Information Statement

Institute of Food Science & Technology Trust Fund

Cyclospora

The Institute of Food Science & Technology has authorised the following Information Statement dated May 2008, replacing that of March 2003**.**

SUMMARY

Cyclospora cayetanensis ***is a human-specific protozoan parasite that causes a prolonged and severe diarrhoeal illness known as cyclosporiasis. This infection emerged in North America in 1996 as the cause of over two thousand cases of food borne disease, with no reported deaths. Further outbreaks that occurred in the USA and Canada in 1997 and 1998 were linked to consumption of the fresh spring crop of raspberries from Guatemala; no cases were identified in Europe. The US Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC) worked with producers in Guatemala to prevent contamination and control this disease. Smaller outbreaks in the US, also in 1997 and 1998, were linked to consumption of mesclun (salad greens, spring mix, field greens or baby greens) and basil, probably grown in the US.***

Cyclosporiasis is endemic to many developing countries and, apart from the outbreaks described above, almost all cases in Europe and North America are confined to travellers returning from tropical countries. The microbiological safety of fresh fruit and salad vegetables depends on the avoidance of contamination with pathogenic microorganisms at all stages of production, most particularly in the field. Increasing importation, into Northern Europe and North America, of soft fruits and salad vegetables from developing countries has the potential to provide a greater risk of contamination by foodborne pathogens, including Cyclospora.

INTRODUCTION

A hitherto relatively obscure protozoan parasite, *Cyclospora cayetanensis* jumped into prominence as an important foodborne human pathogen in May 1996 when 1,465 cases of cyclosporiasis were reported over a few weeks in the United States and Canada. 1.5% of the victims were treated in hospital but there were no deaths (CDC, 1996; Herwaldt et al. 1997). The first *Cyclospora* infection in humans in the USA was diagnosed in 1977 and sporadic outbreaks were reported between 1977 and 1996 (Herwaldt, 2000). These were almost exclusively associated with travellers returning from tropical countries; 4 or 5 cases were reported in New York annually. In the UK, *Cyclospora* was first encountered in 1986 in a patient returning from Pakistan (Bendall & Chiodini, 1995); 43 cases were reported in 1994, 40 of these were returning travellers.

The multiple outbreaks starting in May 1996 in the USA and Canada were mostly attributed to imported raspberries from Guatemala and other imported fruits and vegetables where contaminated water had been used on crops (CDC, 1996; Herwaldt et al. 1997).

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Guatemalan berries may have been responsible for previous cases and outbreaks, including Florida in 1995.

BIOLOGY OF CYCLOSPORA

Cyclospora is a genus of protozoans (subclass Apicomplexa) parasites, which were first described in moles in 1881. *Cyclospora cayetanensis* is specific to humans and was named after Professor Cayetano at the University of Lima in Peru. An outbreak of diarrhoea (21 illnesses) occurred among staff physicians in a Chicago hospital in July 1990. Tap water in a physicians' dormitory was implicated as the cause (Soave, 1996). Doubt was later expressed as to the accuracy of this observation.

Little is known about the biology of *Cyclospora,* partly because of the paucity of material on which to work, since Cyclosporiasis is rarely reported in temperate countries and the organism cannot be cultured outside human tissue. Following ingestion of the transmissive stage (the oocyst) infection develops within gut epithelium cells by asexual multiplication. This is followed by a sexual stage resulting in the production of oocysts which are passed out in the faeces. Oocysts are not immediately infectious and a temperature-dependent maturation (two weeks at 25^0C or up to six months at 4^0C) is necessary (Sterling & Ortega, 1999).

Human sewage potentially contains *C. cayetanensis* oocysts and, where contamination of drinking water occurs, total removal cannot be guaranteed by the use of coagulants, rapid filtration or chemical disinfection with chlorine. The oocysts do not withstand drying or freezing.

IDENTIFICATION

An interest in *Cryptosporidium parvum* and pathogenic enteric protozoa probably contributed to the discovery of *Cyclospora*. Acid-fast stains (e.g. the modified Ziehl-Nielsen technique) sometimes used to detect *Cryptosporidium* oocysts (4-5 microns in diameter) in faeces also stained the larger *Cyclospora* oocysts (8 to 10 microns in diameter). Unstained Cyclospora oocysts auto-fluoresce blue-green under UV light, whereas related *Coccidia*, *Cryptosporidium* spp and *Isospora belli* do not. Procedures for detection by the Polymerase Chain Reaction (PCR) have been described. No commercially available immunological reagents exist for *Cyclospora* (Orlandi et al. 2002*)*.

It is important to remember that not all cases of cyclosporiasis are either detected or reported. Cases go undetected because not all patients with diarrhoea either seek medical attention or have a faecal specimen examined for enteric pathogens. Under-reporting can occur because not all specimens are examined for *Cyclospora* oocysts (some laboratories will not look at all, and some only examine patients with a recent history of foreign travel) although, as stated above, one of the procedures for detection of *Cryptosporidium* oocysts will also detect *Cyclospora* oocysts. Diarrhoeal diseases are greatly under-reported, worldwide (Sharp & Reilly, 2000).

The standard of diagnostic parasitology in the UK is monitored by the National External Quality Assessment Scheme (NEQAS) (Bendall & Chiodini, 1995). In September 1993, a

faecal suspension containing oocysts of *Cyclospora* was distributed to 315 laboratories; 28% of the laboratories identified it correctly, in many cases without previously having encountered the organism, while 45% failed to see any oocysts. In August 1994, fixed faecal smears containing *Cyclospora* were distributed with a request to examine for parasites. Out of the 286 participating laboratories, 236 (82%) detected *Cyclospora;* 36 reported an unexpected parasite while 33 identified it as *Cryptosporidium*. Further evaluation of the testing proficiency of laboratories was carried out in 1996 and 2000, when 299 laboratories took part in the UK's National External Quality Assurance Scheme for microbiology (Cann et al. 2000).

The incidence of the organism is very low in Europe and North America. An examination of 1,042 stool specimens of patients presenting with diarrhoea in Chicago and 6,525 in Burlington, Mass. showed *Cyclospora* in 0.5% and 0.3%, respectively. It was also found in 0.1% of 1,333 stool specimens from healthy individuals in the UK (Clarke & McIntyre, 1994). Cyclosporiasis is endemic in many tropical and sub-tropical areas including Central America, Nepal, Haiti, Peru, the Indian Sub-Continent and parts of Africa (Shields & Olsen, 2003).

CYCLOSPORIASIS

The first *Cyclospora* infection in humans was diagnosed in 1977 and sporadic outbreaks were reported between 1977 and 1996 (Herwaldt, 2000). These were almost exclusively associated with travellers returning from tropical countries; 4 or 5 cases were reported in New York annually. In 1996 and 1997, there were many outbreaks involving hundreds of people in the USA and Canada (1.5% were treated in hospital; no deaths) mostly attributed to imported raspberries from Guatemala and other imported fruits and vegetables, where water contaminated with human faeces had been used on crops. As noted above, Guatemalan berries may have been responsible for previous outbreaks including that in Florida in 1995.

Cyclosporiasis in humans is characterised by long-lasting diarrhoea (average of 43 days), relapsing and cyclical diarrhoea, sometimes alternating with constipation. In the multiple outbreaks in which raspberries were implicated, the number of berries per serving in infected individuals varied from 1 to 7.5 with a median of 2.5 (Herwaldt, 2000). Females and males were equally susceptible. Number of stools per day varied from 1 to 48 with a median of 6. Duration of diarrhoea varied from 1 to 60 days with a median of 10; ten patients noted bloody stools. Fatigue, weight loss (varied from 0.9 to 18.2 with a median of 3.6 kg.), fever (temperature range from 37.2^{0}C to 39.4^{0}C with a median of 38.3^{0}C), cramps and vomiting also occurred. The incubation period ranged from 1 to 14 days with a median of one week. Infection is typically concentrated in the jejunum; in patients with AIDS the bile duct may also be involved. Diarrhoea is usually self-limiting in immunocompetent hosts but can be prolonged in AIDS patients. The oocysts, which do not multiply outside the host (humans and maybe some other primates are the usual hosts) must sporulate before they become infective, which makes direct human-to-human transmission unlikely. The drug of choice for treatment is trimethoprim-sulfamethoxazole (Herwaldt, 2000).

Cyclospora

THE MULTIPLE CYCLOSPORIASIS OUTBREAKS IN NORTH AMERICA

The large number (1,465) of very widespread (over 40 States and Canadian Provinces were implicated) cases and outbreaks recorded in May/June 1966 led to considerable media publicity and public concern. An almost weekly chronicle of events, over the four years that it took to resolve the problem, can be found in Food Chemical News (1996-2001) starting on page 29 of Volume 37.

The New York Times for June 20th 1996 reported that health officials were mystified by the spread of an "exotic microbe". Texas was the first State to report an outbreak. Cyclosporiasis was not reportable in all States at that time but has been since 1999. The Information Collection Rule (ICR) in the United States (designed to assess the threat of *Cyclospora* and similar pathogens in cities with a population over 100,000) should yield more information on the prevalence of *Cyclospora* in the United States. Fresh berries (strawberries and/or raspberries) were incriminated. On June 9th it was announced that Californian strawberries (80% of the strawberries consumed in the USA are Californian) were the cause of the outbreak. The Californian strawberry industry estimated a loss of $40 million arose from this accusation, which was later shown to be unsubstantiated and incorrect. Subsequently, when 37 out of 64 guests at a luncheon in South Carolina became ill with cyclosporiasis, dietary recall showed that raspberries, not strawberries, were responsible; the incriminated raspberries had been imported from Guatemala.

Whereas tropical diseases such as cyclosporiasis used to be confined to travellers returning from tropical countries, the advent of air freight means that potentially-infected products may be now available in any outlet! Raspberries have only been grown in Guatemala since 1987 when the price of the traditional crop - coffee - dropped. The first imports to the USA occurred in 1988. By 1996, Guatemala exported 71,000 tonnes of raspberries (98% of its harvest) to the USA annually.

Exhaustive testing of incoming Guatemalan raspberries failed to find any evidence of *Cyclospora.* Nor were Oocysts detected in the water or on berries in the fields in Guatemala. A *Cyclospora* Advisory Panel was formed, which developed criteria for hygiene in raspberry growing, harvesting and processing. It was suggested that the most likely route of contamination was via the use of sewage-contaminated pesticides that were sprayed on the fruit during the rainy season in Guatemala.

So few *Cyclospora* organisms were available for study (culturing outside human tissue is impossible) that it was not possible to research the biology of the organism, for instance to determine its thermal death rate; how resistant it is to chlorine or other sterilants; how susceptible it is to freezing, etc. It is known that freezing kills the closely related *Cryptosporidium*. At -70^{0}C the organisms die quickly; at -20^{0}C they live for up to 24 hours, while at -15^{0}C they live for a couple of days. Pulsed light kills high levels of *Cryptosporidium*. Ozonisation is a possible safeguard; high (>9) pH is ineffective. Irradiation within the limits allowed for fresh produce is effective against both *Cryptosporidium* and *Cyclospora* and also doubles the shelf life of the berries at chill temperatures (Osterholm, 1999). *Cyclospora* is probably resistant to chlorine: an outbreak has been reported from Nepal where a chlorinated water supply was in use (Shields & Olson, 2003). *Cryptosporidium* is known to be resistant to chlorine at the levels commonly used in potable water (Cann et al. 2000).

Raspberries are covered in thick hairs, which make recovery of any contamination difficult. Spiking experiments showed that only 5 to 15% of *Cyclospora* oocysts were recovered. There was no appreciable difference in recovery rates using distilled water, ethanol, surfactants and enzymes. Five years were to elapse after the US outbreak described above before a much higher recovery rate (70%) was achieved (Ortega, 2001).

Despite efforts to clean up the farms, 7 event-associated clusters of cyclosporiasis were reported in California, Texas, Florida and New York during April and early May 1997. 80 cases were laboratory confirmed but no source was positively identified. Guatemala voluntarily suspended shipments at the end of May, CDC having determined Guatemalan berries to be the most likely source. In 1997, more than 1,300 cases of cyclosporiasis were reported in the USA and a cruise ship departing from Florida on March 29 also suffered 220 clinically defined cases (Food Chemical News, 1997). Also in 1997, a cluster of cases in Tallahassee was associated with mesclun (raw salad greens, spring mix, field greens or baby greens) (Herwaldt, 2000). A further source emerged when 231 people who consumed fresh basil and basil products (the origin of the basil was not traced) from a gourmet store in Washington D.C. were diagnosed with cyclosporiasis (Calvin et al. 2000).

Early in 1998, the United States Food and Drug Administration (FDA) agreed to allow 4 to 6 Guatemalan berry growers, selected as the best of the low risk operations, to export, providing precautions additional to those instituted in 1997 were put in place. FDA determined *Cyclospora* to be a water-borne faecal contaminant and demanded that all water used in fumigation, cleaning and sanitation be filtered to remove all material greater than 0.5 microns. The scheme was known as the MPE - "Model Plan of Excellence for the Export of Raspberries" - and has been recommended as having the potential to serve as a model policy document for other produce-trading partners to limit the risk of contaminating crops with *Cyclospora* or other pathogens.

Despite these precautions, 18 clusters of cyclosporiasis occurred in Canada during May of that year. By the end of the summer, concern arose in the USA that cyclosporiasis might be becoming endemic in the USA. It was thought to be likely to largely affect children since in Peru, where *Cyclospora* are endemic, it is confined to children under 12, suggesting immunity may be built up (Herwaldt, 2000). An outbreak of cyclosporiasis occurred at a conference in West Palm Beach in August 1999; 119 people were reported as becoming ill. Guatemalan raspberries had not been served, but suspicion fell on blackberries from Guatemala, Chilean fruits and some USA produce (Food Chemical News, 1999).

The FDA announced a major breakthrough in analysis using an improved PCR technique when *Cyclospora* was detected in a salad, containing basil, that infected more than 60 people in an outbreak in Missouri in 1999 (Lopez et al. 1999). In 2001, Dr. Ynes Ortega in the Center for Food Safety at the University of Georgia reported greatly improved recovery rates - up to 70% - of *Cyclospora* from spiked product. In 2002, Guatemalan farms approved by FDA and inspected by PIBA (a Guatemalan authority) were allowed to export raspberries to the USA from March 15th through to August 15th.

CYCLOSPORA IN EUROPE

As stated above, not all cases of cyclosporiasis are either detected or reported. Not all specimens are examined for *Cyclospora* oocysts (some laboratories will not look at all, and

some only examine patients with a recent history of foreign travel), although one of the procedures for detection of *Cryptosporidium* oocysts also detects *Cyclospora* oocysts. In a 1998 UK survey, it was found that 68% of the public health laboratories questioned "actively looked for the protozoan" (Cann et al. 2000). However, only about half of these laboratories were using methods to concentrate oocysts. In external quality assurance tests, only 58% of the participating laboratories correctly identified the organism in preparations supplied to them. The conclusions drawn from these results are that the correct procedure for identifying the organism in specimens needs to be generally applied; that identification should be confirmed by a reference laboratory; and that all patients who exhibit watery diarrhoea for more than one week should be examined for the presence of the organism (Cann et al. 2000). There is no reason to believe that the UK and other European countries are not at risk from foods imported from areas, usually in the tropics, in which *Cyclospora* may be endemic. The consumption of such foods is increasing (some Guatemalan raspberries have been imported to the UK (Chalmers et al. 2000) and it is not clear if the absence of reports of infections from consuming such foods reflects an absence of infection and/or an absence of testing for and identification of any such infections. In the USA, Cyclospora caused 14,638 reported cases (0.1%) of the 2.5 million reported cases annually ascribed to food and beverage-borne disease (Orlandi et al. 2002). This compares with a total of 7% for parasites, 13% for bacterial and 80% for viral caused infections (Mead et al. 1999). This suggests that food parasitology is becoming increasingly important.

An outbreak of 34 cyclosporiasis cases was reported in Germany, where a significant association was found with lettuce imported from Southern Europe (Döller et al., 2002). This demonstrates the importance of having controls in place to prevent sewage contamination of all ready-to-eat products, irrespective of the source.

CONCLUSIONS

- Care must be exercised in implicating any source of food or water as the source of any pathogen before the pathogen has both been identified and shown to occur in the incriminated vehicle.
- Both national and international reporting and communicating cases/outbreaks of food or water borne disease need to be improved.
- Every importer of fresh products (i.e. not pasteurised by heat/irradiation or other means), especially if these come from tropical countries, should be alert to the possibility that hazards, little known in the developed world, may be present and ensure that best practice is followed throughout the chain from farm to table.
- Chlorination may be an inadequate safeguard against some such contaminants.
- Routine laboratory analysis may be inadequate to identify all pathogens, some of which call for unusually sophisticated analytical techniques.

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