryptosporidiosis were highest for cases of European ethnicity (25.6 per 100,000; 312) followed by Other (16.0 per 100,000; 40 cases) and Maori (13.7 per 100,000; 72). Pacific Peoples had the lowest notification rate (7.0 per 100,000; 14 cases) (ESR, 2006).

The notification rate for females was slightly higher than for males in 2005: females 24.8 per 100,000; males 22.2 per 100,000 (ESR, 2006).

Risk factor information collected from cases includes; 342/593 (57.7%) had farm animal contact, 171/492 had consumed untreated water, 95/475 (20%) had contact with sick animals, 130/383 (33.9%) consumed food from retail premises, 178/557 (32%) had recreational water contact (147/557 of which was swimming pools), 146/543 had faecal contact and 137/543 (25.2%) had contact with other symptomatic people during the incubation period.

Figure 2: Cryptosporidiosis notifications by year 1996-2005

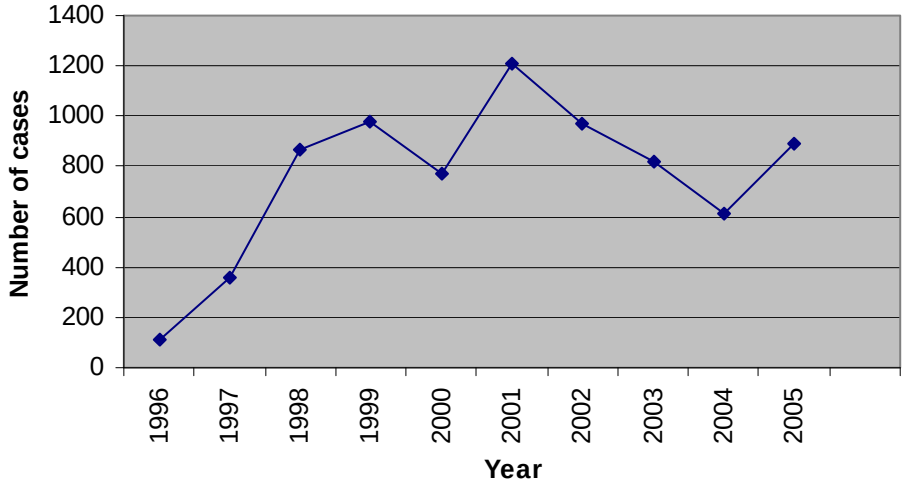
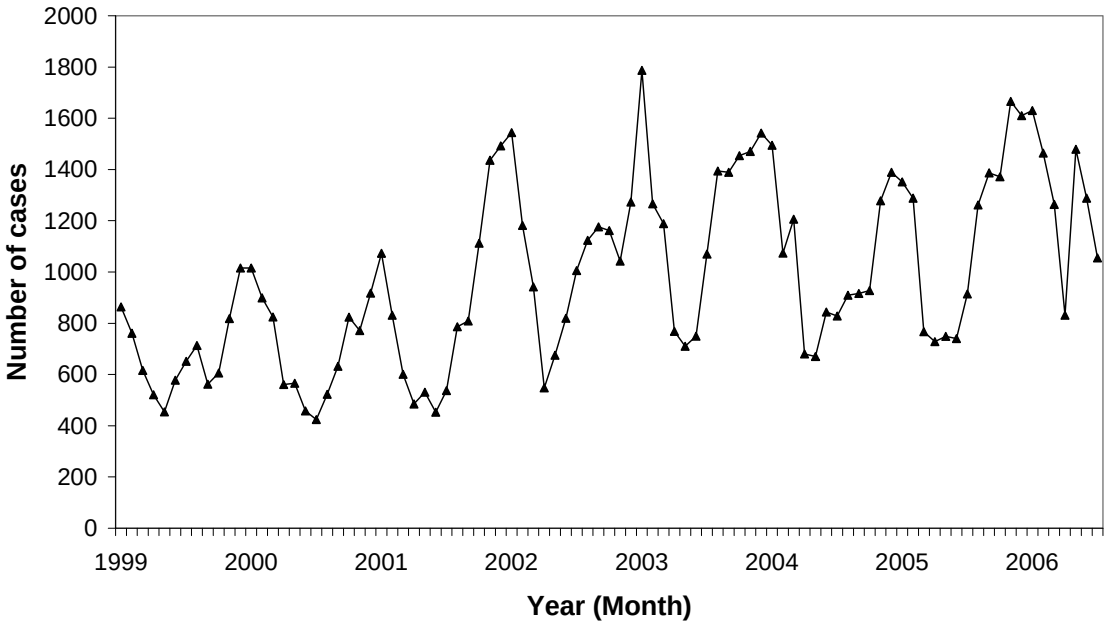


Figure 3: Cryptosporidiosis notifications by month June 1996 – November 2006



Prepared from ESR data (ESR, 2006; Perera 2006)

New Zealand's reported rate of cryptosporidiosis is high by developed world standards (overseas range 1.43 – 16.95 per 100,000 per annum), as shown in Section 6.2.1. However,

such comparisons must be made with caution, as reporting practices may differ between countries.

6.1.2 Clinical consequences of *C.parvum* and *C. hominis* infection

Hospitalisation and fatality rates for notified cases of cryptosporidiosis in New Zealand are given in Table 18. These outcomes are not always reported for each case, so percentages are expressed in terms of the number of cases for which outcomes are known.

Table 18: Outcome data for cryptosporidiosis in New Zealand, 1997 - 2005

Year	Hospitalised cases	Fatalities	Reference
1997	16/329 (4.9%)	0/329	Galloway *et al*., 1998
1998	44/800 (5.5%)	0/800	Perks *et al.*, 1999
1999	47/846 (5.6%)	0/846	Kieft *et al.*, 2000
2000	52/665 (7.8%)	0/665	Lopez *et al.*, 2001a
2001	40/964 (4.1%)	0/964	Sneyd *et al.*, 2002
2002	40/859 (4.7%)	0/859	Sneyd and Baker, 2003
2003	45/697 (6.5%)	0/697	ESR, 2004a
2004	24/517 (4.6%)	0/1517	ESR, 2005a
2005	47/750 (6.3%)	0/750	ESR, 2006a

A search of the Episurv database (cryptosporidiosis and shellfish) found a total of 7 cases where filter feeding shellfish were reported as having been consumed although none of these cases had laboratory confirmation that the implicated food was involved. The cases are shown in Table 19

Table 19: Cases of cryptosporidiosis implicated but not laboratory confirmed in New Zealand, 1999- 2006

Case No.	Food implicated	Comments
1999WK00418	Mussels	From Coromandel, cooked on BBQ
2001CB01443	Oysters	From supermarket
2003GS 00164	Mussels	
2004NL00444	Mussels	
2004OT00859	Mussels	BBQ
2006OT00381	Cockles	Case collected cockles from beach, were cooked. Beach "probably contaminated by septic tanks"
2006WN02544	Mussels	Marinated, purchased from market

6.1.3 Outbreaks

There have been no outbreaks of cryptosporidiosis in New Zealand associated with shellfish.

The first documented outbreak of cryptosporidiosis in Zealand occurred in Hutt Valley. The use of one particular public swimming pool was strongly associated with illness and *Cryptosporidium* oocysts were found in the pool water (Baker *et al*., 1998).

The New Zealand data summarised in Table 20 show that *Cryptosporidium* is identified as the causative agent in around 2 - 7% of reported outbreaks. The average number of cases per outbreak was 8 (calculated from 1997 to 2005). It should be noted that these figures represent all outbreaks of cryptosporidiosis

Table 20: Total number of reported outbreaks and cases for which *C. parvum or C. hominis* was identified as the causative agent in New Zealand 1997-2005

Year	No. of outbreaks	Percent	No. of cases	Percent	Reference
1997	2	1.9	9	0.6	Galloway *et al.,* 1998
1998	7	2.2	152	7.1	Naing *et al.*, 1999
1999	10	2.8	116	4.9	Perks *et al*., 2000
2000	7	2.4	66	2.9	Lopez *et al*., 2001b
2001	27	6.9	147	6.3	ESR, 2002
2002	15	4.5	122	4.2	Boxall and Ortega, 2003
2003	7	2.1	102	3.7	ESR, 2004b
2004	5	1.5	19	0.5	ESR, 2005b
2005	25	7.2	108	4.4	Perera, 2006

There have been no notified outbreaks of cryptosporidiosis associated with shellfish filterfeeders (Trevor Margolin, personal communication, ESR January 2007).

Table 21 shows the transmission routes associated with outbreaks of cryptosporidiosis for the year 2005 in New Zealand (Perera, 2006). The total number of outbreaks do not balance with Table 18 above because there is often more than one vehicle associated with an outbreak.

Table 21: Transmission routes associated with outbreaks of cryptosporidiosis – year 2005

Outbreak source/transmission route	Total no. outbreaks (% of total outbreaks)	Total no. cases (% of total cases in outbreaks)
Water-borne	5 (18.5)	17 (9.2)
Environmental	3 (13.6)	33 (8.0)
Foodborne	1 (0.5)	3 (0.4)
Zoonotic	2 (18.2)	18 (18.6)
Person-to-person	22 (12.9)	97 (5.6)

For previous years, this information is not always reported. In 2004, outbreaks were associated with: water-borne - untreated supply (1 outbreak, 8 cases); person-to-person (2 outbreaks, 6 cases); environmental (1 outbreak, 2 cases) and zoonotic (2 outbreaks, 11 cases). The number of outbreaks of cryptosporidiosis associated with food are not stated. In 2003, again food as a vehicle is not reported. Waterborne (1 outbreak, 4 cases); person-to-person (none); environmental (2 outbreaks,73 cases); zoonotic (none).

6.1.4 Case control studies and risk factors

No case control studies have been located that are specific to consumption of shellfish and cryptosporidiosis.

A risk assessment has been carried out for treated wastewater effluent discharged via an ocean outfall into Pegasus Bay discharging 1.5 km offshore (Ball, 2003). Based on the assumptions used in this risk assessment, there was a very small risk of *Cryptosporidim* infection (maximum infection rate of 1 per 1000; mean = 0.001/1000) from the consumption of filtering-feeding shellfish collected at the shoreline.

6.1.5 Types causing human disease in New Zealand

In New Zealand, 423 human faecal samples from cases were obtained from the different regions between August 2000 and August 2003. The samples were examined by PCR. One rural and one urban region provided the majority of samples. In total, *C. hominis* accounted for 47% and *C. parvum* (bovine) 53% of the isolates. *C. hominis* was dominant in cases from urban regions whereas *C. parvum* was dominant in cases from rural areas (Learmonth *et al*., 2004). A further novel species with a close relationship to *C. canis* was detected in two unrelated children from different regions. Disease caused by *C. parvum* was most prevalent during the spring months and this was attributed to the calving and lambing activity at this time. *C. hominis* was dominant during the autumn months. The geographic distribution of *Cryptosporidium* spp. in cryptosporidiosis cases in New Zealand can be found in Table 22. Note that recent surveillance of *Cryptosporidium* has shown a decline in the autumn peak of *C. hominis*, with its total disappearance in some years (Moriarty and Learmonth, 2007).

Table 22: The geographical distribution of *C. hominis, C. parvum and C. canis* from human cases in New Zealand

Region	*C. hominis*	*C. parvum*	Novel (*C. canis*)	Total*
Wellington	109	13		122
Hamilton	40	72	1	113
Hawkes Bay	21	15		36
Southland	1	34		35
Bay of Plenty	6	27		33
Hutt Valley	12	12	1	25
All others	9	50		59

* Only those referring more than 20 faecal specimens are shown

When incidence data overall is reviewed, there seems to be a trend towards high springtime notification rates and higher rates in the under 9 year olds. District Health Boards with higher notification rates tend to be largely rural.

6.2 Adverse Health Effects Overseas

6.2.1 Incidence

Table 23 compares the rate of cryptosporidiosis incidence between countries.

Table 23: Comparison of cryptosporidiosis incidence between countries

Country	Period	Rate /100,000	Reference
USA (FoodNet)	2006	1.91	CDC, 2007
	2005	2.95	CDC, 2006
	2004	1.43	CDC, 2005
Belgium	1998	7.4	Duncanson *et al*., 2000
Canada	2000	2.67	Health Canada, 2006
	2001	7.47	
	2002	2.46	
	2003	2.61	
	2004	1.85	
Ireland	2004	11.0	Garvey and McKeown, 2005
England and Wales	1999	9.03	WHO, 2000
	2000	10.95	WHO, 2000
	2004	6.8	Garvey and McKeown, 2005
Scotland	1999	11.68	WHO, 2000
	2000	16.95	WHO, 2000
	2004	9.1	DEFRA. 2006; Garvey & McKeown, 2005
Northern Ireland	1998	10.7	Duncanson *et al.,* 2000
Wales	2005	15.61	CDSC Wales, 2006
	2006#	6.23	

Data up to 29/10/2006

The figures given for the USA are collected from 10 US States in a network collectively known as FoodNet. The surveillance population from 1996 to 2005 has grown from 5% of the US population in 5 States to 15% of the population in 10 States. Surveillance for cryptosporidiosis began in 1997 but comparing 2005 data with the baseline data collected 1997-1998 shows that the estimated incidence of cryptosporidiosis infections has not changed significantly (CDC, 2006). There has been no National Health Objective set for cryptosporidiosis.

Putting cryptosporidiosis into context, an estimated 2.5 million (7%) of foodborne diseases in the USA have been attributed to parasites (Slifko *et al*., 2000). Of these, 300,000 cases were associated with *C. parvum*. The percentage of foodborne cryptosporidiosis transmissions was estimated at 10%.

Ten percent of the cryptosporidiosis cases are also estimated to be foodborne in Australia, which equates to 25,000 cases (Australian Government, 2005).

Work in Tasmania found that 2.4% of diarrhoeal specimens examined contained oocysts and that most cases were less than 5 years old. An association was made in this study with consumption of raw milk or contact with animals.

In Ireland, cryptosporidiosis became a notifiable disease for all age groups in 2004, it is the most common protozoal gastrointestinal pathogen in that country based on notification data.

Cryptosporidiosis is not a notifiable disease in Sweden nor is it notifiable in animals. Approximately 40 cases are reported in humans annually although this will probably not reflect the true occurrence.

Cryptosporidiosis is not a notifiable disease in Denmark, only cases with histories of persistent diarrhoea or recent travel are routinely tested. By this screening, 66, 84, 38, 58, 30 cases were diagnosed in 2001 - 2005 respectively (Anonymous, 2002, 2003, 2004, 2005, 2006). An additional 99 patients were recorded in connection with the outbreak associated with carrots – described below.

In terms of prevalence; Ungar (1990) (cited in Fayer 2004) has carried out a thorough review of geographic distribution and prevalence of oocysts and seroprevalence in humans. Based on the number of oocysts detected in human faeces, the following estimates of the prevalence of human cryptosporidiosis have been made; Africa 2.6 – 21.3%; Central and Southern America 3.2 – 31.5%, Asia 1.3 – 13.1%. These figures are greater than those estimated for Europe 0.1 – 14.1% and North America 0.3 – 4.3%. Better sanitation and cleaner potable water in industralised countries are some of the reasons given for the difference.

Over 100 studies on the prevalence of cryptosporidiosis in more than 40 countries were reviewed by Current and Garcia (1991). Prevalence of the illness in industralised countries were much lower than in the Ungar study. For example North America and Europe were between 1 and 3%. In developing countries the prevalence estimates were 5% in Asia to 10% in Africa, and increases were noted with warmer and/or wetter months. In general, the prevalence rates are lower than the incidence rates as would be expected.

Table 24 below summarises the reported outbreaks (and cases) overseas that have been associated with a food. Note there are no reports of outbreaks associated with shellfish.

Table 24: Reported cryptosporidiosis outbreaks (or cases) overseas associated with food

Country	No. of cases	Food type associated	Reference
Australia (Queensland)	8	Raw cow's milk	Harper *et al.*, 2002
Australia	2	Raw goat's milk	WHO, 1984
Denmark	99 (12 laboratory confirmed)	Carrots (human carrier related)	Anonymous, 2006
Mexico	1	Salad from street vendor	Sterling *et al*., 1986
Mexico	22	Possible milkborne	Elsser *et al.*, 1986
UK	50 (school	Cow's milk, on-farm	Gelletlie *et al.*, 1997

Country	No. of cases	Food type associated	Reference
	children)	pasteuriser faulty	
UK	1	Frozen tripe	Nichols and Thom, 1985
Wales	19	Sausage (positive correlation)	Casemore *et al.,* 1986
USA	154 (284 exposed)	Unpasteurised apple cider (windfalls on ground grazed by livestock)	Millard *et al*., 1994
USA	31	Unpasteurised apple cider, processed with well water	Peng *et al.,* 1997
USA	15 (26 exposed)	Chicken salad (food handler related)	CDC, 1996
USA	54 (62 exposed)	Green onions (unwashed)	CDC, 1998
USA	148	Fruits/vegetables (food handler related)	Quiroz *et al.,* 2000

Source: Millar *et al*., 2002; Duffy and Moriarty, 2003

In the Danish outbreak which occurred in a company canteen, the carrots were kept in a large bowl of water, it was thought that a human carrier contaminated the water (Anonymous, 2006; Eurosurveillance 2005).

There has been one case of cryptosporidiosis in a Swiss HIV positive patient who had eaten raw oysters while in France (Baumgartner *et al.,* 2000). However, contamination of the oysters by *Cryptosporidium* was not examined, and the potential for contamination was inferred from other studies.

6.2.2 Contributions to outbreaks and incidents

The number of cryptosporidium outbreaks by transmission route has been collated for the years 1983 to 2005. This work was carried out for the UK (Nichols *et al*., 2006). This information is summarised in Table 25 below.

Table 25: Summary of *Cryptosporidium* outbreaks in the UK by transmission route: 1983 - 2005

Outbreak source/transmission route	Total no. outbreaks	Total no. cases (lab positive)
Public drinking water supply	55	7097 (5821)
Private drinking water supply	6	176 (30)
Swimming pool	43	799 (490)
Interactive water features	3	191 (66)
Paddling pools	2	13 (6)
Other recreational water	2	27 (12)
Animal contact	16	936 (294)
Farm (transmission route unknown)	3	25 (19)

Outbreak source/transmission route	Total no. outbreaks	Total no. cases (lab positive)
Foodborne	4	140 (81)
Person-to-person	10	276 (111)
Unknown	5	148 (141)
Total	149	9851 (7071)

Source: Nichols *et al*., (2006)

In terms of foodborne outbreaks, the authors note the difficulty in detecting outbreaks due to the technicalities of finding oocysts in food and lack of routine isolate typing. In addition, outbreaks in tourist resorts were also considered unlikely to be detected through normal surveillance procedures.

6.2.3 Case control studies overseas

A number of case control studies have been conducted, particularly in relation to public drinking water supplies (Nichols *et al*., 2006). None have been conducted for consumption of shellfish because, to date, no cases have been reported with laboratory confirmation that the disease is linked to shellfish.

6.2.4 Risk assessment and other activity overseas

A quantitative assessment of viable *C. parvum* oocysts in commercial oysters in the Chesapeake Bay (Maryland, USA) has recently been published (Graczyk *et al.,* 2007) and suggests reasons for the lack of human cryptosporidiosis associated with shellfish. The authors explain that there are three key factors in a risk assessment, these are;

- *Cryptosporidium*- specific;
- consumer related; and
- environmentally-derived.

The interaction of these three factors may explain the discrepancy between the widespread prevalence of shellfish and environmental contamination by *Cryptosporidium*, and lack of associated human illness.

Thirty market size oysters were collected from each of 53 commercial harvesting sites and quantitatively tested by the IFA method – in groups of six. These samples were later retrospectively retested for viable oocysts by the combined fluorescent in situ hybridisation method (FISH) then IFA. Lower numbers of oocysts were enumerated by the combined FISH and IFA, due possibly to the elimination of dead oocysts, oocysts of other *Cryptosporidium* species and elimination of small unicellular algae detected by the IFA method.

The mean number of oocysts in six oysters was 42.1 ± 4.1. This was significantly higher than the number of viable oocysts (28.0 ± 2.9) for reasons given above. Of the 265 oyster groups, 221 (83.4%) contained viable oocyts, and of those total viable oocysts, a mean of 23% (range 10-32%) originated in the haemolymph as opposed to the gill washings. The amount of viable oocysts was not related to the oyster's size (range 92.8g to 216g) nor to the quality of water at the sampling site (i.e. number of faecal coliforms).

Taking this assessment further, the authors considered the worst case scenario. Firstly *C. parvum* isolates differ in their virulence to humans. Three isolates; TAMU, IOWA and UCP varied from 9-18, 87-190 and 1042-2980 oocysts respectively to achieve a median infective dose in healthy volunteers. Even when oysters are contaminated with the most virulent isolate (i.e TAMU at ID_{50} = 9), 205/265 (77.4%) of the oysters in this study would have been potentially able to cause infection in 50% of exposed individuals. This translates to 265 oyster meals potentially causing infection in 100 people assuming they are all immunocompetent and have no pre-existing anti-*C. parvum* serum immunoglobulin Ig. If people with partial immunity (previous exposure) were exposed, the ID_{50} becomes more than 20 fold higher. Therefore the results from the Graczyk *et al.* (2007) study suggest that infections are not likely in oyster consumers who are partially immune, even when they consume the most virulent isolate of *C. parvum* in a raw oyster.

Even the most heavily contaminated oysters did not contain more viable oocysts than the intermediate ID_{50} value, i.e. the ID_{50} value for IOWA is 190 oocysts.

While immuno-compromised populations are at greater risk from infection, they may also be more acutely aware of the potential health hazards of consuming raw shellfish and therefore likely to avoid this food.

It was notable that 23% of the viable oocysts in this assessment originated from the haemolymph. When an oyster is shucked, both sides of the abductor muscle are severed. As this muscle is a haemolymph rich organ, leakage is inevitable. Therefore the authors conclude that the actual number of viable oocysts ingested would be lower than estimated from total counts.

Other risk attenuating factors cited were that oocysts enter the human gastrointestinal tract while deeply buried within oyster tissue, preventing or delaying the stimuli to trigger oocyst excystation. In mouse bioassays, used to assess viability, the oocysts are delivered purified from the oyster tissue and directly into the gastric region. In addition, false-negative results would be returned for *C. hominis* oocysts in this animal model. The authors suggest piglets as a more relevant animal model especially if fed whole oyster meat (simulating human conditions).

The authors concluded that although oysters are frequently found to be contaminated with oocysts, the actual number of viable oocysts is significantly less and may be too low to frequently cause cryptosporidiosis in healthy individuals.

They further conclude that IFA methods should not be used for oocyst enumeration because of the overestimation effects (dead oocysts, other species and algae). Whatever detection technique is used, recovery will not be 100%. Previous studies by the author demonstrated a 51.1% recovery rate of oocysts, that calculates to a detection limit of 19 oocysts /0.7 ml of mussel tissue homogenate (Graczyk *et al.,* 1999b), therefore the number of viable oocysts reported in this assessment are likely to be underestimated.

The size of the oyster was also discussed. Even though the filtering capacity of an oyster is proportionate to its size, no correlation between the size of oyster and number of oocysts recovered was observed in this assessment. This suggests some determinant other than size is influential in oocyst concentration. In addition, the assessment confirmed that faecal

coliform numbers do not correlate with oocyst contamination in edible oysters so that monitoring shellfish waters for these coliforms is not suitable to indicate *Cryptosporidium* presence.

6.3 Estimate of Risk for New Zealand

There have been no notified cases or outbreaks of cryptosporidiosis conclusively linked to shellfish consumption either in New Zealand or overseas (although sporadic cases of infection may not be investigated). This is despite extensive evidence for presence in shellfish (overseas) and environmental contamination (New Zealand and overseas). There are suggestions that this may be due to exposure and dose-response factors.

The risk of cryptosporidiosis infection from eating raw or lightly cooked shellfish collected from commercial New Zealand growing waters appears to be very low due, supported by the sanitary measures in place. The risk from feral shellfish gathering is probably higher, but nevertheless this food has not featured in surveillance data to date, and the risk remains low.

6.4 Risk Categorisation

Cryptosporidium was included in the severity rating in the risk categorisation system in the Appendices to other Risk Profiles. The proportion of severe outcomes (hospitalisation, long term sequelae, and death) resulting from *Cryptosporidium* is approximately < 0.5 % placing this disease in the lowest severity category (3).

No incidence estimate is possible.

7 RISK MANAGEMENT INFORMATION

7.1 Relevant Food Controls

In New Zealand, the Microbiological Reference Criteria (Ministry of Health, 1995) category for ready-to-eat foods (with some components not cooked during manufacture), best describes raw shellfish. The APC criterion is n = 5, c = 2, m = 10^5 and M = $5x10^5$. There is no criterion in respect of *Cryptosporidium* oocysts.

7.1.1 HACCP guide for the Seafood Industry

In the HACCP guide for the seafood industry (MAF, 1997), a generic model for frozen half shell mussels and pottled fresh oysters has been produced. The guide is available at the following website address;

http://www.nzfsa.govt.nz/animalproducts/seafood/guidelines/haccp/seahaccp.pdf.

In the case of the oysters, shelf life and storage conditions given are 7 days at chilled temperature maintained at 4°C or cooler, with labelling instructions in accordance with IAIS 004 and IAIS 002. Food Safety Objectives are stipulated for the product are as follows;

1. APC/g n=5, c=1, m=10^5, M=$5x10^5$ and *E. coli*/g n=5, c=1, m=2.5, M=7,
2. shellstock are sourced from approved growing areas, open at the time of harvest, and
3. to minimise the presence of shell pieces.

7.1.2 Bivalve Molluscan Shellfish Regulated Control Scheme

On 1 June 2006, the Animal Products (Regulated Control Scheme-Bivalve Molluscan Shellfish) Regulations 2006 and the Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006 came into effect.

http://www.nzfsa.govt.nz/animalproducts/legislation/notices/animal-material-product/shellfish/bmsrcsspecv-16_2_signed.pdf

The Notice covers sanitary surveys, bacteriological standards, growing and classification areas, harvesting, conditions for relaying, wet storage, marine biotoxin control, transport, personnel health, handling, microbiological risk management and laboratory requirements. The Notice does not cover further processing which is subject to risk management programmes developed by individual businesses under the Animal Products Act 1999.

Under the interpretation of terms in the Notice, pathogens include parasites (with *Cryptosporidium* given as an example). This is the only mention of this organism.

7.1.3 Animal Products Act 1999

Risk Management Programmes (RMPs) under the Animal Products Act 1999 are part of the emerging food assurance system in New Zealand. These are similar to Food Safety Programmes (FSPs) required by the Food Act 1981.

The Animal Products Act 1999 reforms the New Zealand law that regulates the production and processing of animal material and animal products to:

- manage associated risks; and
- facilitate overseas market access.

The Animal Products Act requires all animal products traded and used to be "fit for intended purpose". This means they must meet New Zealand animal product standards. The New Zealand animal product standards are contained in Part 1 of the Animal Product Regulations 2000.

The risk management system potentially applies anywhere in the value chain from production, through processing to the market. The risk management system comprises the following main types of controls:

- risk management programmes;
- regulated control schemes; and
- controls relating to the export of animal material and animal products.

The Animal Products (Ancillary and Transitional Provisions) legislation has enabled a staggered implementation of RMPs under the Act. This schedule was developed by NZFSA. All animal product primary processing businesses are required to have a RMP except those exempt under the Act or exempt under the Animal Products (Exemptions and Inclusions) Order 2000.

A risk management programme is a documented programme to identify and manage biological, chemical and physical hazards. The programme is to be based on the principles of Hazard Analysis and Critical Control Point (HACCP): identifying the hazards, the systems of control, and demonstrating that the controls are effective. Risk management programmes are to be designed by individual businesses for the animal materials used, the processes performed and the product range produced.

7.1.4 Post harvest treatments: depuration and relaying

In New South Wales, depuration in tanks prior to sale appears to have reduced the number of oyster associated outbreaks of gastroenteritis although this measure may not be completely effective (Fleet *et al.*, 2000). Disinfection of the depuration water by UV radiation is favoured over other methods because few residuals are left in the water. Ozone, chlorine and iodophors are also used. A review of depuration has been carried out by Jackson and Ogburn (1999). The effectiveness of standard UV depuration at inactivating *C. parvum* in Pacific oysters was recently published (Sunnotel *et al.*, 2007). The authors reported a 13-fold inactivation of recovered viable oocysts at standard UV levels (50 W per pass) and a 9-fold inactivation in half-power tanks (25 W per pass, (water circulation 1,200 litres/min, water temperature <15°C). However low numbers of viable oocysts were still recovered following these treatments.

Relaying of shellfish to clean growing waters is an approved treatment (under the Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006). It is currently

employed by Northland oyster farmers where the oyster farming waters have been classified as "restricted" following the detection of norovirus in oysters. It is interesting to note that the waters in this area have met statutory requirements for bacterial contamination.

Depuration in New Zealand is allowed under the Animal Products (Specifications for Products Intended for Human Consumption Notice 2004. The depuration facility must have a Risk Management Programme registered by NZFSA. However, depuration is not considered an effective risk management strategy for the removal of enteroviruses with only two facilities registered in New Zealand, one for cockles and one for surf clams.

7.1.5 Consumers

Other than physically posting risk communication signs at popular collection sites for feral shellfish, very little could be found in relation to risk communication to the public and *Cryptosporidium* oocysts in shellfish. In New Zealand, health protection agencies have issued web-based consumer information factsheets concerning commercial and private shellfish gathering. For example, Otago and Southland Public Health Unit information can be found at; http://www.phsouth.co.nz/prot_shellmonitor.html.

A study into the behaviour of Pacific Islanders living in Auckland found that 50% (n=55) reported ignoring warning signs at shellfish collection sites, deciding that the shellfish was fit for human consumption (Fakalago, 2001).

In the USA, the USFDA website contains recommendations to prevent and control cryptosporidiosis. It is noted that under the foods to be avoided section, there is no mention made of seafood
http://www.cdc.gov/ncidod/dpd/parasites/cryptosporidiosis/factsht_cryptosporidiosis.htm#12

7.1.6 Environment

7.1.6.1 Sewage

An example of how the discharge of sewage by commercial and recreational boats affects oysters was demonstrated during investigations into the outbreaks of Norwalk-like virus in the Auckland area (Simmons *et al*., 2001). Sewage was suspected of causing the contamination of commercial oyster growing areas. Provisions in the Resource Management (Marine Pollution) Regulations 1998 and subsequent amendments aimed to reduce the impact of such contamination. From July 2002, the discharge of 'Grade A treated' sewage from boat holding tanks is permitted beyond 100 metres from marine farms, and 'Grade B treated' or untreated sewage discharge is permitted beyond 500 metres from marine farms or mataitai reserves in water depths greater than five metres. A rule may only be included in a regional coastal plan or a proposed regional coastal plan relating to the discharges under this regulation if the rule increases the distances seaward or increases the depth specified. Most recreational boats discharge either untreated or grade B treated sewage.

Pressures from population growth and incidents of pollution from on-site wastewater systems, have led the Far North District Council to introduce a bylaw on wasterwater disposal systems. The bylaw, that came into force in July 2006, aims to minimise the effect of septic tank leachate on public health, the environment at large and marine environments in

particular. This bylaw appears to go some way towards mitigating the risk from this particular source (Mike Wright, personal communication, Far North District Council, August 2007). The bylaw (Chapter 28: Control of onsite wastewater disposal systems) requires owners to desludge and provide full onsite assessment reports of their septic tank systems at intervals of not more than 3 years.

7.1.6.2 Urine

In Sweden, the survival of *C. parvum* in urine (separated at source) was investigated as part of a wider study into sustainable agriculture and the reuse of human urine, a Swedish practice is to store urine for 6 months before use in agriculture (Hoglund and Stenstrom, 1999). Nitrogen present in urine is largely in the form of ammonia and pH value around 9. The effects of ammonia, cattle manure and pH have been discussed already in this document, the activity of free ammonia having been shown to have a significant effect on the viability of oocysts. The authors found that *C. parvum* oocysts in untreated urine were inactivated below the detection limit (1/300) within 63 days, the authors further found that the antiprotozoan effect of urine was assisted by factors besides pH.

7.1.7 Risk management options/ studies overseas

A list of international and USA regulations and guidelines for seafood can be found on the following website address: http://seafood.ucdavis.edu/guidelines/international.htm and http://seafood.ucdavis.edu/guidelines/usguidelin.htm.

In Australia, a Proposal for a Primary Production and Processing Standard for Seafood (P265) was first raised by Food Standards Australia New Zealand in December 2002. The final Standard (FSANZ, 2005) applies to Australia only and not to New Zealand (Standard 4.2.1). The Standard came into effect on 26 May 2006. The proposal is for a set of national standards to protect public health and safety by implementing a set of basic safety provisions for medium and low risk categories, and specific measures to manage higher risk products (particularly oysters and bivalve molluscs). An interpretative guide to Standard 4.2.1 has been produced, entitled Safe Seafood Australia (FSANZ, 2006).

Division 3 of the guide (FSANZ, 2006) sets out specific requirements for bivalve molluscs, terms are defined and food safety management systems are discussed. Appendix 1 also sets out harvesting (approved, conditionally approved, approved as remote or offshore), harvesting for depuration or relaying and post-harvest temporary wet storage requirements.

The New Zealand Submission to the Draft Assessment Report is available at the NZFSA website; http://www.nzfsa.govt.nz/labelling-composition/publications/submissions-to-fsanz/p265.htm.

In terms of screening shellfish routinely with commercially available reagents, the process would be laborious and expensive. In addition, there is the possibility of false-positive identification (Fayer *et al.,* 1997). Currently this does not appear to be economically viable option.

7.2 Economic Costs

No estimates for the cost of cryptosporidiosis for New Zealand have been located. An assessment of the total medical costs and productivity losses was made following the Milwaukee, Wisconsin cryptosporidiosis outbreak (waterborne) in 1993 (Corso *et al*., 2003). The outbreak affected an estimated 403,000 residents with a total cost of outbreak-associated illness being $96.3 million ($31.7 million medical and $64.6 million productivity). This means that the average total costs for persons with mild, moderate and severe illness were $116, $475 and $7807 respectively. Figures quoted are in US dollars.

7.3 Other Transmission Routes

7.3.1 Other transmission routes: food

Foods associated with outbreaks include raw milk, carrots (salad), chicken salad, frozen tripe, sausage, unpasteurised apple juice, green onions, fruits and vegetables. In these events, there has either been a food handler issue or contact of fruit with faeces from orchard grazing livestock/wildlife.

7.3.2 Other transmission routes: environment

Duncanson *et al.,* (2000) investigated 915 cases of notified cryptosporidiosis in New Zealand. For 790 of the cases, drinking water distribution zones were identified. The cases were categorised according to the gradings given to the water supplies. 633 cases (80%) lived in drinking water zones that complied with the New Zealand drinking water standard while 138 (17%) had unsatisfactory drinking water supplies. The geometric mean annual rate of cryptosporidiosis was 48.6 per 100,000 (range 2.0-1304.3) in complying water supply zones and 184.8 (12.3-5000) in unsatisfactory supply zones. The difference was statistically significant. Therefore the lowest mean rates of reported cryptosporidiosis occurred in communities served by drinking water supplies with completely satisfactory public health gradings. Although confounding variables made the quantification of the risk unviable, the authors state that improvements in drinking water quality were likely to effect health benefits at the population level. It was also noted that a high proportion of cases still occurred in areas where the drinking water supply was "satisfactory" highlighting the importance of non-drinkingwater modes of transmission.

Overseas, drinking water outbreaks have accounted for a number of large scale outbreaks involving thousands of people. Some of the larger outbreaks have occurred in Texas (2006 cases), Georgia (12,960), Oregon (15,000), Ontario (>1000), British Columbia (14,500), British Columbia (2097), Japan (>9000) and the UK (>4321). The largest water-borne outbreak ever recorded was in Milwaukee, Wisconsin in April 1993. Cryptosporidiosis affected an estimated 403,000 people and was associated with a potable public water supply. Fifty-four associated deaths were recorded during the two years following the outbreak (Fayer, 2004).

Rainfall and runoff events are major factors determining microbial loading of oocysts in surface waters and reservoirs (Fayer, 2004).

Swimming is a risk factor for the illness because of the contamination of recreational waters used for swimming. In public swimming pools, outbreaks have also occurred due to faecal accidents, resistance to chlorine disinfection, low infectious dose and high density of swimmers. Transmission increases where pools are used by preschool children and incontinent people (Fayer, 2004).

Another recognised transmission route is hand to mouth infection of children following educational visits to petting farms. For example, an outbreak of 20 confirmed cases (19 under the age of 7 years) was reported in the Wellington region following a 2 day educational

farm event. The most likely route of infection was hand to mouth transfer after touching an infected animal (Stefanogiannis *et al*., 2001).

8 CONCLUSIONS

8.1 Description of Risks to Consumers

8.1.1 Risks associated with shellfish

Bivalve filter feeding molluscs have been frequently found contaminated with oocysts overseas. Given the low sensitivities of detection methods, the numbers reported may be an underestimation. Conversely, there is potential for false-positives to be reported due to the mistaken identification of other similar sized organisms. Some detection methods may also enumerate empty oocysts that are clearly unviable.

A quantitative risk assessment on oysters in the United States concluded that the actual number of viable oocysts consumed is significantly less than previous estimates, for a variety of reasons, and may be too low to frequently cause cryptosporidiosis in healthy individuals.

The lack of reported human cryptosporidiosis associated with shellfish must be a key consideration when interpreting the literature on the subject of *Cryptosporidium* oocysts in shellfish. Despite reports of shellfish contamination overseas, the ineffectiveness of depuration, survival of the oocyst in seawater and many shellfish being consumed raw or lightly cooked, the expected disease burden in the human population is either not being detected or not occurring in the first instance.

Feral shellfish gathered from areas impacted by human sewage or livestock runoff may be contaminated with oocysts but data is not available to quantify this risk, and there is no evidence for illness from surveillance data. Although coliform testing is not appropriate as an indicator for *Cryptosporidium* contamination in marine waters, the risk management of human or animal faecal contamination associated with such testing will reduce the risk of contamination in commercial shellfish beds.

The evidence from surveys of freshwaters, sewage influents and effluents, livestock, meat works effluents, and human faecal samples indicates that *Cryptosporidium* contamination is widespread in New Zealand. The association between high levels of contamination in shellfish overseas with rainfall is consistent with agricultural runoff being an important contributing factor. Nevertheless, this likely contamination of shellfish in New Zealand is not being reflected in the notified disease or outbreak data.

Although reporting systems will differ, New Zealand apparently has a higher notified cryptosporidiosis rate than other developed countries. The available information from outbreaks and analysis of notified cases suggest that waterborne transmission, both potable water and recreational water (especially swimming pools) are more important than other sources. Analysis of *Cryptosporidium* types found in livestock and human faecal samples suggest that zoonotic contact also plays an important role.

Overall the risk of *Cryptosporidium* infection from consuming shellfish in New Zealand appears to be very low. The reasons for this are unclear, as contamination of at least feral shellfish appears likely. Local marine conditions may play a role, or exposures may be too low to cause infection.

8.1.2 Risks associated with other foods

Outbreak data for New Zealand suggest that foodborne transmission make a very small contribution to the overall burden of cryptosporidiosis. Outbreaks overseas have usually been caused by contaminated raw milk or produce.

8.1.3 Risk assessment options

At this stage a quantitative risk assessment on this food:hazard combination would not be possible due to incomplete data. The apparent burden of disease suggests that such an assessment is unnecessary.

8.2 Commentary on Risk Management Options

Many studies have made an association with the density of grazing ruminants, particularly cattle, farm practices, rainfall events and the distribution of *C. parvum* oocyst around river mouths and estuaries environments.

The control of contamination of molluscan shellfish in the growing environment is currently managed by monitoring growing waters and shellfish. None of this monitoring includes mandatory testing for *C. parvum* or *C. hominis* (Animal Products (Specifications for Bivalve Molluscan Shellfish) Notice 2006.

Relaying of product from 'restricted' growing areas to clean waters is an option that is occasionally practised in New Zealand where pollution events have occurred. Relaying of shellfish to clean growing waters is an approved treatment that has been shown to be successful for virus removal (Greening *et al.*, 2003) although there are mixed scientific views on whether depuration works in the case of oocysts. Relaying requirements in New Zealand are based upon the EU Directive 91/492 for depuration (this Directive is bacterial based rather than oocyst or virus based). The Directive specifies that, for shellfish grown in heavily contaminated waters, a minimum two months relaying period in clean waters is required and that the relay area must be a minimum of 300 metres from any production area. The NZFSA has approved relaying as a process for virus cleansing, there is no specific mention of *Cryptosporidium* oocyst cleansing. Requirements are that the relay period be a minimum of eight weeks and the relay area be a minimum of 300 metres from other production areas (Greening *et al.*, 2003).

There are regulations in place prohibiting sewage discharges from boats within certain defined areas of marine farms and reserves, how effective these provisions are in protecting shellfish from oocyst contamination is unclear.

8.3 Data gaps

- There is very little evidence to support the use of faecal coliforms to indicate the presence of *Cryptosporidium* spp. This may be an area for further research;
- Immunity status of the population;
- Prevalence and quantitative data on *C. parvum* or *C. hominis* oocysts in shellfish, particularly in feral shellfish areas impacted by sewage and agricultural runoff;

- Prevalence and quantitative data on *C. parvum* or *C. hominis* oocysts in New Zealand marine waters, particularly in feral shellfish areas impacted by sewage and agricultural runoff;
- *Cryptosporidium* oocyst carriage rates in waterfowl and sea-gulls in New Zealand; and
- Effect of the different salinities and temperatures in New Zealand seawaters on the uptake of *Cryptosporidium* oocysts and retention by bivalve filtering feeding shellfish. In addition, the viability and infectivity of oocysts in the New Zealand marine environment.

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APPENDIX 1: CATEGORIES FOR RISK PROFILES

The assignment of a category for a food/hazard combination uses two criteria: incidence and severity.

1. Incidence

The incidence is an estimate of the proportion of the foodborne disease rate due to an individual hazard, that is transmitted by a single food or food group.

The overall rate of foodborne disease caused by individual hazards can be derived from information in the published estimate of foodborne disease (Lake *et al*., 2000). This estimate has been updated to reflect more recent notifications rates for the 12 months to June 2001, but still using 1996 census figures (3,681,546 population). Rates include estimates for unreported cases who do not present to a GP.

Disease/organism	Food rate (/100,000 population) Calculated for 12 months to June 2001	Food rate (/100,000 population) Calculated for 12 months to December 1998
Campylobacteriosis	1320	2047
Listeriosis	0.4	0.4
VTEC/STEC	1.9	1.4
Salmonellosis	176	230
Yersiniosis	38	62
Shigellosis	7	7
NLV*	478	478
Toxins*	414	414
Typhoid*	0.3	0.3
Hepatitis A*	0.4	0.4

* not recalculated.

These are **total** foodborne rates, so it is probably safe to assume that in most cases the rates associated with a particular food are likely to be an order of magnitude lower. For instance, a category of ">1000" would only be assigned if it was decided that all campylobacteriosis was due to a single food/food type.

The following categories are proposed for the rates attributable to a single hazard/food (or food group) combination:

Category	Rate range	Comments/examples
1	>100	Significant contributor to foodborne campylobacteriosis Major contributor to foodborne NLV
2	10-100	Major contributor to foodborne salmonellosis Significant contributor to foodborne NLV
3	1-10	Major contributor to foodborne yersiniosis, shigellosis
4	<1	Major contributor to foodborne listeriosis

A further category, of "no evidence for foodborne disease in New Zealand" is desirable, but it was considered more appropriate to make this separate from the others. Also separate is another category, of "no information to determine level of foodborne disease in New Zealand".

The estimation of the proportion of the total foodborne disease rate contributed by a single food or food group will require information from a variety of sources including:

- exposure estimates
- results from epidemiological studies (case control risk factors)
- overseas estimates

For illnesses where the rate is <1 per 100,000 the ability to assign a proportion is unlikely to be sensible. For such illnesses it may be more useful to consider a Risk Profile across the range of all high risk foods, rather than individual foods or food groups.

2. Severity

Severity is related to the probability of severe outcomes from infection with the hazard. The outcomes of infectious intestinal disease are defined in the estimate of the incidence (Lake *et al*, 2000) as:

- death
- hospitalised and long term illness (GBS, reactive arthritis, HUS)
- hospitalised and recover
- visit a GP but not hospitalised
- do not visit a GP

The first three categories of cases were classed as severe outcomes. Some hospitalisations will result from dehydration etc. caused by gastrointestinal disease. However, for infections with *Listeria* and STEC hospitalisation will result from more severe illness, even if recovery is achieved.

The proportion of severe outcomes resulting from infection with the hazards can be estimated from the proportion of cases hospitalised and recover, hospitalised and long term illness, and deaths (Lake *et al.*, 2000).

Disease/organism	**Percentage of outcomes involving death or long term illness from foodborne cases**
Campylobacteriosis	0.3
Listeriosis	60.0
VTEC/STEC	10.4
Salmonellosis	1.0
Yersiniosis	0.4
Shigellosis	2.7
NLV	Assumed to be <0.5%
Hepatitis A	15.4
Typhoid	83.3
Toxins	Assumed to be <0.5%

Categories for the probability of severe outcomes are suggested as follows:

Severity Category	Percentage of cases that experience severe outcomes	Examples
1	>5%	listeriosis, STEC, hepatitis A, typhoid
2	0.5 – 5%	salmonellosis, shigellosis
3	<0.5%	campylobacteriosis, yersiniosis, NLV, toxins

There are a number of hazards for which the incidence of foodborne disease is uncertain. These have been assigned to the above severity categories as follows:

Severity category 1:

Bacteria

Clostridium botulinum

Protozoa

Toxoplasma

Severity category 3:

Bacteria

Aeromonas/Plesiomonas
Arcobacter
E. coli (pathogenic, other than STEC)
Pseudomonas
Streptococcus
Vibrio parahaemolyticus

Viruses

Others (e.g. rotavirus)

Protozoa

Giardia
Cryptosporidium
Cyclospora
Others (e.g. *Entamoeba*)

Proposed Category Matrix

Incidence	>100	10-100	1-10	<1
Severity 1				
Severity 2				
Severity 3				

Alternatives:

No evidence for foodborne disease in New Zealand

No information to determine level of foodborne disease in New Zealand