REPORT FROM THE PUBLIC HEALTH LABORATORY SERVICE ON EMERGING PATHOGENS AND THE DRINKING WATER SUPPLY

Report prepared under contract to the Department of the Environment (Contract Number EPG 1/9/72)

Report compiled and edited by Dr J de Louvois
Head, PHLS Environmental Surveillance Unit
PHLS Headquarters
London NW9 5DF

EMERGING PATHOGENS AND THE DRINKING WATER SUPPLY

Department of the Environment Contract No. EPG 1/9/72

Executive Summary

Under this contract the Public Health Laboratory Service (PHLS) was required to consider whether *Escherichia coli* 0157, astrovirus, calicivirus, microsporidia cyclospora and acanthamoeba might be considered as emerging pathogens according to the US Department of Heath and Human Services' definition. In addition adenovirus 40/41, Hepatitis A and E, other VTEC producing *E.coli*, aeromonas and yersinia were considered in varying degree. The study concluded that it is unlikely that a previously undescribed organism could gain access to, and survive within, a well controlled water distribution system and result in a previously unknown disease. *E.coli* 0157 VTEC had, on the evidence available, to be considered an emerging pathogen, which has on occasions been transmitted by water. The importance of VTEC other than *E.coli* has yet to be determined. Existing epidemiological and microbiological information leads to the conclusion that microsporidia and cyclospora are not currently significant as causes of infections in the UK population. It is most unlikely that the small number of infections reported arise from distributed water. Acanthamoeba must be considered as an emerging pathogen. However, its significance in the overall population is not great and infection could be controlled by better contact lens hygiene. Astroviruses, calicivirus and the viruses of Hepatitis A and E show no signs of becoming emergent pathogens.

The most appropriate way to address the potential problem of emerging pathogens is not by over zealous microbiological examination of water samples but by the diligent and detailed surveillance of cases of human infection and the investigation of all outbreaks which may be attributed to water. Because of the systems which the PHLS already has in place there is reason to believe that were a pathogen to emerge in the water distribution system it would be identified well before any problems became significant.

CONTENTS

Section 1

INTRODUCTION

Dr Craig Mackerness
Scientific Programmes Division
Headquaters, Public Health Laboratory Service
London NW9 5DF

1.1 Definition of an emerging pathogen

The definition of an emerging pathogen used by the contractor is from the US Department of Health and Human Services, who define these pathogens as those microorganisms, '...whose incidence in humans has increased within the past two decades or threatens to increase in the near future'.

There are other definitions of emerging pathogens, however, such as, '...infections that have newly appeared in a population or have existed but are rapidly increasing in incidence or geographic range' (Morse, 1995). The US Institute of Medicine's Committee on Emerging Microbial Threats to Health considered emerging infectious diseases as, '...clinically distinct conditions whose incidence in humans has increased.' (Lederberg *et al*, 1992).

In these definitions an increase in human infection is a requirement for a disease to be classified as emerging or, in some instances, re-emerging. The emerging pathogens may not be new at all but may have existed for some time in isolated human populations, or they may be zoonoses, and therefore the increase in incidence may be due to a change in human behaviour. Improved methods for the isolation of fastidious bacteria will increase the number of isolates/infections reported, and these may give the false impression of an emerging pathogen. It is possible, although unlikely, that emerging pathogens are newly evolved. It is more likely that they already exist in nature and that either they have been identified or recognised as a human pathogen. The existing infectious agents are becoming better characterised by the application of new and superior techniques, and further divided into identifiable subgroups. This happens more often than the emergence of truly new organisms. The problem is how to recognise a pathogen which is genuinely emerging from the background of constant change.

Although specific agents are usually associated with individual diseases, historically it is the clinical presentation that is recognised first. With improved techniques for the identification of microorganisms, however, this situation is changing. The causative agents for many newly emergent diseases are often discovered virtually simultaneously with, or in some cases before, their associated disease syndromes.

Emergence or re-emergence may be due to:

- the introduction of a new agent
- the recognition of a disease that had been previously undetected or not previously known to be of microbial aetiology
- the reappearance of a known disease after a decline in incidence
- a change in the environment that provides an epidemiologic 'bridge'
- arrival of a pathogen into a new (*i.e.* susceptible) host population
- cross-species transfer of infectious agents
- the acquisition by the microorganism of an identifiable characteristic
- the development of techniques which permit further division of existing species and strains

The appendices give examples of microorganisms considered by the US Institute of Medicine to be emerging (Lederberg *et al.* 1992). Appendix I lists organisms directly or indirectly associated with water, and Appendix II lists organisms with no association with water.

1.2 Factors related to the emergence of new pathogens

The factors identified as being related to the emergence of new pathogens are:

- human demographics and behaviour
- advances in technology and industry
- economic development
- increased land use or a change in land use
- increased international travel
- microbial adaptation and change
- breakdown of public health measures
- farming practices
- widespread use of antibiotics

- increases in the number and type of organ transplants
- increasing number of at risk patients, such as the elderly or those with HIV infection, who are more susceptible to opportunistic diseases

Although it is difficult, if not impossible, to predict the emergence of truly new infectious diseases or agents, it is helpful to understand the factors that facilitate the emergence and spread of infectious diseases in general. It is, therefore, important to focus on what is currently known: the infectious diseases that will emerge or re-emerge are likely to do so through one or more of these 'facilitative pathways'. An awareness of this system of pathways constitutes the first step to reasoned prevention and control of infectious diseases. Many of the diseases caused by new pathogens have emerged because of a combination of these factors. This is not surprising given the often complex interactions of microbes, their human and animal hosts, and the environment.

Re-emergence is often the result of:

- lapses in public health measures possibly as a result of complacency
- changes in human behaviour that increase person-to-person transmission of an infectious agent (*e.g.* sexual behaviour)
- changes in the ways humans interact with their environment

The incidence of a number of known infectious diseases is increasing, including some that were once considered under control. The reasons for the increase vary but include the waning effectiveness of some approaches to disease control and treatment, changes in the ways human beings interact with the environment and the fact that certain individuals have become more susceptible to infection. These circumstances explain the re-emergence of, among other diseases, malaria and tuberculosis. Although it is impossible to predict the emergence, in time and place, of individual new microbial agents capable of causing human infection, it is to be expected that further new pathogens will emerge.

1.3 Mechanisms of emergence

Microorganisms are exceedingly numerous and diverse, but only a small fraction are capable of causing disease in animals or humans To survive, most microbial species, whether pathogenic or not, must be well adapted to a particular ecological niche and must compete

effectively with other microorganisms. The small size and high surface-to-volume ratios of microorganisms facilitate their rapid growth and extensive impact on their environment. Microbial pathogens can colonise animals, humans, and arthropods because they have acquired (or evolved) a number of genes and gene products that enable them to do so. These gene products are extremely varied and involve factors concerned with:

- transmission from one host to another
- cell-surface attachment and invasiveness
- countering or suppressing specific and non-specific host immune responses
- persistence or survival inside and outside a host organism
- resistance to antimicrobial drugs

Non pathogens can potentially become pathogenic, and low-virulence pathogens are able to become highly virulent through gene mutation, recombination and transfer of genetic material.

Microbial pathogens can evolve and adapt very quickly due to:

- the relatively small amount of DNA or RNA, or both, that they carry
- their rapid growth rate
- their large population size

These evolutionary mechanisms allow microorganisms to:

- adapt to new host cells or host species
- produce "new" toxins
- bypass or suppress inflammatory and immune responses
- develop resistance to antimicrobial drugs and antibodies

This ability to be highly adaptable is required for the successful competition and evolutionary survival of any microorganism, but it is particularly crucial for pathogens, which must cope with host defences as well as microbial competition. There are, for example, a number of determinants that can exert an influence on viral evolutionary events, and these can be considered as either restrictive or propitious.

1.4 Format and scope of the Report

1.4.1 The microorganisms included in this report are summarised in Section 1.6. These organisms occur naturally and could potentially contaminate the drinking water supply. The report does not include microorganisms which may be deliberately introduced.

1.4.2 This study does not include:

a) water associated organisms which are already established as significant human pathogens and whose role in human infection is reasonably well understood. Microorganisms in this group include:

- *Vibrio cholera*
- *Cryptosporidium parvum*
- *Entamoeba histolytica*
- *Giardia lamblia*

b) water associated organisms that are known not to be transmitted by water in distribution, such as:

- *Legionella pneumophila*

c) organisms which exert a toxic, rather than an infectious effect on man, such as the Cyanobacteria

d) human pathogens, whether emerging or not, which are not associated with water:

- *Salmonella enteritidis* phage type 4
- *Salmonella typhimurium* DT104
- *Salmonella typhi*

- Methicillin Resistant *Staphylococcus aureus* (MRSA)

The current methods of water treatment used in the UK have usually proved to be sufficient for the protection of public health from the threat posed by these organisms.

1.4.3 The study will address the situation as it exists in the United Kingdom and countries with similar climatic conditions. It will be confined to those organisms identified by the contractor and such additional organisms that experts within the Public Health Laboratory Service consider to be worthy of attention in the context of water in distribution. These are shown in Table 1, Section 1.8.

1.4.4 The available information on the organisms listed Section 1.8 has been reviewed with regard to:

- human pathogenicity
- human infectivity
- clinical treatment
- laboratory diagnosis
- incidence of confirmed illness
- extent and frequency of outbreaks
- the potential for secondary spread (*i.e.* person-to-person spread)
- resistance to water treatment processes
- environmental resistance
- environmental occurrence

1.4.5 The study will examine the incidence data available, where possible, for the last ten years to determine whether there is a real increase in the number of infections due to the organisms in Section 1.8. An assessment will be made of the risk to public health of those organisms with the potential to become emerging pathogens. For reasons explained in Section 2 reliable data do not exist for infections caused by these organisms prior to 1985.

1.5. Mechanisms of emergence

1.5.1 Viruses

The Evolution of New Viruses: Constraints and Opportunities

Constraints

- Extreme viral alterations are lethal to the virus

- Requirements for the co-evolution of viral cellular proteins
- Virus survival requires a critical level of virulence
- Propagation in alien hosts tends to be attenuating
- Adaptation to ecological niches is highly specific
- Penetration of the human immune system usually requires major antigenic change
- Infection with non-human viruses (*i.e.* zoonoses) is sometimes - but not always - contagious

Opportunities

- High mutation rates
- Interviral genetic interaction
- Ecological changes increase the opportunity for contact between man and viruses or their vectors (*e.g.* arthropods)
- Changes in human behaviour
- Altered behaviour of viruses in immunocompromised hosts

(Kilbourne, 1991)

RNA viruses present a paradox. On the one hand, their mutation rates are extraordinarily high, because unlike DNA viruses, RNA viruses have no mechanisms for correcting errors made during replication, and on the other hand the clinical expressions of the diseases they cause (*e.g.* poliomyelitis, measles) have remained constant for centuries. Nevertheless, analysis of RNA virus genomes reveal that each "virus" comprises a heterogeneous mixture of mutants in variable proportions. Thus, any given strain or isolate is, in fact, polymorphic or represents a subset of the quasi-species (Domingo *et al*, 1978). A virus, then, could be considered as a consensus that reflects the predominating mutant(s) in a mixed population: because predominating mutants seldom change, only unusual selective pressure by the host's immune response (or other factors, such as host adaptation) will allow new mutants to gain ascendancy. It has to be concluded that the consequence of this will be the emergence of distinguishably new viruses some of which may be pathogenic to man. This is, however, considered to be an extremely rare event and as such is not a significant consideration in the context of this report.

1.5.2 Bacteria

One mechanism by which bacteria are able to cause disease is by the production of virulence factors. These virulence factors have several important roles in bacterial pathogenesis:

- they allow bacteria to resist non-specific host clearance mechanisms
- they aid bacteria in the acquisition of the nutrients necessary for growth and survival
- they help the bacteria to resist specific host immune mechanisms
- they can provide bacteria with a competitive advantage by the inhibition of the growth of other microorganisms in the host

The evolution, *ab initio*, of virulence through mutation would require an extraordinarily complex chain of events and it is more often inferred as a natural process, rather than one that can be observed in the laboratory. The development of resistance to antibiotics and to serum bactericidal factors can be observed experimentally. Virulence factors vary from organism to organism and can be transferred among receptive bacteria either by bacteriophages (*i.e.* viruses that infect bacteria) or plasmids (*i.e.* extra chromosomal DNA which is carried by bacteria in addition to and separate from the genome). The transfer of genetic material is one way that bacteria are able to adapt to changes in their environment and a means by which new strains emerge.

Bacteria may possess more than one virulence factor which include:

- toxins (neuro-, entero-, endo-, cyto-, erythrogenic, *etc.*)
- enzymes
- colonisation factors
- adhesins
- bacteriocins
- hemolysins
- cell invasion factors
- drug resistance factors

1.6 The prevention and control of emerging infectious diseases

Alone or in combination, economic collapse, war, and natural disasters, among other disruptions in society have, and will again, cause the breakdown of public health measures and the emergence, or re-emergence, of a number of human diseases. Timely recognition of

emerging infections requires surveillance systems capable of detecting these diseases so that investigations and preventive measures can be rapidly initiated before they create major public health problems.

Surveillance and the rapid response to identified disease threats are at the core of preventive medicine. Surveillance can take many forms and is often a passive process that is based on reports of unusual or particularly contagious human illnesses. The active monitoring of factors such as: population growth and migration; insect vector populations; developments that disturb the natural environment; and environmental factors (*e.g.* temperature and rainfall) are essential components of surveillance and their understanding can help to control the spread of emerging infectious diseases and increase the effectiveness of efforts to control them.The importance of surveillance, in its broadest context, for the detection and control of emerging microbial threats cannot be overemphasised.

1.7 Potential public health threats from the water supply

In many parts of the world subsets of the population do not have access to clean potable water. In others, where public water supplies are available, system failures can place entire communities at risk. One such incident occurred in the spring of 1993 in the United States. Contamination of a municipal water supply with cryptoporidium in Milwaukee. Wisconsin, caused the largest outbreak of waterborne illness ever reported in the USA, with over 400,000 people affected and over 4,000 admitted to hospital. Many other outbreaks associated with drinking water have been reported, yet cryptosporidium - a coccidian protozoan parasite - was only identified as a human pathogen in 1976 (Nime *et al*, 1976; Rush *et al*. 1988). This organism can cause diarrhoeal illness in immunocompetent as well as immunocompromised individuals (Casemore, 1990).

Water that is untreated or that does not receive adequate processing or is contaminated post-treatment can transmit infectious agents. Potentially a large number of microorganisms are able to be transmitted in water. Examples of infectious agents which have been transmitted via water include:

Bacteria

- *Vibrio cholerae*
- *Salmonella typhi*
- enterotoxigenic *Escherichia coli*

- *Legionella pneumophila*

Viruses

- Hepatitis A virus
- Norwalk agent and related viruses, such as the small round structured viruses

Parasites

- *Giardia lamblia*
- *Cryptosporidium parvum*
- *Entamoeba histolytica*

1.7.1 Individuals susceptible to waterborne disease

An increasing proportion of the population of many countries are elderly and a growing number of persons are immunosuppressed as a result of a variety of factors including HIV infection and various therapies. These populations are at increased risk of food- and waterborne disease which may not present a major problem for the general population but which can have devastating consequences for vulnerable subgroups. In many countries, including the UK, there are increasing numbers of people in residential care and there is an increase in the use of nurseries for children. These environments present the opportunity for the secondary spread of pathogens that may have been introduced initially by food or water borne routes.

1.8 Microorganisms considered as potential emerging pathogens in the water supply

The report focuses only on emerging pathogens in water distribution and does not attempt to examine the issue of emerging pathogens associated with other modes of potential spread (table 1.1).

Table 1.1

Microorganisms considered in this report

- Verocytotoxin-producing *Escherichia coli* O157 and non O157
- *Aeromonas* spp.
- *Yersinia* spp.
- Astrovirus

- Calicivirus (SRSV (ie Norwalk-like) and classic calicivirus
- Hepatitis A
- Hepatitis E
- Adenovirus 40 and 41
- Microsporidia
- Cyclospora
- *Acanthamoeba* and related organisms

1.8.1 Bacteria

Escherichia coli O157

Vero cytotoxin-producing *Escherichia coli* (VTEC), also termed enterohaemorrhagic *E. coli*, is an enteric pathogen which is associated with a spectrum of clinical conditions ranging from mild diarrhoea to haemorrhagic colitis and haemolytic uraemic syndrome. This organism has emerged as a major enteric pathogen in parts of North America and there has been increasingly isolated in the UK from sporadic cases and a few outbreaks of infection. The majority of reported VTEC infections are with strains belonging to the serotype O157:H7 or O157:H- but VTEC of many other serotypes have now been identified.

The majority of infections of *E. coli* O157 have been associated with transmission by food, although non-food-borne vehicles have also been implicated (Chinyu and Brandt, 1995). Water-borne transmission has been implicated in two outbreaks in Missouri, USA (Swerdlow *et al*, 1992) and Grampian, Scotland (Dev *et al*, 1991).

Diagnosis

- identification of antibodies to the O157:H7 serotype of *E. coli*
- demonstration of VTEC or free Vero cytotoxin (VT) in the faeces of patients
- a significant serological response to *E. coli* O157 lipopolysaccharide
- detection of antibodies with VT-neutralising ability

Mode of transmission

- ingestion of contaminated food or water, typically poorly cooked beef and raw milk

Prevention and Control

- proper cooking of meat
- hand washing

- proper sewage and water treatment

Aeromonads

Aeromonads are ubiquitous in the environment, and in certain situations can cause human infection. *Aeromonas hydrophila*, *A. veronii* subsp. *sobria* and *A. caviae* can cause diarrhoea and *A. hydrophila*, *A. schubertii* and A. *veronii* subsp. *sobria* have been associated with non-enteric infections, including necrotic wound infections and septicaemia. Wound infections can be caused by contamination with surface waters.

Aeromonas spp. are normally present in sewage and surface waters. Infection with aeromonas has been associated with untreated water and 25% of patients with diarrhoea caused by aeromonas are reported to have recently returned to the UK from abroad. *Aeromonas* spp. have been isolated from chlorinated drinking water supplies in the USA, Sweden, Denmark and Holland (LeChevalier *et al*, 1982; Krovacek *et al*, 1992; Knochel and Jeppesen, 1990; Havelaar *et al*, 1992).

Cases of aeromonas-associated diarrhoea in Australia were related to the isolation of *Aeromonas* spp. from a chlorinated water supply, with both being more common in the summer months (Burke *et al*, 1984a). In contrast, studies on the unchlorinated domestic supply to another Australian population showed that both clinical and environmental isolations continued in the winter (Burke *et al*, 1984b). From these reports it was concluded that the incidence of clinical infection was related to bacterial counts in drinking water rather than some other variable. In one report from Holland the majority of clinical isolates from patients were recovered during the spring and summer months (van der Kooij, 1988). The depletion of free chlorine residuals in drinking water has been associated with increasing aeromonas isolations (van der Kooij, 1988). In addition, there is evidence of regrowth of total coliforms and aeromonads in fully treated water where there has been no evidence of accidental faecal contamination of the supply concerned (Payment *et al*, 1988).

Diagnosis

- identification of the organism in patient's faeces or in wound secretions

Mode of transmission

- ingestion of contaminated water

- entry of the organism through a break in the skin

Prevention and Control

- proper treatment of drinking water
- predisposed individuals should avoid aquatic environments

Yersinia

Although identified as a human pathogen in 1939, *Yersinia enterocolitica* was not recognised as a foodborne pathogen until the mid 1970s. The organism can grow at temperatures as low as $0^{o}C$ and the increased use of refrigeration for the preservation of food may play a part in the increase in the prevalence of this organism in food (Doyle, 1990). Different serotypes of *Y. enterocolitica* are associated with human infections in different regions of the world. In Europe, Canada and Japan, sporadic infections are predominantly caused by serovar O:3 and to a lesser extent O:9, whereas in the US sporadic cases, which are not frequently reported, are associated with multiple serovars where outbreaks usually involve serovar O:8.

Both pathogenic and non-pathogenic strains of yersinia can be found in drinking water systems and infections caused by yersinia have been associated with drinking water, well water and surface water. There are no reports of infection from treated drinking water, probably because yersinia are sensitive to chlorine (Paz *et al*, 1993) and therefore should not survive in distribution systems where an adequate residual chlorine concentration is maintained.

Diagnosis

- culture of a faecal swab onto appropriate culture media

Mode of transmission

- food and untreated drinking water

Prevention and Control

- chlorination of drinking water

Other bacteria

For individuals with HIV infection many environmental organisms have become opportunistic pathogens and the possibility of waterborne transmission exists for *Pneumocystis carinii* and *Mycobacterium avium intracellulare*.

1.8.2 Viruses

A number of viruses have previously been recognised as emerging pathogens, either in a global context, such as the human immunodeficiency virus (HIV), or within national populations. The classification of viruses is complex and subject to repeated revision. The current classification for the viruses being considered in this report is shown in Table 1.2.

The technology for the isolation of viruses is not fully developed. As a result there are many examples in which the number of reported cases of infection increases year on year due not to the emergence of a new pathogen but simply to improved methods of detection. It is understandable that improved technology leads to increased awareness and enhanced ascertainment. These situations are not mutually exclusive and the problem is, therefore, to recognise a genuine emerging pathogen in the context of improving technology, enhanced surveillance *etc.*

The Caliciviruses

The caliciviruses considered in this report are: SRSV, (ie Norwalk-like virus) and classic calicivirus. During the 1970s a number of viruses associated with acute gastroenteritis were discovered. Transmission by the food or waterborne route has been documented for astroviruses and caliciviruses, *i.e.* human calicivirus and Norwalk-like viruses (Kapikian *et al*, 1972; Hedberg and Osterholm, 1993). Norwalk-like viruses which in the UK are known as SRSV have become recognised as important causes of both sporadic and epidemic gastroenteritis. Norwalk virus is a prototype of human calicivirus causing acute gastroenteritis and was first identified in 1972. Study of these viruses has been difficult: until recently they were not cultivatable. In addition, there is no animal model and over 50% of the viral antigen in faeces consists of soluble protein. Improvements in technology, such as cryo electron microscopy, has allowed the structure of the virus to be determined.

Table 1.2

Current classification of the viruses considered in the report

DNA viruses

Family	Genus	Pathogenic species
Adenoviridae	Mastadenovirus	Adenovirus 40 and 41

RNA viruses

Family	Genus	Pathogenic species
Picornaviradae	Enterovirus	Hepatitis A
Caliciviridae	Hepevirus	Hepatitis E
Caliciviridae	Calicivirus	Small round structured virus (SRSV) eg Norwalk virus (which is the prototype of SRSV classic human calcivirus)
Astroviridae		Astrovirus

Diagnosis

- reverse transcriptase polymer chain reaction
- electron microscopy of faecal sample

Mode of transmission

- person to person
- contamination of drinking water by sewage
- eating shellfish contaminated by or grown in water contaminated by sewage

Prevention and Control

- proper treatment of sewage for discharge into receiving waters
- chlorination of treated drinking water

Hepatitis A and E

Hepatitis A virus (HAV), also known as enterovirus 72, causes infectious hepatitis and is spread by the faecal-oral route. HAV infection may be caused by the consumption of contaminated shellfish, water or food. In the US approximately 40% of acute hepatitis cases are caused by HAV.

Epidemics of hepatitis due to non-A non-B virus occurred in India in the 1950s mainly among young adults and resulted in a high mortality in pregnant women. The likely etiological agent of these epidemics, *i.e.* hepatitis E, was only recognised in 1990 (Krawczynski, 1993). The natural history of infection is similar to that of hepatitis A. Epidemics caused by hepatitis E have been recognised in Asia, Africa, Peru and Mexico, and these have often been linked to faecal contamination of drinking water (Gust and Purcell, 1987). There were ten cases of hepatitis caused by hepatitis E virus in the UK in 1995. All of them were imported.

Diagnosis

- serological tests

Mode of transmission

- faecal-oral route

Prevention and Control

- unknown

Adenovirus

Adenoviruses were first isolated in 1953 in human adenoid cell culture. Since then approximately 100 serotypes, at least 42 of which infect humans, have been identified. Adenovirus serotype 40/41 is a major cause of acute viral gastroenteritis, accounting for 15% of hospitalised cases in the US. The serotypes 40, 41 and 42 have been grouped as the enteric adenoviruses and are responsible for episodes of acute diarrhoea in infants.

Diagnosis

- serological tests
- electron microscopy

Mode of transmission

- person to person
- contaminated water

Prevention and Control

- chlorination of drinking water

1.8.3 PROTOZOA

Microsporidia

Microsporidia are a group of protozoal parasites which may cause human infection particularly in immunocompromised individuals (Weber *et al*, 1994). The exact modes of transmission have yet to be established. Water and food borne routes have not been ruled out.

Diagnosis

- light and electron microscopy of biopsy specimen

Mode of transmission

- unknown. May be by the ingestion of contaminated food or water. Spores of some species can survive in the environment for up to four months.

Prevention and Control

- unknown

Cyclospora

Infection with *Cyclospora* spp. (also known as cyanobacteria-like bodies) is associated with diarrhoea and was first described in travellers returning to the US from Haiti and Mexico (Soave *et al*, 1986). *Cyclospora* spp. have been found in both immunocompetent (Bendall *et al*, 1993) and immunodeficient (*i.e.* AIDS) patients (Long *et al*, 1990).

Diagnosis

- identification of oocysts by a modified Ziehl-Neelsen stain
- phase contrast microscopy

Mode of transmission

- contaminated drinking water (Hoge *et al*, 1993)

Prevention and Control

- unknown

Acanthamoeba

The genus *Acanthamoeba* was first recognised in 1931, but it was not until 1973 that it was established that this organism could cause a severe and potentially blinding infection of the cornea (Nagington *et al*, 1974). Although Acanthamoeba keratitis was previously considered a rare ocular infection, from 1985 there has been a marked increase in the number of isolates, particularly among wearers of contact lenses with poor hygiene techniques. While the majority of infections have been reported from the USA, approximately 90 cases have occurred in the UK - 63 in the period 1990-1992 - and most of these were contact lens wearers (Kilvington and White, 1994). Although a comparatively rare disease the incidence of Acanthamoeba keratitis in the UK is rising. On rare occasions *Acanthamoeba* may attack the central nervous system causing granulomatous amoebic encephalitis (GAE) (Martinez, 1985). Patients are usually immunosuppressed from chemotherapy, alcohol abuse, or chronic disease (Martinez, 1985; Martinez, 1991). GAE has been reported as a primary cause of death in AIDS patients (Visvesvara, 1990).

Diagnosis

- clinical presentation
- culture
- histological staining of corneal tissue

Mode of transmission

- tap water, swimming baths, hydrotherapy and spa pools

Prevention and Control

- improved lens cleaning hygiene by contact lens wearers
- advice that contact lenses should not be worn when swimming and that following contact with any environmental matter they should be removed and disinfected
- contact lens storage cases should be cleaned and disinfected every week

1.9 Waterborne disease outbreaks

A primary source of many of the pathogens in water is the faecal contamination by animals, birds or man, of source or raw water that is subsequently inadequately treated, or similar

contamination of potable water after treatment. The leakage of wastewater from septic tanks and other sewage disposal facilities into groundwater can also transmit microorganisms. Fortunately, most water used in the UK is effectively processed and treated prior to consumption.

In some instances, however, water intended for drinking is not treated. This is often the case with water from private wells or from natural springs. In 1989, for example, there was an outbreak of 900 cases of gastroenteritis in a new resort community in north-central Arizona. An investigation revealed that the source of the outbreak was tap water obtained from a deep well on the resort property. A Norwalk-like virus was apparently introduced into the well from a faulty sewage treatment facility nearby. It was concluded that untreated sewage passed through fractures in the sandstone and limestone fields surrounding the well (Lawson *et al*, 1991).

An outbreak of cryptosporidiosis in Texas, described by D'Antonio *et al*,(1985), was preceded by an outbreak believed to be due to Norwalk virus. The outbreaks occurred in a community supplied from an artesion well which received low level chlorination shortly before distribution, suggesting intermittent contamination coupled with inadequate treatment.

Wastewater treated to remove organic and inorganic contaminants is now being used for irrigation, industrial processing, and nonpotable residential (watering gardens, toilet flushing) and commercial (watering golf courses) applications. Inadvertent ingestion of reclaimed water, which has undergone the same basic treatment as potable water (including disinfection), is not likely to cause waterborne disease. Reclaimed water used for drinking is of concern because of residual contamination.

1.10 References

Anasari, S.A., Springthorpe, V.S. and Sattar, S.A. Survival and vehicular spread of human rotaviruses: possible relation to seasonality of outbreaks. *Review of Infectious Diseases*. 13: 448-61. 1991.

Bendall, R.P., Lucas, S., Moody, A., Tovey, G. and Chiodini, P.L. Diarrhoea associated with cyanobacteria-like bodies: a new coccidian enteritis of man. *Lancet*. 335: 169-70. 1993.

Burke, V., Robinson, J., Gracey, M., Peterson, D. and Partridge, K. Isolation of *Aeromonas hydrophila* from a metropolitan water supply: seasonal correlation with clinical isolates. *Applied and Environmental Microbiology* 48: 361-66. 1984a.

Burke, V., Robinson, J., Gracey, M., Peterson, D., Meyer, N. *et al,*. Isolation of *Aeromonas* spp. from an unchlorinated domestic water supply. *Applied and Environmental Microbiology*. 48: 367-70. 1984b.

Casemore, D.P. Epidemiological aspects of human cryptosporidiosis. *Epidemiology and Infection*. 104: 1-28. 1990.

Chinyu, S. and Brandt, L.J. *E. coli* O157:H7 infection in humans. *Annals of Internal Medicine*. 123: 698-714. 1995.

Dev, V.J., Main, M. and Gould, I. Waterborne outbreak of *E. coli* O157 [Letter]. *Lancet*. 337: 1412. 1991.

Domingo, E., Sabo, D., Taniguchi, T. and Weissman, C. Nucleotide sequence heterogeneity of an RNA phage population. *Cell*. 13:735-44. 1978.

Doyle, M.P. Pathogenic *Escherichia coli*, *Yersinia enterocolitica* and *Vibrio parahaemolyticus*. *Lancet*. 336: 1111-5. 1990.

Elder, G.H., Hunter, P.R. and Codd, G.A. Hazardous freshwater cyanobacteria (blue green algae). *Lancet*. 341: 1518-9. 1993.

Gust, E.D. and Purcell, R.H. Waterborne non A non B hepatitis. *Journal of Infectious Diseases*. 156: 630. 1987.

Hamilton, W.D., Axelrod, R. and Tanese, R. Sexual reproduction as an adaptation to resist parasites: a review. *Proceedings of the National Academy of Sciences*. 87: 3566-3. 1990.

Havelaar, A.H., Schets, F.M., van Silfout, A., Jansen, W.H., Wieten, G. and van der Kooij, D. Typing of Aeromonas strains from patients with diarrhoea and from drinking water. *Journal of Applied Bacteriology*. 72: 435-44. 1992.

Hedberg, C.W. and Osterholm, M.T. Outbreaks of food-borne and water-borne viral gastroenteritis. *Clinical Microbiology Review*. 6: 199-210. 1993.

Hoge, ChW., Shlim, D.R., Rajah, R., Triplett, J., Shear, M., Rabold, J.G. and Echevarria, P. Epidemiology of diarrhoeal illness associated with coccidian-like organisms among travellers and foreign residents in Nepal. *Lancet*. 341: 1175-9. 1993.

Hunter, P.R. Cyanobacteria and human health. *Journal of Medical Microbiology*. 36: 301-2. 1992

Kapikian, A.Z. and Chanock, R.M. Rotaviruses. In: Fields, B.N., Knipe, D.M. and Chanock, R.M., eds. Virology. London, England: Raven Press. 1353-1404. 1990.

Kapikian, A.Z., Wyatt, R.G., Dolin, R., Thornhill, T.S., Kalica, A.R. and Chanock, R.M. Visualisation by immune electron microscopy of a 27nm particle associated with acute infectious non-bacterial gastroenteritis. *Journal of Virology*. 10: 1075 - 81. 1972.

Kilbourne, E.D. New viruses and new diseases: mutation, evolution and ecology. *Current Opinion in Immunology*. 3:518-24. 1991.

Kilvington. S. and White, D.G. *Acanthamoeba*: biology, ecology and human disease. *Reviews in Medical Microbiology*. 5: 12-20. 1994.

Knochel. S. and Jeppesen, C. Distribution and characteristics of aeromonas in food and drinking water in Denmark. *International Journal of Food Microbiology*. 10: 317-22. 1990.

Krawczynski, K. Hepatitis E. *Hepatology*. 17: 932-41. 1993.

Krovacek. K., Faris, A., Baloda. S.B., Lindberg, T., Peterz, M. and Mansson, I. Isolation and virulence profiles of *Aeromonas* spp. from different municipal water supplies in Sweden. *Food Microbiology*. 9: 215-2. 1992.

Lawson. H.W., Braun. M.M., Glass, R.I.M., Stine, S.E., Monroe, H.K., Atrash, L.E., Lee, L.E. and Englender, S.J. Waterborne outbreak of Norwalk virus gastroenteritis at a southwest US resort: role of geological formations in contamination of well water. *Lancet* 337: 1200-4. 1991.

LeChevalier, M.W., Evans, T.M., Seidler, R.J., Daily, O.P., Merrel, B.R., Rollins, D.M. and Joseph. S.W. *Aeromonas sobria* in chlorinated drinking water supplies. *Microbial Ecology*. 8: 325-33. 1982.

Lederberg, J., Shope. R.E. and Oaks Jr, S.C. eds. Emerging Infections: Microbial Threats to Human Health in the United States. National Academy Press, Washington DC. 1992.

Long, E.G., Ebrahimzadeh, A., White, E.H., Swisher. B. and Callaway, C.S. Algae associated with diarrhoea in patients with acquired immunodeficiency syndrome and in travellers. *Journal of Clinical Microbiology*. 28: 1101-4. 1990.

Martinez. A.J. Free-living amebas: natural history, prevention, diagnosis, pathology and treatment of disease. Boca Raton. Florida: CRC Press Inc. 1985.

Martinez, A.J. Infections of the central nervous system due to *Acanthamoeba. Review of Infectious Diseases*. 13: S399-420.1991.

Morse, S.S. Factors in the emergence of infectious diseases. *Emerging Infectious Diseases*. 1: 7-15. 1995

Nagington, J., Watson, P.C. and Playfair, T.J. Amoebic infections of the eye. *Lancet*. ii: 1537-40. 1974.

Nime, F.A., Burek, J.D., Page, D.L., Holscher, M.A. and Yardley, J.H. Acute entercolitis in a human being infected with the protozoan Cryptosporidium. *Gastroenterology*. 70: 592-8. 1976.

Payment, P., Gamache, F. and Paquette, G. Microbiological and virological analysis of water from two water filtration plants and their distribution systems. *Canadian Journal of Microbiology*. 34: 1304-9. 1988.

Paz, M.L., Duaigues, M.V., Hanashiro, A., D' Aquino, M. and Santini, P. Antimicrobial effects of chlorine on *Yersinia enterocolitica*. *Journal of Applied Bacteriology*. 75: 220-5. 1993.

Rush, B.A., Chapman, P.A. and Ineson, R.W. Cryptosporidium and drinking water. *Lancet*. ii. 632-3. 1988.

Soave, R., Dubey, J.P., Ramos, L.J. and Tummings, M. A new intestinal pathogen. *Clinical Research*. 34: 533A. 1986.

Swerdlow, D.L., Woodruff, B.A., Brady, R.C., Griffin, P.M., Tippen, S. and Donnell, H.D. Jr. A waterborne outbreak in Missouri of *E. coli* O157: H7 associated with bloody diarrhoea and death. *Annals of Internal Medicine*. 116: 812-9. 1992.

van der Kooij, D. Properties of Aeromonads and their occurrence and hygienic significance in drinking water. *Zentralblatt für Bakteriologie und Hygiene* B 87: 1-17. 1988.

Visvesvara, G.S. and Stehr-Green, J.K. Epidemiology of free-living amoeba infections. *Journal of Protozoology*. 37: 25S-33S. 1990.

Weber, R., Bryan, R.T., Schwarz, D.A. and Owen, R.L. Human microsporidial infections. *Clinical Microbiology Reviews*. 7: 426-61. 1994.

Section 2

THE ROLE OF EPIDEMIOLOGY AND SURVEILLANCE IN RECOGNISING EMERGING PATHOGENS

Dr Patrick Wall
Epidemiology Division
PHLS Communicable Disease Surveillance Centre
London NW9 5EQ

2.1 Introduction

Information on the pathogens possibly associated with drinking water in England and Wales is generated from two principle sources:

a) National surveillance scheme for laboratory confirmed infections

This system produces information on laboratory confirmed infections in England and Wales. It has the ability to detect an increasing incidence or the emergence of a pathogen, however, it does not contain information on the mode of transmission. Therefore this system would not detect whether the infections were waterborne. Routine surveillance data do however highlight problems which are then subjected to in-depth investigations. It is at this stage that an association between infection and drinking water would be found.

b) National surveillance scheme for general outbreaks of infectious intestinal disease

A general outbreak is one effecting residents of more than one household or of an institution distinguishing it from a family outbreak which only affects the residents of one household (Department of Health 1994). This surveillance system rapidly detects general outbreaks of infectious intestinal disease, including those caused by the consumption of drinking water and captures the relevant epidemiological information

Both systems are unique to the UK and reduce the possibility of an organism which causes human illness, emerging without being recognised at an early stage.

2.2 National surveillance scheme for laboratory confirmed infections

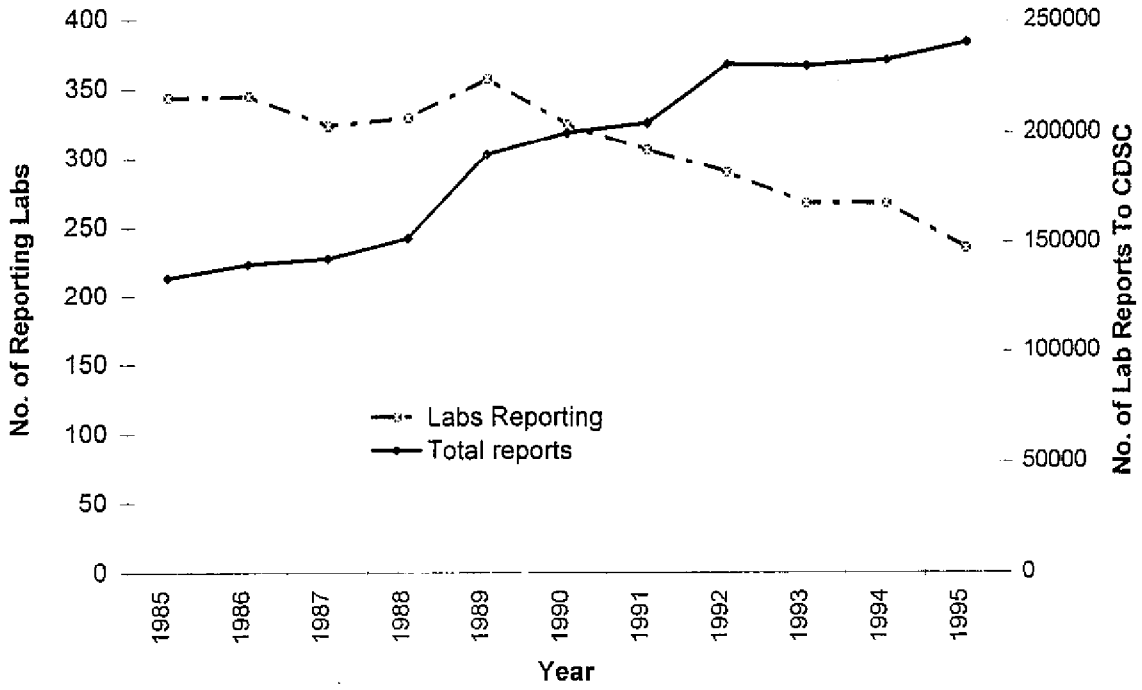
Reports of laboratory confirmed infections have been collected, summarised and published for over 50 years by the PHLS and its predecessor the Emergency Public Health Laboratory Service (Williams, 1985; Handysides, 1996). The original weekly summary of these reports has evolved to become today's Communicable Disease Report (CDR), containing up-to-date surveillance data, outbreak reports and articles about communicable disease control. In addition, since January 1996, to increase timeliness and disseminate the information more

widely these are available, via the Internet, on the PHLS Communicable Disease Surveillance Centre (CDSCs) world wide web home page (Handysides, 1996).

The CDSC, the epidemiology unit of the PHLS, receives reports of laboratory confirmed human infections from the 53 public health laboratories and the reference laboratories of the PHLS network as part of its core function and voluntarily from over 200 NHS laboratories throughout England and Wales. In 1995 the Strategic Review of Pathology Services recommended that, 'all pathology contracts should refer to the necessity for prompt reporting of data relevant to the epidemiology of communicable disease, both to the Consultant in Communicable Disease Control (CCDC) and to the PHLS CDSC; this should include the production and reporting of relevant data which may not be essential for the immediate management of the individual patients (Anon, 1995). The reports of laboratory confirmed infections are stored on a central database at CDSC and include data on: source laboratory; reference laboratory; patient's name or laboratory identification number; date of birth; sex; date of onset of symptoms; specimen type; clinical and epidemiological features: the name of the organism/toxin and the tests used to identify it and if appropriate whether the infection contributed to the patient's death. Most of the Public Health and some of the NHS laboratories are electronically linked to CDSC and download their reports weekly (Grant *et al*, 1993). The remaining laboratories send paper reports to CDSC where they are manually entered onto the central database. There is an ongoing initiative to have all laboratories reporting electronically (Henry *et al*, 1995).

The laboratory reports received by CDSC have been stored in a computer database, known as LabBase, since 1975. LabBase contains reports on nearly 2,000 species, subspecies and types of organism *e.g. Mycobacterium avium intercellulare*, *Neisseria meningititis* group B and the gastrointestinal pathogens such as *Salmonella enteritidis* PT4, *Salmonella typhimurium* DT104 and *Cryptosporidium parvum*. The number of reports sent to CDSC has increased by a factor of three since 1975 and by almost a factor of two since 1985 to a current level of over 240,000 reports per year. The number of reporting laboratories has fallen by one third since 1985 possibly as a result of mergers of hospital laboratories (Figure 2.1). Reports originating in the PHLs account for about 50% of the total.

Figure 2.1 A comparison of laboratory reports received by CDSC and laboratories reporting to CDSC (England and Wales 1985-1995)



The surveillance of salmonella is unique among the intestinal pathogens in that nearly all initial human isolates from England and Wales are forwarded to the national reference laboratory, the Laboratory of Enteric Pathogens (LEP), at the PHLS Central Public Health Laboratory (CPHL) for confirmation and further identification. The LEP has an electronic link with LabBase and updates the salmonella figures daily. In addition, most human isolates of *E.coli* O157 from England and Wales are referred to LEP for definitive typing and the national returns on the incidence on *E. coli* O157 VTEC are based on LEP data. It is planned that in the near future the salmonella model for data entry into LabBase will be adopted for all organisms that are typed by LEP, including *Shigella sonnei* and campylobacter for which a new reference facility has been established is the centre for typing and/or toxin testing.. Food Hygiene Laboratory (FHL) at the PHLS CPHL for *Bacillus cereus*, *Clostridium perfringens*, *Listeria monocytogenes* and *Staphlococcus aureus*. These are used to compliment data in LabBase from the source laboratories. In addition the FHL is the sole source of information on marine biotoxin poisoning such as scombrotoxin.

The staff of CDSC have access to LabBase from their desks and the data are used to monitor current trends, for retrospective analysis and to detect outbreaks. A sophisticated computer

software package is used to scan the incoming data and detect increases in the number of reports over what would be expected from historical patterns (Farrington *et al*, 1993). The software produces a print out for epidemiologists highlighting the pathogen and age-groups affected. Additionally, it plots the cases on a map of England and Wales to enable the identification of geographical clusters.

The reports of laboratory pathogens represents only a proportion of the true incidence of these pathogens as not all infected individuals seek medical attention and only a subset of these have a sample submitted for examination (Feldman *et al*, 1994). Not all of these will have a pathogen identified, this is particularly true for those less common pathogens requiring special media and enrichment techniques for isolation. Finally, not all pathogens identified are reported to CDSC. The exact proportions in the different categories remains unclear. The PHLS is currently collaborating with the Medical Research Council, the London School of Hygiene and Tropical Medicine and the Department of Health in a large study of infectious intestinal disease in the community which should clarify the situation (Roderick 1995).The results of this study will become available in 1997.

2.3 Surveillance of possible emerging pathogens

2.3.1 The interpretation of trends in emerging pathogens is difficult since for many, diagnostic methods have only recently been developed, improved or become widely used. Trends must be interpreted with caution as often apparent increases, especially when the numbers are small, can be explained by:

- the introduction of new diagnostic tests
- more widespread use of tests
- special interest by one or more microbiologists
- one or two outbreaks which skew the figures

Many of the emerging pathogens are reported to CDSC as broad groups of organisms rather than as particular strains. Without more definitive typing the incidence of one particular strain may increase without being recognised. For example, within the salmonellae, serotyping and phage typing, enabled the identification of *S. enteritidis* PT4 as an emerging pathogen in the 1980s (Baird-Parker, 1990; WHO, 1989). Similarly, the addition of antibiograms and plasmid profile analysis has enabled the identification of multidrug resistant

S typhimurium DT104 as the emerging salmonella of the 1990s. (Threlfall *et al*, 1994; Wall *et al*, 1994).

For those pathogens which affect subgroups of the population, such as the immunosuppressed, only those laboratories processing specimens from these patients will report to CDSC and the number of reports are small.

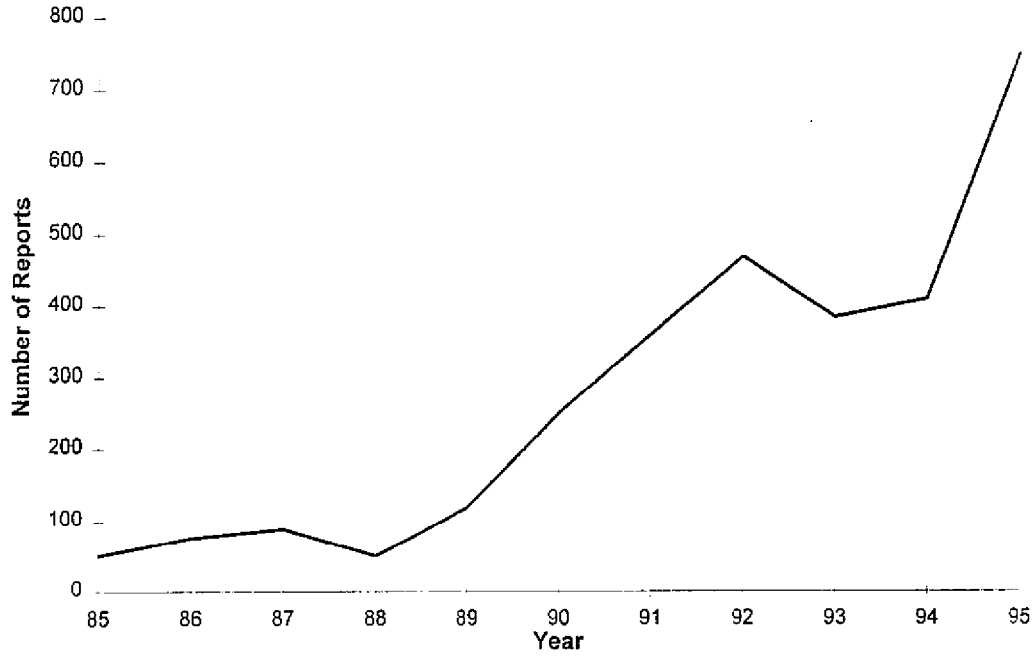
2.3.2 Verocytotoxin-producing *Escherichia coli* O157 (VTEC)

Verocytotoxin producing *Escherichia coli* O157 (VTEC) was first identified as a human pathogen in 1982 in two outbreaks associated with the consumption of hamburgers from a fast food chain in the United States (Riley, 1983). VTEC are different from other *E. coli* and require a special medium for their identification, such as, sorbitol MacConkey agar or improved derivatives (Chapman, 1994). Non-sorbitol fermenting colonies are tested for agglutination with an O157 antiserum or with an O157 latex agglutination kit (Smith, 1993). These newer diagnostic methods have only become more widely used in the 1990s. The immunomagnetic separation technique was developed in the PHLS in 1993 (Chapman, 1994) and is currently becoming the accepted standard for the isolation of VTEC O157.

In 1995 the VTEC working group of the Advisory Committee on the Microbiological Safety of Food recommended that all diarrhoeal stools should be tested for VTEC O157 (Advisory Committee on the Microbiological Safety of Food, 1995). Before this time many laboratories only looked for VTEC O157 in children with haemorrhagic colitis. As only one or two laboratories in England and Wales were initially looking for VTEC O157 the graph of reports to CDSC starts in 1985 (Figure 2.2). The increase in the late 1980s and early 1990s is most likely due to increased ascertainment as the more sophisticated diagnostic methods have become more widely used. The more recent increase is believed to reflect both an increased ascertainment and a genuine increase in incidence. Waterborne outbreaks have been reported (Dev, 1991; Brewster, 1994; Keene, 1994; Hildebrand, 1996) but no outbreak related to drinking water has occurred in England and Wales. However, one such outbreak occurred in Scotland in 1995 (Reilly, personal communication) emphasising the need for vigilance.

Figure 2.2

Laboratory reports of E coli O157 VTEC England and Wales 1985-1995

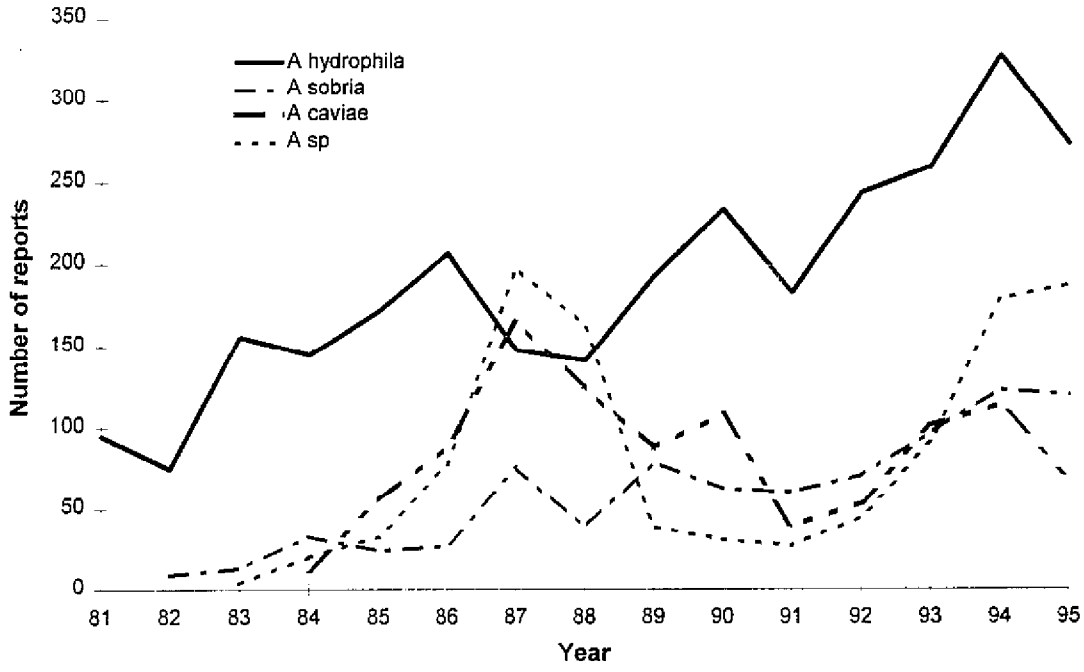


2.3.3 Aeromonas

Aeromonas sp. are Gram negative aerobic bacilli, and are ubiquitous inhabitants of fresh and brackish water (McGowan, 1995). Initially four species were recognized (*A. salmonicida, A. hydrophila, A. sobria* and *A. caviae*)(Krieg *et al*, 1984). These have now been subdivided and there are currently 14 genospecies of which 12 are named (Carnahan, 1991). Understanding of their biology and ability to cause human disease has only expanded in the past decade (Janda, 1991). A number of reports from diverse geographical locations have associated *Aeromonas* spp. with diarrhoeal disease in humans and in some locations they have been reported as commonly as *Campylobacter* spp. (Holmberg *et al*, 1986; Gluskin *et al*, 1992; Challapalli, *et al*, 1988; King, 1992). There is debate ongoing as to whether this association is a causal one. Evidence supporting a causative role in diarrhoeal disease includes: (i) a higher carriage rate in symptomatic compared to asymptomatic individuals, (ii) absence of other enteric pathogens in most symptomatic patients harbouring *Aeromonas* sp. (iii) Identification of aeromonas exotoxins (although the absence of an animal model has hampered efforts to directly link toxin production with disease), (iv) improvement of diarrhoea with antibiotics active against *Aeromonas* spp. and (v) evidence of a specific secretory immune response to infection with *Aeromonas* spp. (McGowan, 1995; Namardi, *et al*, 1990a; Namardi, *et al*, 1990b; Jiang, *et al*, 1991). The majority of aeromonads isolated

from diarrhoeal stools are *A. caviae* (McGowan, 1995). *A. hydrophila* is the most common species producing soft tissue infection and trauma followed by exposure to contaminated water commonly precedes infection (Calleaus, *et al*, 1993). In England and Wales three species are identified, *A. hydrophilia*, *A. sobria*, and *A. caviae* with *A hydrophilia* being the most common. The number of reports, however, are under 300 per year.

Figure 2.3 Laboratory reports of Aeromonas England and Wales 1981-1995

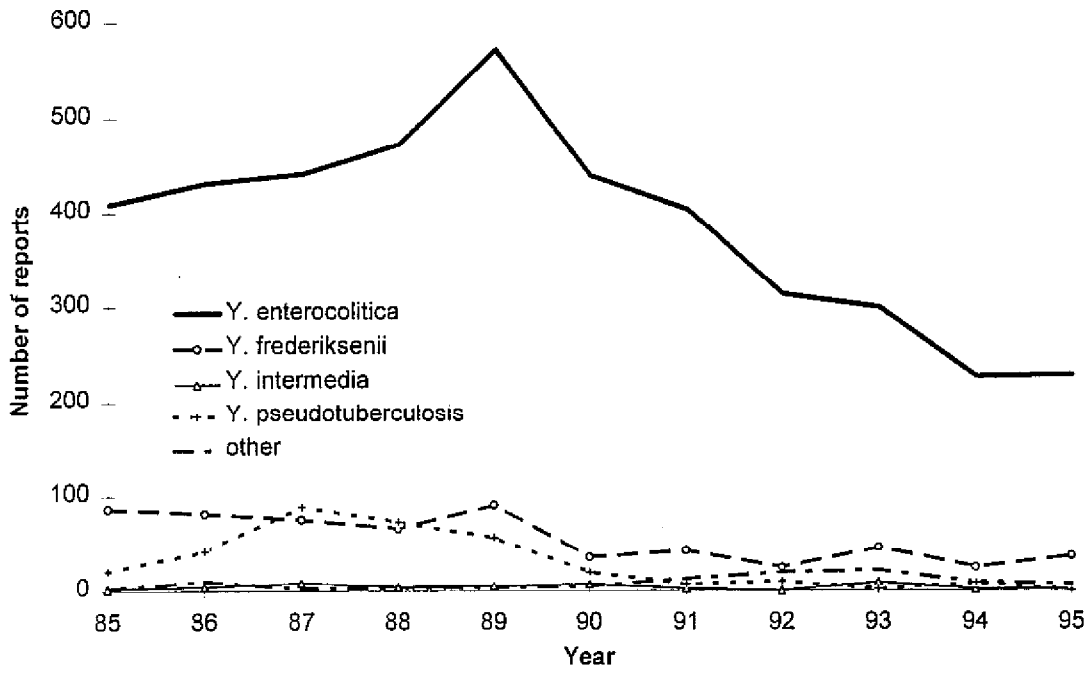


2.3.4 Yersinia

The genus Yersinia includes the pathogens *Y. pestis*, *Y. enterocolitica*, *Y. pseudotuberculosis*, *Y. frederikseni* and *Y. intermedia*. These are zoonotic infections that affect predominantly rodents, pigs and birds. Humans are accidental hosts for infection. *Yersinia pestis* is the cause of plague and does not occur in the UK, most cases being reported from developing countries where people come into contact with rodents and rodent fleas. Only rarely during epidemics of pneumonic plague is the infection passed directly from person to person. *Y. enterocolitica* is transmitted by the ingestion of contaminated food or water and less commonly by direct contact with infected animals or patients. It is a relatively infrequent cause of diarrhoea in the United Kingdom (Figure 2.4) but is more common in other European countries where ingestion of incompletely cooked pork and contamination of other foods by pork are important in disease transmission (Tauxe *et al*, 1987).

Figure 2.4

Laboratory reports of Yersinia England and Wales 1985-1995

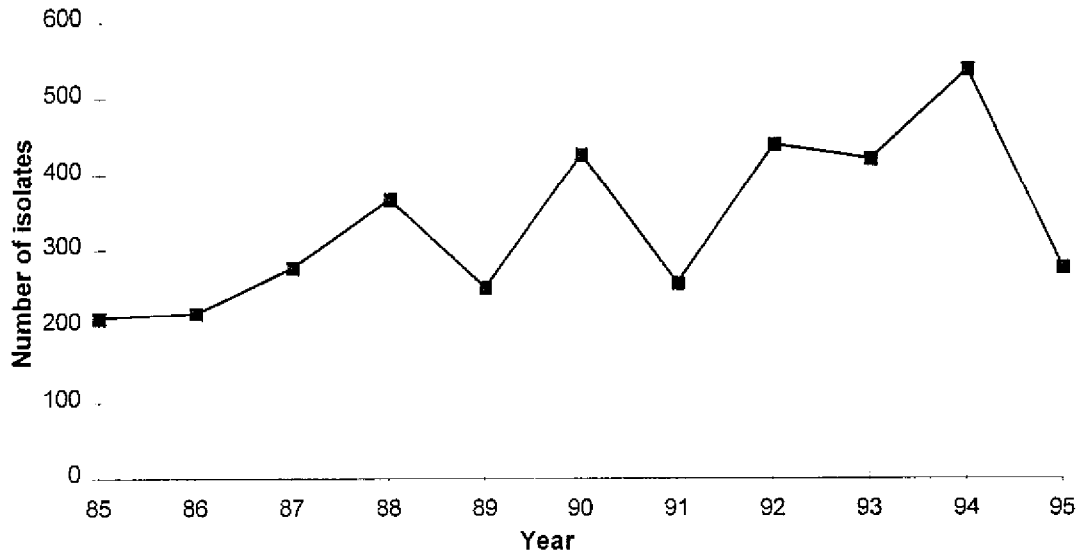


2.3.5 Astrovirus

In addition to the caliciviruses, SRSV and classical human caliciviruses, the most important viral agents causing food/waterborne outbreaks are astroviruses. (Hedberg, 1993). Astroviruses were first described in 1975 (Madely, 1976). The genome organisation lead to the establishment of the family Astroviridae (Monroe, 1993; Carter, 1994), with human astrovirus 1 as the type species (Murphy, 1995). Seven serotypes of human astroviruses have been reported (Kurtz, 1994; Lee, 1994). Electron microscopy is the method of choice for diagnosis. Astroviruses may be seen from one day before the onset of diarrhoea and may persist in faeces for two to four days (Kurtz, 1994). Only about 10% of particles show the star -like appearance which results in an underestimation of astrovirus incidence because these particles will be labelled as SRSV. Astrovirus does not grow in conventional tissue cultures. It is only since the late 1980s that outbreaks have been described in family groups, in nurseries and in paediatric wards, some outbreaks have been associated with the consumption of oysters or contaminated water (Kurtz, personal communication). The number of laboratory reports of astrovirus in England and Wales has remained constant at over the last ten years between 200 and 400 reports per year (Figure 2.5).

Figure 2.5

Laboratory Reports of Astrovirus England and Wales 1985-95



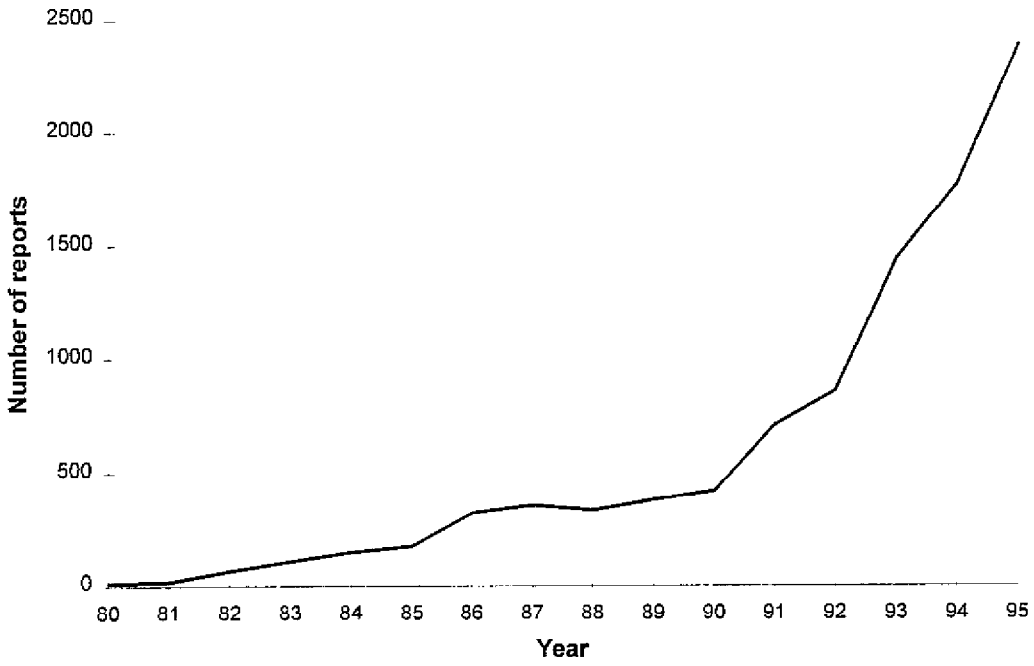
2.3.6 Small Round Structured Viruses and other Caliciviruses

Until twenty five years ago the causes of acute non-bacterial gastro-enteritis were unknown, even though Zahorsky, an American paediatrician, described a syndrome which he named winter vomiting disease in 1929 (Zahorsky, 1929; Alder, 1969). A viral agent was first described in 1972 following and outbreak of gastro-enteritis in Norwalk, Ohio, and was named Norwalk virus (Kapikian, 1972). Other isolates with similar features were later designated Norwalk- like viruses and named after the places of isolation *eg* Hawaii agent (Thornhill, 1977). It was only in 1982 that an interim classification scheme for small round viruses based on electron microscopic features was introduced, where viruses with an amorphous surface and ragged outline within a size range of 30-35 nm and a buoyant density of 1.36 to 1.41 g/cm^3(*e.g.* Norwalk virus) were classified as small round structured viruses (SRSV) (Caul, 1982). American authors refer to these viruses as Norwalk or Norwalk-like viruses, whereas British authors refer to them as SRSV. Following the classification and nomenclature of viruses by the International Committee on Taxonomy of Viruses, Norwalk virus and Norwalk-like viruses are now classified within the family of Caliciviridae in the genus Calicivirus, and the species human calicivirus (Murphy, 1995 and Table 1.2, in Section 1.8.2). The small round structured viruses are not one strain and contain many different pathogens. As the classification of SRSV was only developed in 1980 and became more

widely used in the mid 1980s the initial increase in laboratory reports was due to increased ascertainment whereas the latter increase reflects a genuine increase in incidence (Figure 2.6).

Figure 2.6

Laboratory reports of SRSV England and Wales 1980-1995

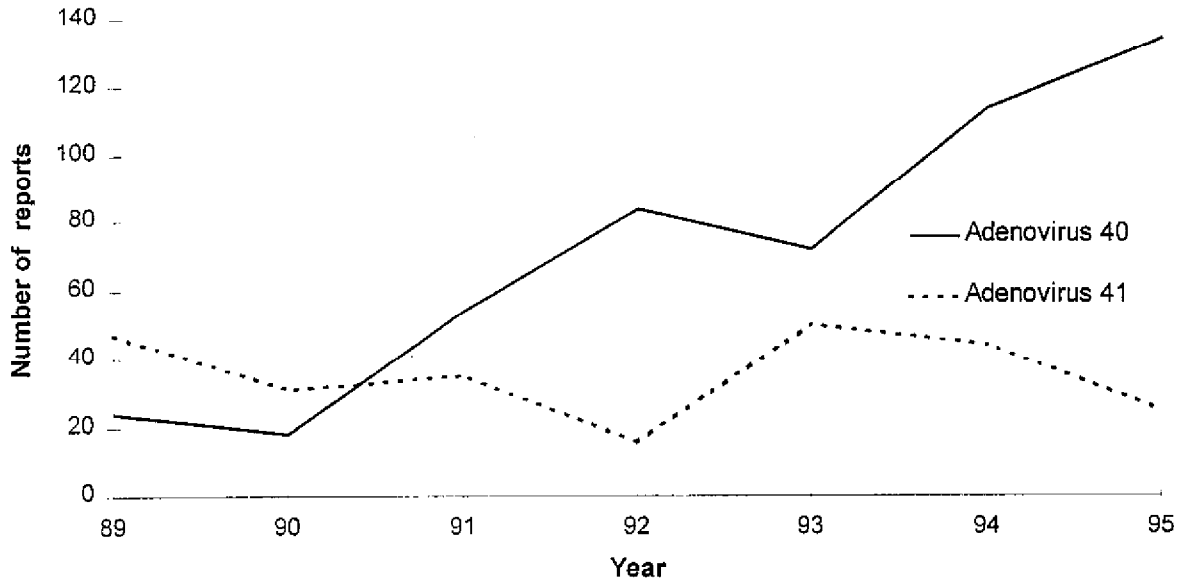


2.3.7 Adenovirus

Of the 47 serotypes at present known, subgenus F serotype 40 and serotype 41 are established as a cause of viral gastro-enteritis (Wadell, 1994). Outbreaks occur mainly in settings with susceptible children (*e.g.* wards and day care centres) and infection probably leads to long term immunity (CDC, 1990). Adenovirus may also contribute to illness in immunocompromised hosts (Shaw, 1993). It is not possible to explain why serotype 40 is more prevalent than 41 from CDSC data (Figure 2.7).

Figure 2.7

Laboratory reports of Adenovirus 40 and 41
England and Wales 1989-95



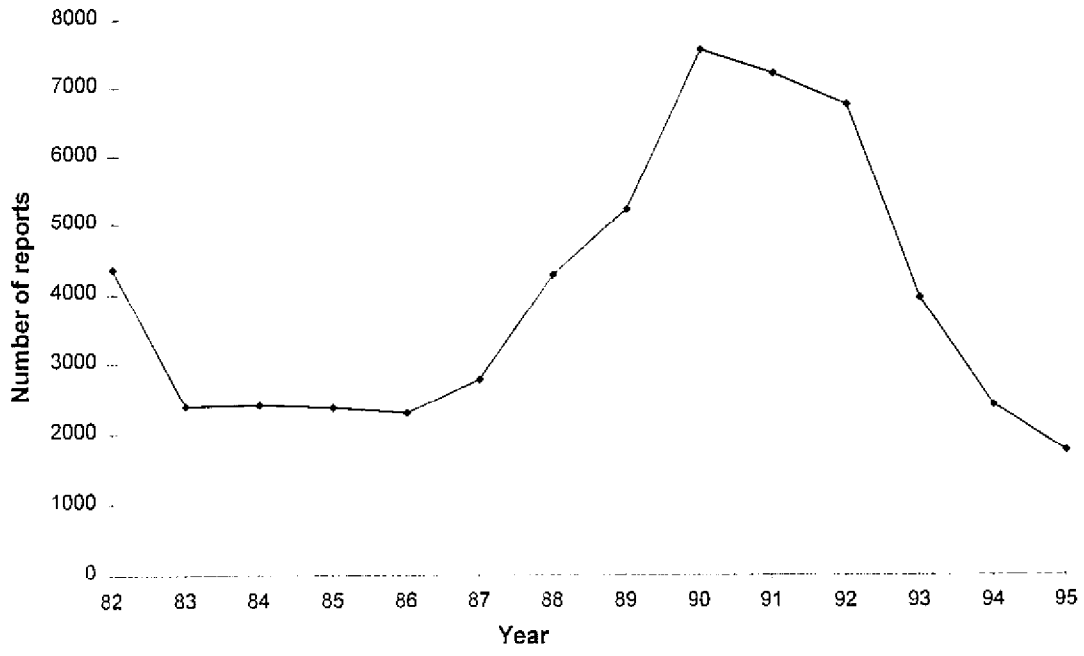
2.3.8 Hepatitis A

Hepatitis A virus has a world-wide distribution and like other enteric infectious diseases it is typically an infection of childhood, relating to conditions of crowding and poor hygiene. The decrease in the incidence of hepatitis A in developed countries is probably associated with the high quality of water supplies, good personal hygiene and good facilities for handling and treating human waste. Cyclical patterns of disease prevalence with peaks every 5-10 years have been noted in developed countries (Gust *et al*, 1988) and the pattern in the UK is similar (Figure 2.8). The first water-borne epidemic of hepatitis A was described in 1920 and since then many other episodes have been documented (Gust *et al*, 1988; Mosley 1959). However, waterborne outbreaks of hepatitis A are uncommon in developed countries. Heavily contaminated water can be rendered safe by the addition of high levels of chlorine or by boiling. In addition it is possible to acquire hepatitis A by swimming in contaminated water. Hepatitis A (as infective jaundice) was made notifiable in England and Wales to the Office of Population Censuses and Surveys (OPCS) in 1968. However, laboratory reporting of positive results of anti-hepatitis A IgM tests to CDSC only began in 1980. The increase in reports seen in 1990 and 1991 was primarily in school age children and young adults (Maguire *et al*,.1992) Figure 2.8. A travel history is provided for only a third of laboratory reports and the proportion of hepatitis A cases reported to CDSC with a history of foreign

travel varies each year from 20 to 50% (PHLS Working Group, 1991). Most of the outbreaks reported to CDSC are small family outbreaks and are not investigated further. Apart from cases acquired by visitors from the UK to endemic areas, infection has been acquired by person to person spread (Rajaratnam *et al*, 1991), contaminated food (Warburton *et al*., 1991) and by the consumption of shellfish harvested in waters contaminated by human sewerage (O'Mahony *et al*,1983). Shellfish are particularly likely to transmit hepatitis A because they filter large quantities of water to obtain adequate supplies of food and oxygen. They may serve as reservoirs of infection by concentrating the virus. Shellfish are often eaten raw or after gentle steaming which is sufficient to cause the shell to open but inadequate to inactivate the virus (Koff *et al*, 1967). Although hepatitis A has been around for many years it has the potential to re-emerge as a problem hence the need for ongoing surveillance.

Figure 2.8

Laboratory reports of Hepatitis A England and Wales 1982-1995



2.3.9 Hepatitis E

Waterborne epidemics of non A non B hepatitis were recognised in India in the late 1950s (Wong *et al*, 1980; Balayan *et al*, 1983). However it was only in 1990 that the causal agent hepatitis E was first identified and so hepatitis E is a new pathogen by recognition rather than by prevalence (Desselberger, 1995). Hepatitis E is transmitted primarily by the faecal -oral

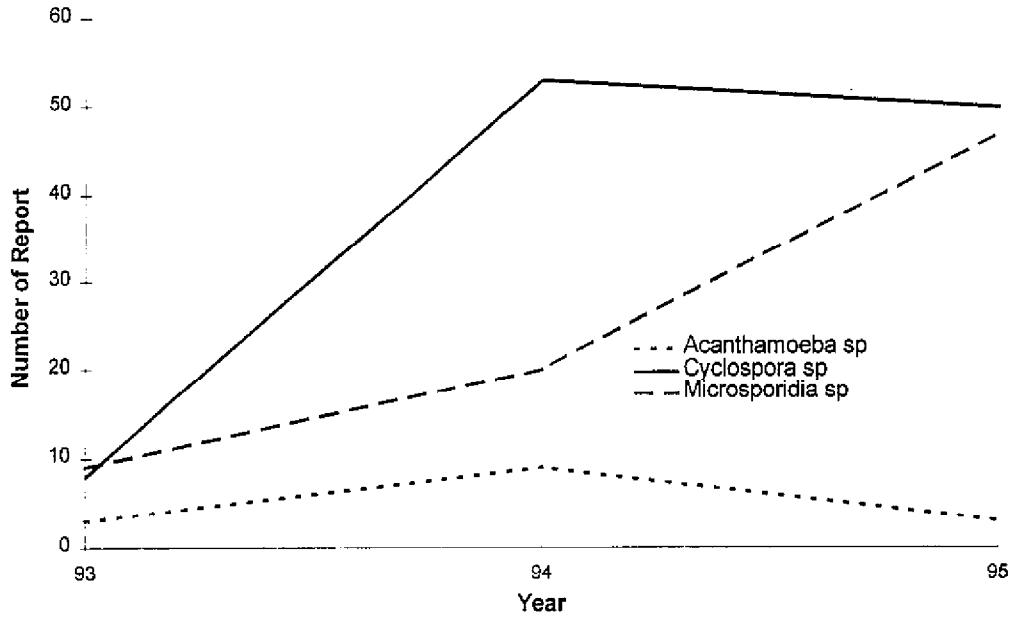
route and contaminated drinking water has figured prominently in epidemic reports (Skidmore *et al*, 1992; Jothikumar *et al*, 1993). Thus the incidence of hepatitis E is very dependent on the overall level of public health sanitation and in particular the quality of potable water. The geographical distribution of the virus is largely confined to developing regions and the few cases in England and Wales (<10 per year) occur in visitors to endemic regions.

2.3.10 Acanthamoeba

Acanthamoeba are a group of free living amoebe, they have been isolated from soil, water, and air from diverse geographical locations (Sotelo-Avila, 1987; Martinez, 1985). *Acanthamoeba* spp. were cultured from pharyngeal swabs of 39 of 2,289 individuals during a study of respiratory viruses in healthy families (Butt, 1966), and serological surveys have detected serum antibodies directed against acanthamoeba in healthy people (Cursons, 1977). Despite such evidence of common exposure without disease manifestations *Acanthamoeba* spp. have been reported to cause disease predominantly in debilitated or immunosuppressed individuals (Martinez, 1980). Amoebic encephalitis has been described in fewer than 200 patients world-wide (Marciano-Cabral, 1995) and the granulomatous form in only seventeen patients, two of whom had AIDS (Gordon, 1992; Gonzalez, *et al*, 1986; Wiley *et al*,1987). In addition. *Acanthamoeba* spp. have been reported to cause keratitis (Visvesvara, 1990) in immunocompetent people particularly wearers of contact lenses (Stehr-Green. *et al*,1987). Acanthamoeba can survive in many contact lens solutions which may result in transmission of disease (Nauheim. *et al*, 1990). Distinct from other pathogenic protozoa by nature of their free living existence, there are no known insect vectors, no human carrier states of epidemiological importance and little relationship of poor sanitation to the spread of infection (Marciano-Cabral, 1995). Interest in *Acanthamoeba* spp. as a pathogen in England and Wales has

only developed in the 1990s and the number of reports are less than ten per year (Figure 2.9).

Figure 2.9
Laboratory reports of Acanthamoeba Cyclospora and Microsporidia In England and Wales 1993-1995



2.3.11 Microsporidia

The term microsporidia is a nontaxonomic designation commonly used to describe organisms belonging to the order Microsporidia of the phylum Microspora. Contained in this phylum are over 100 genera and almost 1.000 species (Sprague *et al*, 1992). These unique protozoa should not be confused with the more familiar coccidia (*e.g. Cryptosporidium* spp. and *Isospora* spp.) which belong to the phylum Apicomplexa (Bryan *et al*, 1991). Their role as important emerging pathogens in humans is being increasingly recognised (Weber *et al*, 1994; Lederberg, 1992). Most recognised human cases have occurred in persons infected with HIV, most commonly associated with severe immunosuppression (Bryan, 1995) and many authors believe that clinically apparent human microsporidial infections may be limited to persons with compromised immunity or to infections of the cornea in persons with normal immunity (Shadduck 1989; Cali *et al*, 1991; Canning, 1991). These are extremely rare in England and Wales with less than 10 reports in 1993 and less than 50 in 1995 (Figure 2.9). Faecal-oral and urinary-oral transmission appear likely, Microsporidia can survive for prolonged periods in the

environment in a spore form. It is possible that waterborne disease could occur in susceptible individuals (Bryan, 1995).

2.3.12 Cyclospora

Organisms in human faeces, previously, and mistakenly named blue-green algae (cyanobacterium-like-bodies) or large cryptosporidium have recently been identified as belonging to the coccidian genus Cyclospora (Bendall, *et al*, 1993; Ortega, 1993). Although reported in HIV infected individuals (Wurtz *et al*, 1993), infection with *Cyclospora* spp. has been primarily documented in immunocompetent adults and children, especially among travellers (Bendall, *et al*, 1993; Ortega, 1993; Wurtz, *et al*, 1993; Shlim, *et al*, 1991). Infection is frequently linked to contaminated water, occurs world-wide but is particularly notable in Nepal where rainy season outbreaks have been reported (Hoge, *et al*, 1993). The organism resembles *Cryptosporidium parvum* but is roughly twice the size (Ortega, 1993). These organisms are extremely rare in England and Wales with less than 10 reports in 1993 and less than 50 in 1995 (Figure 2.9).

2.4 National surveillance scheme for general outbreaks of infectious intestinal disease

CDSC becomes aware of possible general outbreaks of infectious intestinal disease from a variety of sources, including the national laboratory reporting scheme, Consultants in Communicable Disease Control (CCDC), Environmental health officers (EHOs), microbiologists and others. A structured questionnaire is then dispatched to the appropriate CCDC with the request that the form be completed by the lead investigator on completion of the outbreak investigation. The questionnaire seeks a minimum data set on all general outbreaks, including details of the setting in which the outbreak occurred, mode of transmission, causative organism, and details of laboratory and epidemiological investigations. To improve the surveillance of waterborne disease the existing system has been adapted to ensure that relevant data are received in a timely fashion and thus permit the generation of useful output. To this end a new questionnaire specific for waterborne outbreaks has been developed and standard definitions for the strength of association between illness and water have been adopted for classifying waterborne outbreaks. A formal procedure for handling information on outbreaks of waterborne infection has been put in place and became operational on January 1 1996. The commonest pathogen causing waterborne outbreaks in England and Wales is *Cryptosporidium parvum*. Only one waterborne outbreak has been identified and reported by the present outbreak surveillance system as having been caused by the emerging

pathogens under review. This was a general outbreak of waterborne astrovirus which occurred in the South Western Regional Health Authority in May 1994, four people became ill after consuming contaminated water.

2.5 Conclusions

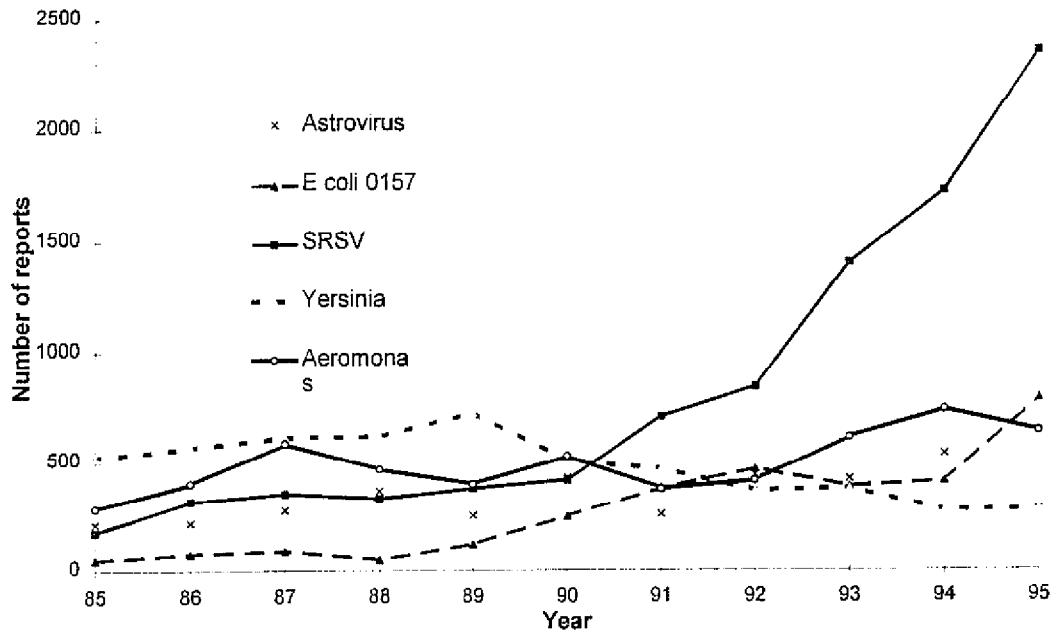
Surveillance is defined as the ongoing systematic collection, collation and analysis of data and the prompt dissemination of the resulting information to those who need to know so that an action can result. Information from the two surveillance systems described can highlight emerging or re-emerging pathogens and, in the case of general outbreaks, the faults contributing to waterborne disease and so facilitate the targeting of appropriate interventions.

It must be stressed that the incidence of these pathogens is low and the problem must be viewed in the context of the more common pathogens such campylobacter and salmonella at over 40,000 and over 30,000 isolates per year respectively. Figure 2.10 shows the most common of these possible emerging pathogens and highlights that although SRSV has the highest incidence there are less than 2,500 reports per year.

Surveillance data are crude data and do not represent the true incidence as both under-reporting and underdiagnosis exist, however, they serve to highlight areas deserving of further investigation by in depth studies.

Figure 2.10

Possible emerging pathogens in England and Wales 1985-1995



2.6 References

Adler I, Zickl R. Winter vomiting disease. J Infect Dis 1969; 119: 668-673
Advisory Committee on the Microbiological Safety of Food. Report on Verocytotoxin-producing Escherichia coli. London: HMSO, 1995.

Anon. Strategic Review of Pathology Services. NHS Executive London HMSO 1995.
Baird-Parker AC. Foodborne salmonellosis. *Lancet* 1990;336:1231-1235.
Balayan MS, Andzhaparidze AG, Savinskaya SS *et al*,. Evidence for a virus in nonA, non B hepatitis transmitted via the faecal oral route. Intervirology 1983;20:23-31.

Brewster DH, Brown MI, Robertson D, Houghton GL, Bimson J, Sharp JCM. An outbreak of Escherichia coli O157 associated with a children's paddling pool. *Epidemiol Infect* 1994;**112**:441-7.

Bryan RT, Visvesvara GS. Coccidia and microsporidia. *Ann Intern Med* 1991;114:343.
Butt CG. Primary amoebic meningoencephalitis. N Engl J Med. 1966;274:1473-6.
Cailleaux V, Dupont MJ, Hong B *et al*,. Why did infection with Aeromonas hydrophilia occur when water contains so many other microorganisms? *Clin Infect Dis*. 1993;16:174.

Cali A, Meisler D, Lowder CY *et al*,. Corneal microsporidiosis, characterization and identification. J Protozool 1991;38:105S-111S.

Canning EU, Hollister WS. In vitro and in vivo investigations of human microsporidia. J Protozool 1991;38:631-5.

Carnahan AM, Behram S, Joseph SW. Aerokey II; a flexible key for identifying clinical Aeromonas species. J Clin Microbiol 1991;29:2843-9.

Caul EO, Appelton H. The electron microscopical and physical characteristics of small round human fecal viruses: an interims scheme for classification. J Med Virol 1982; 9: 257-65

Centers for Disease Control. Comprehensive plan for epidemiologic surveillance: Centers for Disease Control, August 1986. Atlanta, GA:CDC, 1986

Challapalli M, Tess BR, Cunningham DG *et al*,. Aeromonas associated diarrhoea in children. Pediatric Infect Dis J 1988;7:693-8.

Chapman PA. Isolation, identification and typing of Verocytoxin-producing Escherichia coli O157. *PHLS Microbiology Digest* 1994;11:13-7.

Cursons RTM, Brown TJ, Keys EA. Immunity to pathogenic free living amoeba. *Lancet* 1977;2:875-6.

Department of Health. Management of outbreaks of foodborne illness. London: Department of Health. 1994.

Desselberger U. Emerging infections. *PHLS Microbiology Digest* 1995;12:141-4.

Dev VJ, Main M, Gould IM. Waterborne outbreak of Escherichia coli O157. Lancet 1991;337:1412.

Farrington CP, Beale AD. Computer-aided detection of temporal clusters of organisms reported to the communicable Disease Surveillance Centre. *Communicable Disease Report* 1993;3:R78-R82.

Feldman RA, Banatvala N. The frequency of culturing stools from adults with diarrhoea in Great Britain. *Epidem Inf* 1994;113:41-44.

Gluskin I, Batash D, Shoseyov D *et al*,. A 15 year study of the role of Aeromonas spp in gastroenteritis in hospitalized children. J Med Microbiol 1992;37:315-8.

Gonzalez MM, Gould E, Dickenson G, *et al*,. Acquired immunodeficiency syndrome associated with Acanthamoeba infection and other opportunistic organisms. Arch Pathol Lab Med 1986;110:749-51.

Gordon SM, Steinberg JP, Dupuis MH *et al*,. Culture isolation of Acanthamoeba species and Leptomyxid amebas from patients with amebic meningoencephalitis, including two patients wit AIDS. *Clin Infect Dis* 1992;15:1024-30.

Grant AD, Eke B. Application of information technology to the laboratory reporting of communicable disease in England and Wales. *Communicable Disease Report* 1993;3:R78-R82.

Gust IS, Feinstone SM. Epidemiology. In: Hepatitis A. Boca Raton,FL:CRC Press;1988:163-92.

Handysides S. CDR Weekly and CDR Review: five years old. *Communicable Disease Report* 1996;6:R25-6.

Hedberg CW, Osterholm MT. Outbreaks of food-borne and waterborne viral gastroenteritis. *Clin Microbiol Rev* 1993; 6:199-210

Henry R, McGuirk S. COSERV:CDR at your fingertips. PHLS Annual Conference 1995 (Abstract 3/27) p178.

Hildebrand JM, Maguire HC, Holliman RE, Kangesu E. An outbreak of Escherichia coli O157 infection linked to paddling pools. *Communicable Disease Report* 1996; 6:R33-R36.

Holmberg SD, Schell WL, Fanning GR *et al*,. Aeromonas intestinal infection in the United States. *Ann Intern Med* 1986;105:683-9.

Janda JM. Recent advances in the study of the taxonomy, pathogenicity and infectious syndromes associated with the genus Aeromonas. Clin Microbiol Rev 1991;4:397-410.

Jiang ZD, Nelson AC, Mathewson JJ *et al*,. Intestinal secretory immune response to infection with Aeromonas species and Plesiomonas shigelloides among students from the United States in Mexico. *J Infect Dis* 1991;164:979-82.

Jothikumar N, Aparna K, Kamatchiammal S *et al*,. Detection of hepatitis E virus in raw and treated wastewater with the polymerase chain reaction. *Appl Environ Microbiol* 1993;59:2558-62.

Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, Chanock RM. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J Virol 1972; 10: 1075-1081

Keene WE, McAnulty JM, Hoesly FC, Williams LP Jr, Hedberg PHK, Oxman GL *et al*,. A swimming associated outbreak of hemorrhagic colitis caused by Escherichia coli O157:H7 and Shigella sonnei. *N Engl J Med* 1994;331:579-84.

King GE, Werner SB, Kizer KW. Epidemiology of Aeromonas infections in California. Clin Infect Dis 1992;15:449-52.

Koff RS, Sear HS. Internal temperature of steamed clams. N Engl J Med 1967;276:737-9.

Krieg NR, Holt JG. Bergey's Manual of Systematic Bacteriology. Baltimore: Williams and Wilkins;1984.

Lederbeg J, Shope RE, Oaks SC eds. Emerging Infections:Microbial Threats to Health in the United States. Washington, DC:National Academy Press 1992.

Maguire H, Heptonstall J, Begg NT. The epidemiology and control of hepatitis A. Communicable Disease Report 1992;2:R114-117.

Marciano_Cabral F, Petri WA. Free living Amoeba. Chapter 252 pp 2408-2415 in Principles and practices of Infectious Diseases 4 edition eds Mandell GL, Bennett JE, Dolin R. Churchill Livinstone New York 1995.

Martinez AJ. Free-Living Amebas: Natural Histroy, Prevention, Diagnosis Pathology and Treatment of Disease. Boca Raton, FL:CRC Press;1985.

Martinez AJ. Is Acanthamoeba encephalitis an opportunistic infection? *Neurology* 1980;30:567-74.

Mosley JW. Waterborne infectious hepatitis. N Engl J Med 1959;261:703-8.

Murphy FA, Fauquet CM, Bishop DHL, Ghabrial SA, Jarvis AW, Martelli GP, Mayo MA, Summers MD, (eds.). Virus Taxonomy. Classification and Nomenclature of Viruses. Sixth Report of the International Committee on Taxonomy of Viruses. *Arch Virol* 1995; Suppl 10: p 359-367, 409-411.

Namardi H, Bottone EJ. Cytotoxin and enterotoxin production as factors delineating enteropathogenicity of Aeromonas caviae. J Clin Microbiol 1990;28:1796-8.

Namardi H, Bottone EJ. Microbiologic and clinical evidence supporting the role of Aeromonas caviae as a pediatric enteric pathogen. J Clin Microbiol, 1990;28:837-40.

Nauheim RC, Brockman RJ, Stopak SS *et al*,. Survival of Acanthamoeba in contact lens rinse solutions. *Cornea* 1990;9:290-3.

Rajaratnam G, Patel M, Parry JV, Perry KR, Palmer SR. An outbreak of hepatitis A:school toilets as a sourcve of transmission. *J Publ Hlth Med* 1991;14:72-7.

Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR. Hemorrhagic colitis associated with a rare Escherichia coli serotype. N Engl J Med 1983;308:681-5.

Roderick P, Wheeler J, Cowden JM *et al*,. A pilot study of infectious disease in England. *Epidem Inf* 1995;114:227-288.

Shadduck JA. Human microsporidiosis and AIDS. Rev Infect Dis 1989;11:203-7.

Skidmore SJ, Yarbough PO, Gabor KA *et al*,. Hepatitis E virus: the cause of a waterborne hepatitis outbreak. *J Med Virol* 1992;37:58-60.

Smith HR, Scotland SM. Isolation and identification methods for Escherichia coli O157 and other verocytotoxin producing strains. *J Clin Pathol* 1993;46:10-7.

Sotelo-Avila C. Naegleria and Acanthamoeba. Free living amoebas pathogenic for man. *Perspect Pediatr Pathol* 1987;10:51-85.

Spraque V, Becnel JJ, Hazard EI. Taxonomy of the phylum Microspora. *Crit Rev Microbiol.* 1992;18:285-395.

Stehr-Green JK, Bailey TM, Brandt FH *et al*,. Acanthamoeba keratitis in soft contact lens wearers. A case control study. JAMA 1987;258:57-60.

Tauxe RV, Vandepitte J, Wauters G *et al*,. Yersinia enterocolitica infection and pork:The missing link. *Lancet* 1987;1:1129-32.

The present state of hepatitis A infection in Engalnd and Wales. Report of a PHLS working group. *PHLS Microbiology Digest* 1991;8:122-6.

Thornhill TS, Wyatt RG, Kalica AR, Dolin R, Chanock RM, Kapikian AZ. Detection by immune electron microscopy of 26-27 nm virus-like particles associated with two family outbreaks of gastroenteritis. J Infect Dis 1977; 135:20-27

Threlfall EJ, Frost JA, Ward LR, Rowe B. Epidemic in cattle of S typhimurium DT104 with chromosomally integrated multiple drug resistance. *Vet Rec* 1994;134:577.

Visvesvara GS, Stehr-Green JK. Epidemiolgy of free living amoeba infections. *J Protozool.* 1990;37:25S-33S.

Wall PG, Morgan D, Lamden K, Ryan M, Griffin M, Threlfall EJ, Ward LR, Rowe B. A case control study of infection with an epidemic strain of multiresistant Salmonella typhimurium DT104 in England and Wales. Comm Dis Rev 1994;4:R130-R135.

Warburton ARE, Wreghitt TG, Rampling A, Buttery R, Ward KN, Perry KR *et al,*. Hepatitis A outbreak involving bread. *Epidemiol Inf* 1991;106:199-202.

Weber R, Bryan RT, Schwartz DA *et al,*. Human microsporidial infections. *Clin Microbiol* Rev 1994 **check vol and p**

Wiley CA, Safrin RE, Davis Ce, *et al,*. Acanthamoeba meningoencephalitis in a patinet with AIDS. J Infect Dis. 1987;155:130-3.

Williams REO. Microbiology for the Public Health. London: Public Health Laboratory Service, 1985.

Wong DC, Purcell RH, Sreenivasan MA *et al,,*. Epidemic and endemic hepatitis A in India: Evidence for a non-A, non-B hepatitis virus aetiology. Lancet 1980;2:876-9.

World Health Organisation. Consultation on epidemiological emergency in poultry and egg salmonellosis. Geneva:WH) 1989.

Zahorsky J. Hyperemesis hiemis or the winter vomiting disease. Arch Pediat 1929; 46: 391-395.

Section 3

BACTERIA IMPLICATED AS POTENTIAL EMERGING PATHOGENS

3.1 Vero cytotoxin producing Escherichia coli VTEC belonging to serogroup 0157 and other serogroups

Dr Henry Smith
Laboratory of Enteric Pathogens
PHLS, Central Public Health Laboratory
London NW9 5HT

3.1.1 Introduction

Certain strains of *Escherichia coli* are an important cause of diarrhoeal disease. Several classes of diarrhoeagenic *E. coli* are now recognised and these have been defined by the possession of distinct virulence factors. The first three classes to be described were the enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC) and the enteroinvasive *E. coli* (EIEC). A further class, the Vero cytotoxin-producing *E. coli* (VTEC) was defined in 1977.

VTEC produce a cytotoxin active on Vero cells which are African green monkey kidney cells grown in tissue culture. Although VTEC were first described in 1977 it was not until 1982 that these organisms were recognised as important aetiological agents of human disease. In particular, VTEC of serogroup O157 (O157 VTEC) were reported for the first time and since 1982 these organisms have become one of the most important emerging human pathogens. Individuals of all ages can be infected but young children in the age group from one to four years have the highest incidence of infection.

This report will concentrate on O157 VTEC as an emerging pathogen in relation to the drinking water supply but some information on VTEC of other serogroups will be included.

3.1.2 Human pathogenicity

Infections with VTEC in man are associated with a wide spectrum of symptoms ranging from a mild non-bloody diarrhoea to severe bloody diarrhoea (haemorrhagic colitis) and in some patients there is progression to haemolytic uraemic syndrome (HUS) (Karmali, 1989; Griffin and Tauxe, 1991; Su and Brandt, 1995). However, some individuals carry VTEC but without development of clinical symptoms. Haemorrhagic colitis, is usually preceded by abdominal cramps and watery diarrhoea and in some cases there is pyrexia. HUS is defined by three clinical features: acute renal failure, microangiopathic haemolytic anaemia and

thrombocytopenia. The typical form of HUS is associated with a prodromal diarrhoea that is often bloody. The pathogenesis of VTEC is not yet clearly understood although a number of potential virulence factors have been identified (for review, see ACMSF Report 1995). These include production of Vero cytotoxins, specific adhesins, formation of attaching and effacing lesions and production of an enterohaemolysin.

In addition to serogroup O157, VTEC belong to many different *E. coli* O serogroups. Two major classes of Vero cytotoxin, VT1 and VT2, are now recognised although several variants of both VT1 and VT2 have been identified. VT1 is very closely related to Shiga toxin, produced by *Shigella dysenteriae* 1. The Vero cytotoxins and Shiga toxin are proteins composed of one enzymatically active A subunit and five receptor binding B subunits. The A subunits of the toxins cause an irreversible inhibition of protein synthesis in eukaryotic cells. The B subunit is involved in binding of the toxin to glycolipids which are considered the functional receptors on cells. The glycolipid, globotriaosyl ceramide is highly expressed in the cortex of the human kidney.

Properties other than VT production may be essential for the full virulence of VTEC strains. Some VTEC, including those of serogroup O157, produce a characteristic attaching and effacing (AE) lesion and this property is also shown by the enteropathogenic *E. coli*. However, some VTEC lack the ability to cause AE lesions but may possess other adhesive properties that play a role in pathogenesis. Production of an enterohaemolysin has been described in many VTEC including *E. coli* O157 strains but the role of this putative virulence factor requires further investigation.

3.1.3 Human infectivity

The number of O157 VTEC organisms needed to produce disease in man appears to be low. Studies following a large outbreak in the United States suggested that as few as 40 organisms may have produced disease (USDA Report). In Britain analysis of a raw beefburger tested following an outbreak in Gwent showed that approximately 2 organisms per 25g were present (Willshaw *et al*, 1994). However, it should be noted that higher levels of contamination of beefburgers (40-930 organisms per gram) have been reported (Roberts *et al.* 1995). Other evidence for a low infective dose is circumstantial. Some O157 VTEC infections have been traced to vehicles such as raw milk and water supplies which are likely to be associated with a small number of organisms (Griffin and Tauxe, 1991). In addition, person to person spread is

an important means of transmission of infection. In this respect O157 VTEC infections resemble shigellosis which is known to have a low infective dose and there is often transmission from person to person.

3.1.4 Clinical treatment

At present there is no specific treatment for VTEC infections other than supportive therapy and management of complications such as anaemia and renal failure. The benefit of antimicrobial agents in modifying the course of the illness, affecting the duration of diarrhoea and excretion of the organism or the progression to HUS, is not clearly established (Wood, 1990, Su and Brandt, 1995). There have been recommendations for the avoidance of antimicrobial therapy for VTEC infection based on studies that suggested the duration of symptoms was not reduced and also that there may be an increased risk of development of HUS. Use of antimotility agents has also been suggested as a risk factor for progression of VTEC infection to HUS because their use may allow more time for toxin absorption.

3.1.5 Laboratory diagnosis

Evidence of VTEC infection can be provided by the isolation of VTEC including *E. coli* O157, demonstration of specific VT in faecal specimens or by the presence of antibodies to *E. coli* O157 lipopolysaccharide (ACMSF Report, 1995; Chapman, 1994). Most clinical laboratories have tested for O157 VTEC by plating faecal specimens on MacConkey agar with 1% D-sorbitol (SMAC agar) instead of lactose as, unlike most *E. coli*, O157 VTEC do not ferment sorbitol within 24 hours. Sorbitol non-fermenting colonies on the SMAC agar are tested for agglutination with an O157 antiserum or with an O157 latex agglutination kit. Modifications of this method have led to significant improvements in selectivity. In particular, the incorporation of cefixime and tellurite into SMAC agar, termed CT-SMAC agar, provides improved selection for O157 VTEC. The use of an enrichment culture step before plating has resulted in higher detection rates for O157 VTEC from faecal samples (Hindle *et al*,1995). Two liquid enrichment media have been widely employed; these are a modified trypticase soy broth containing novobiocin and buffered peptone water with added vancomycin, cefixime and cefsulodin. Immunomagnetic separation for *E. coli* O157 has also provided increased sensitivity of detection in faecal, food and environmental samples (Chapman, 1994; Bolton *et al*,1995).

VTEC belonging to serogroups other than O157, can be detected by testing for the presence of VT production or VT genes (ACMSF Report, 1995). VT production is detected by the cytotoxic effect on a monolayer of Vero cells grown in tissue culture. Cytotoxic effects on Vero cells should be confirmed by neutralization using antisera against VT1 or VT2. Several ELISA methods have been described for the detection of VTs and some of these tests can differentiate VT1 and VT2. Recently, ELISAs for the detection of VTs have become available commercially. The presence of VT genes can be detected by DNA probes in hybridization experiments or by amplification using the polymerase chain reaction.

The methods for the isolation of O157 VTEC from environmental samples need to be as sensitive as possible since the number of organisms present may be very small. The following approach has been proposed for the detection of O157 VTEC in water (F.J. Bolton, personal communication). Samples require concentration by filtration followed by enrichment in a liquid medium. This should include a step for recovery of sublethally damaged organisms. *E. coli* O157 can then be selectively detected using immunomagnetic separation followed by plating on a solid medium such as CT-SMAC. Further research is needed to develop and optimize the methods for both detection and enumeration of O157 VTEC in water. These studies should also be extended to VTEC of other serogroups.

3.1.6 Incidence of confirmed illness

The incidence of infections caused by O157 VTEC has been monitored by the PHLS Laboratory of Enteric Pathogens since 1983 (ACMSF Report, 1995; Thomas *et al.* 1993). There has been a very significant increase in the number of O157 VTEC isolates and a rise in the number of outbreaks caused by these organisms reported in Britain (Wall *et al.* 1996, Sharp *et al.*, 1995). The annual totals for England and Wales since 1982 are shown in Figure 1. The observed rises probably result from better surveillance and improved methods for isolation of O157 VTEC, as well as a real increase in the number of infections caused by these organisms. Sporadic cases of O157 VTEC infection have been linked to the drinking of untreated water. Investigation of a case of HUS in the USA showed that a likely source of the infection was consumption of lake water after a fall from a houseboat (Neill, Agosti and Rosen. 1985). In the same report a second case that developed HUS had drunk untreated water from a stream. This water appeared to be a possible source of the infection because the patient was a strict

vegetarian and avoided milk. An enhanced surveillance study in 1992/1993 in Scotland investigated the risk factors in 138 cases of O157 VTEC infection. In 12% of cases a private water supply was a risk factor and in another 12% of cases failure in the water supply was a risk factor (Coia *et al*, 1994).

Risk of infection with O157 VTEC could result from washing of contaminated salad vegetables. Contamination may result from spraying with water containing O157 VTEC as well as other pathogens. The survival of O157 VTEC on raw salad vegetables has been demonstrated (Abdul-Raouf, Beuchat and Ammar, 1993).

3.1.7 Extent and frequency of outbreaks associated with water

Several outbreaks of O157 VTEC infection associated with water have been reported but microbiological confirmation has been lacking. Details of outbreaks linked to water as a vehicle of infection are given below.

Britain

In 1990 four cases of O157 VTEC infection occurred in a village in the Grampian region (Dev, Main and Gould, 1991). Investigation of the drinking water showed heavy contamination with *E. coli* although *E. coli* O157 was not detected. In a period of drought the village supply had been supplemented with water from two reservoirs that had not been used for some time. It was suggested that the supply to one of the subsidiary reservoirs may have been contaminated with cattle slurry but this was not proved by further testing.

In 1992 a community outbreak due to O157 VTEC occurred in South East Scotland (Brewster *et al*, 1994). Six cases, including one asymptomatic, were identified by culture and one child developed HUS. Investigations suggested that a children's paddling pool was the focal point in the transmission of infection. The pool was filled with water from the public mains supply but was probably subsequently contaminated by one of the children. Person to person transmission appears to have been responsible for three of the cases. The water in the pool was discarded before it could be tested for the presence of O157 VTEC.

In 1993 there was an outbreak of O157 VTEC infection affecting six children in a small area in South West London; three of the children had visited the same paddling pool and subsequent investigations showed that the disinfection procedures at the pool were inadequate (Hildebrand *et al*, 1996). Sampling of the water showed the presence of *E. coli* but not *E. coli* O157.

Africa

A large outbreak of haemorrhagic colitis occurred in South Africa and Swaziland in 1992; VT-producing *E. coli* O157 was isolated from an unspecified number of faecal samples (Isaacson *et al,*1993). Epidemiological investigations suggested that the outbreak was likely to be waterborne as a result of contamination of river and dam water by cattle. Several of those infected had drunk untreated water while working in the fields. *E. coli* O157 was isolated from cattle dung and several water samples.

Japan

A large outbreak of O157 VTEC infection occurred in September 1990 in Saitama, Japan (Kudoh *et al,*1994). There were 319 cases and two deaths among children in a kindergarten. Well water which was used for drinking was suspected but not proved microbiologically as being the vehicle of infection in the outbreak.

United States

A large outbreak of VT-producing *E. coli* O157 infection occurred in the town of Cabool in Missouri in 1989/1990; there were at least 243 cases and 4 deaths (Geldreich *et al,*1992; Swerdlow *et al,*1992). The outbreak was confined to people who had been exposed to a municipal water supply and the outbreak probably occurred as a result of sewage contamination of the water distribution system. The town received its drinking water from a non disinfected ground water source. Two large water mains broke during very cold weather over Christmas. The number of new cases declined rapidly after residents were ordered to boil water and the water supply had been chlorinated. *E. coli* O157 was not isolated from any of the water samples but samples were not taken during the peak exposure period.

In 1991 an outbreak of haemorrhagic colitis and HUS caused by O157 VTEC was traced to a lakeside park in Oregon (Keene *et al.*1994). Over a 24 day period there were 21 cases. All of them reported swimming in the lake and many of them had swallowed lake water. *E. coli* O157 was not cultured from the lake water. It was noted that the water temperatures in the swimming area varied from 23°C to 26°C. No additional cases were reported after swimming in the lake was banned. It was reopened after the installation of a pumping system to increase water circulation and further cases have not been reported.

3.1.8 Potential for secondary spread

Person to person transmission is an important means of spread of VTEC infection (ACMSF Report 1995, Griffin and Tauxe, 1991). This occurs particularly in general outbreaks associated with hospitals, nursing homes, nurseries and other institutions. In addition, person to person spread is responsible for many family outbreaks. There is a need for effective hygiene precautions and infection control procedures and guidelines have been published by the PHLS (Subcommittee of the PHLS Working Group on VTEC, 1995; A Working Party of the PHLS Salmonella Committee, 1995).

3.1.9 Resistance to water treatment processes

O157 VTEC strains have been reported as being as susceptible as other *E. coli* to chlorination treatment used for drinking water (Feng, 1995).

3.1.10 Environmental resistance

The survival of two O157 VTEC strains in water has been examined (Rice *et al*,1992: Swerdlow *et al*,1992). The two strains were isolated during outbreaks, one from the waterborne outbreak in Cabool and the second isolate was from an outbreak associated with consumption of beefburgers. The water was collected from a well used as a water source for the city of Cabool where there was an outbreak of O157 VTEC infection (see Section vi)). Tests on the water before inoculation with *E. coli* O157 showed it contained less than one coliform per 100ml and had a heterotrophic plate count of two organisms per ml. The water samples were inoculated with between 10^6 - 10^7 cfu per ml: there were no significant reductions in viable counts of the strains during 7 days in water at 5°C and 20°C. After this time the survival decreased more rapidly at 20°C with a 5 $\log_{10}$ reduction after 35 days. At 5°C there was a 3.5 $\log_{10}$ reduction after 70 days.

3.1.11 Environmental occurrence

There have been few studies to examine environmental samples for the presence of VTEC and in particular *E. coli* O157. A one year study in the Philadelphia area examined water samples from streams and reservoirs in 15 environmental sites as well as clinical samples (McGowan, Wickersham and Strockbine, 1989). Samples (200ml) were centrifuged and the sediment was plated on SMAC agar. VT-producing *E. coli* O157:H7 was isolated in one water sample from a

reservoir 45 kilometres outside the city. Contamination of the water by cattle or humans was not considered likely but there were many deer in the area and these animals were suggested as a possible source of the *E. coli* O157. It should be noted that isolation of O157 VTEC has now been reported from the faeces of cattle, sheep, goats and wild deer.

3.1.12 VTEC belonging to serogroups other than O157

This report has included some information on VTEC belonging to serogroups other than O157. The importance of these organisms in human disease is not clearly established although they have been isolated from cases of haemorrhagic colitis and haemolytic uraemic syndrome. In Britain VTEC of serogroups O26 and O128 have been isolated more frequently than other non-O157 VTEC. Outbreaks of infection caused by VTEC belonging to serogroups O104, O111 and O145 have been reported in countries outside Britain but these outbreaks have been rare compared with outbreaks caused by O157 VTEC. Figures for the incidence of non- O157 VTEC are not available as there are very few laboratories capable of detecting them.

There appears to be very little information on non-O157 VTEC infection in relation to water. Methods for isolation of these organisms from water have not been optimized and should be investigated as there will be some significant differences from the methods developed for O157 VTEC.

3.1.13 Conclusions

1. There has been a significant increase in the laboratory reporting of O157 VTEC in Britain since 1983. This has resulted from a combination of better surveillance, improved methods and an increase in incidence. VT-producing *E. coli* O157 is therefore an important emerging human pathogen. The incidence of VTEC belonging to other serogroups is not known but requires further investigation.

2. Water has been the vehicle of infection for several outbreaks caused by O157 VTEC. One large outbreak was associated with consumption of drinking water possibly as a result of the supply being contaminated with sewage after pipe breakages. Chlorination of the water might have prevented this outbreak. Contaminated water supplies could also result from failure of water treatment or from inadequate levels of residual chlorine.

Other outbreaks have resulted from human contamination of bathing waters. It is likely that there were low levels of O157 VTEC in these waters. Present evidence suggests the infectious dose for this pathogen is low.

3. Private water supplies appear to be an area for concern that requires further investigation. Contamination of such supplies with cattle faeces does occur and therefore may be responsible for human cases of infection particularly in rural areas.

3.1.14 References

Abdul-Raouf, U.M., Beuchat, L.R. and Ammar, M.S. Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. *Applied and Environmental Microbiology* **59**: 1999-2006, 1993.

Advisory Committee on the Microbiological Safety of Food. Report on Verocytotoxin-producing *Escherichia coli*. London: HMSO 1995.

Bolton, F.J., Crozier, L. and Williamson, J.K. Optimisation of methods for the isolation of *Escherichia coli* O157 from beefburgers. *PHLS Microbiology Digest* **12**: 67-70, 1995.

Brewster, D.H., Brown M.I., Robertson D., Houghton G.L., Bimson J. and Sharp J.C.M. An outbreak of *Escherichia coli* O157 associated with a children's paddling pool. *Epidemiology and Infection* **112**: 441-7, 1994.

Chapman, P.A. Isolation, identification and typing of Vero cytotoxin-producing *Escherichia coli* O157. *PHLS Microbiology Digest* **11**: 13-7, 1994.

Coia, J.E., Sharp, J.C.M., Curnow, J. and Reilly, W.J. Ten year's experience of *Escherichia coli* O157 in Scotland (1984-93). In *Recent Advances in Verocytotoxin-producing Escherichia coli infections*. M.A. Karmali and A.G. Goglio, eds., p41-4, Elsevier, 1994.

Dev, V.J., Main M. and Gould, I. Waterborne outbreak of *Escherichia coli* O157. *Lancet* **337**: 1412, 1991.

Feng P. *Escherichia coli* serotype O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerging Infectious Diseases* **1**: 47-52, 1995.

Geldreich, E.E., Fox, K.R., Goodrich, J.A., Rice, E.W., Clark, R.M and Swerdlow, D.L. Searching for a water supply connection in the Cabool, Missouri disease outbreak of *Escherichia coli* O157:H7. *Water Research* **26**: 1127-37, 1992.

Griffin, P.M. and Tauxe, R.V. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli* and the associated hemolytic uremic syndrome. *Epidemiologic Reviews* **13**: 60-98, 1991.

Hildebrand J.M., Maguire, H.C., Holliman, R.E. and Kangesu, E. An outbreak of *Escherichia coli* O157 linked to paddling pools. *Communicable Disease Report* **6**: R33-6, 1996.

Hindle, M.A., Bolton, E.J., Wright, P.A. and Durband, C.A. Improved detection of Vero cytotoxin-producing *Escherichia coli* O157 in faecal samples by enrichment culture. *PHLS Microbiology Digest* **12**: 71-3, 1995.

Isaacson, M., Canter, P.H., Effler, P., Arntzen, L., Bomans, P. and Heenan, R. Haemorrhagic colitis epidemic in Africa. *Lancet* **341**: 961, 1993.

Karmali, M.A. Infection by Verocytotoxin-producing *Escherichia coli*. *Clinical Microbiology Reviews* **2**: 15-38, 1989.

Keene, W.E., McAnulty, M.B., Hoesly, F.C. *et al,* A swimming associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *New England Journal of Medicine* **331**: 579-84, 1994.

Kudoh, Y., Kai, A. Obata, H. *et al,* Epidemiological surveys on Vero cytotoxin-producing *Escherichia coli* infection in Japan. In *Recent Advances in Verocytotoxin-producing Escherichia coli infections*. M.A. Karmali and A.G. Goglio, eds., p53-6, Elsevier, 1994.

McGowan. K.L., Wickersham, E. and Strockbine, N.A. *Escherichia coli* O157:H7 from water. *Lancet i*: 967-8, 1989.

Neill, M.A., Agosti, J. and Rosen, H. Hemorrhagic colitis with *Escherichia coli* O157:H7 preceding adult hemolytic uremic syndrome. *Archives of Internal Medicine* **145**: 2215-7, 1985.

Rice, E.W., Johnson, C.H., Wild, D.K. and Reasoner, D.J. Survival of *Escherichia coli* O157:H7 in drinking water associated with a waterborne disease outbreak of hemorrhagic colitis. *Letters in Applied Microbiology* **15**: 38-40, 1992.

Roberts, C.L., Mshar, P.A., Cartter, M.L. *et al,* The role of heightened surveillance in an outbreak of *Escherichia coli* O157:H7. *Epidemiology and Infection* **115**: 447-54, 1995.

Sharp, J.C.M., Reilly, W.J., Coia, J.E., Curnow, J. and Synge, B.A. *Escherichia coli* O157 infection in Scotland: an epidemiological overview, 1984-94. *PHLS Microbiology Digest* **12**: 134-40, 1995.

Su, C. and Brandt, L.J. *Escherichia coli* O157:H7 infections in humans. *Annals of Internal Medicine* **123**: 698-714, 1995.

Subcommittee of the PHLS Working Group on Vero cytotoxin producing *Escherichia coli* (VTEC). Interim guidelines for the control of infections with Vero cytotoxin-producing *Escherichia coli* (VTEC). *Communicable Disease Report* **5**: R77-81, 1995.

Swerdlow, D.L., Woodruff, B.A., Brady, R.C. *et al,* A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhoea and death. *Annals of Internal Medicine* **117**: 812-9, 1992.

Thomas, A., Chart, H., Cheasty, T., Smith, H.R., Frost, J.A. and Rowe, B. Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O157, associated with human infections in the United Kingdom: 1989 to 1991. *Epidemiology and Infection* **110**: 591-600, 1993.

United States Department of Agriculture, Food Safety and Inspection Service, May 21 1993. Report on the *Escherichia coli* O157:H7 outbreak in the Western States.

Wall, P.G., Adak, G.K., McDonnell, R.J., Cheasty, T., Smith, H.R. and Rowe, B. General outbreaks of Vero cytotoxin producing *Escherichia coli* O157 in England and Wales from 1992 to 1994. *Communicable Disease Report* **6**: R22-9, 1996.

Willshaw, G.A., Thirlwell, J., Jones, A.P., Parry, S., Salmon, R.L and Hickey, M. Vero cytotoxin-producing *Escherichia coli* O157 in beefburgers linked to an outbreak of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in Britain. *Letters in Applied Microbiology* **19**: 304-7, 1994.

Wood, M.J. The use of antibiotics in infections due to *Escherichia coli* O157:H7. *PHLS Microbiology Digest* **8**: 18-22, 1990.

Working Party of the PHLS Salmonella Committee. The prevention of human transmission of gastrointestinal infections, infestations and bacterial intoxications: a guide for public health physicians and environmental health officers in England and Wales. *Communicable Disease Report* **5**, R164, 1995.

AEROMONAS AND YERSINIA AS EMERGING PATHOGENS

Dr Gordon Nichols
Environmental Surveillance Unit
PHLS Headquarters
London NW9 5DF

3.2 Aeromonas as an emerging pathogen

3.2.1 Introduction

Aeromonas spp are ubiquitous in the environment, and in certain situations can cause human infection. *A.hydrophila, A.veronii* subsp *sobria* and *A.caviae* can cause diarrhoea and *A. hydrophila, A.schubertii* and *A.veronii* subsp *sobria* have been associated with non enteric infections including necrotic wound infections and septicaemia, which can be fatal. Wound infections often result from contamination with surface waters. Infections with *Aeromonas* spp have been reported worldwide, although there are questions about its role in diarrhoeal disease.

Aeromonas spp are normally present in sewage and untreated surface waters. Infection has been associated with water and patients with aeromonas diarrhoea are often found to have recently returned to the UK from abroad. *Aeromonas* spp have been isolated from chlorinated drinking water supplies (Le Chevalier *et al*, 1982; Krovacek *et al*, 1992; Knochel & Jeppesen, 1990; Havelaar *et al*, 1992), and the organism may grow on filters, in biofilms or as blooms. The epidemiology of aeromonas infections is not well understood. A seasonal increase in the numbers of aeromonas present in drinking water has been associated with a seasonal incidence of human infections (Burke *et al*, 1984). A case control study found an association between patients with diarrhoea due to *A.hydrophila* and the consumption of untreated water (Holmberg *et al*, 1986).

Both enteric and non enteric aeromonas infection have been reported (Picard & Goullet, 1987); in both immunocompromised and healthy persons when contaminated water has been used for drinking, washing or recreation (Goncalves *et al*, 1992). *Aeromonas* spp. may present a risk of infection to people who are in close contact with contaminated water, including divers (Joseph *et al*, 1979), wind surfers and swimmers.

Case controlled studies have determined carriage and infection rates in both adults (Pitarangsi *et al*, 1982) and children (Deodhar *et al*, 1991; Gracey *et al*, 1982). *Aeromonas* spp have been detected in foods, and have occasionally caused food poisoning (Altwegg *et al*, 1991).

The absence of defined outbreaks, the low levels of infectivity on experimentally infected human volunteers and the high percentage of cases found in people returning from abroad with diarrhoea suggests that people have a relatively high degree of resistance to infection.

3.2.4. Laboratory diagnosis

Havelaar *et al.*., 1987, developed a new selective medium for the isolation of *Aeromonas* spp from environmental samples by membrane filtration. Ten types of media were tested and satisfactory recovery rates were obtained only with mA agar (Rippey and Cabelli, 1979) and dextrin-fuchsin-sulphite agar (Schubert), but neither was sufficiently selective. The positive aspects of both media were combined to produce ampicillin-dextrin agar. Recovery from pure cultures and from environmental samples was optimal at an ampicillin concentration of 10 mg/ml and incubation for 24h at 30^0 C under aerobic conditions. Confirmation was usually >90% and no false negative colonies were observed. The medium can be used for the isolation of *Aeromonas* spp from sea water provided that the vibriostatic agent O/129 is added at 50 mg/l. Recovery was unaffected by varying the pH between 7.1 and 8.3, but colony differentiation was optimal at a higher pH. A final pH of 7.8+/-0.2 at 25^0 C is recommended. Methods for the isolation of *Aeromonas* spp from water include membrane filtration incorporating a combination of anaerobic and aerobic incubation (Cunliffe and Adcock, 1989), and a membrane filtration method which requires a resuscitation step on tryptic soy agar, (Warburton *et al*, 1994). Many of the selective media used for the isolation of *Aeromonas* spp give poor recovery of *A.veronii*, *A.sobria* and *A.schubertii* (Gavreil & Lamb, 1995).

3.2.5. Incidence of confirmed illness

In England and Wales both enteric and systemic aeromonas infection is more common in the second half of the year, with the highest number of cases in September (Unpublished data). Over a five year period (1990-1994) there were 2718 reported cases of human infection with aeromonas, and 24 (0.9%) deaths. Non enteric infections are reported to the Communicable Disease Surveillance Centre at a rate of 65-80 per year, and there are around five deaths per year the majority in patients over sixty years of age.

3.2.6 Outbreaks of Aeronomas Infection

There have been no well documented point source outbreaks of human diarrhoeal infection caused by *Aeromonas* spp. Hospital infection associated with water has been described (Picard, 1983). *Aeromonas hydrophila* from water has been implicated in infection from a home dialysis unit (Ramsay *et al*, 1978), a hospital centralized water softener (Poirier *et al*, 1993), and from a hospital water system (Picard & Goullet, 1987).

3.2.7 Comparison of *Aeromonas* spp found in faeces and drinking water

A.hydrophila, *A.sobria* and *A.caviae* were isolated from both faeces and drinking water. There was some similarity between the strains from drinking water and from faeces and *A.caviae* was the most common species in both

3.2.8 Environmental resistance

A number of factors influence the concentration of *Aeromonas* spp in water. Aeromonads are inhibited by copper ions., particularly in soft water and at pH values below 8 (Versteegh *et al*, 1989. Temperature is also important in the competitive colonisation of water.

Survival of *A.hydrophila* in natural waters altered by thermal effluents discharged from a nuclear production reactor indicated that they survived better in deeper hypolimnetic oxygen depleted waters. The lowest seasonal densities occurred in late summer and late winter (Fliermans *et al*, 1977). Seasonal variation was greater in less polluted water (Monfort and Baleux, 1990).

In source waters the presence of *Aeromonas* spp has been correlated with conductivity, Secchi depth, Relative Trophic Index, dissolved oxygen concentration, total Kjeldal nitrogen, ammonia, chlorophyl α, orthophosphate and total phosphate (Holmes *et al*, 1995). The factors which influence the presence of *Aeromonas* spp in distribution systems include an absence of chlorination in the water treatment, temperatures over 15°C. low residual disinfectant concentrations, a raised carbon content and increased storage time in the distribution system.

3.2.9 Environmental occurrence

Aeromonas spp have been detected from a variety of sources. They may be found in soil, marine and fresh water (Kaper *et al*, 1981; Rhodes & Kator, 1994), and sewage (Montfort &

Baleux, 1990). They are sometimes found in the faeces from humans and animals (Havelaar *et al*, 1992). They have also been reported in household stored water (Simango, 1992), hospital water supplies and centralized water softeners (Poirrier *et al*, 1993).

A survey of chlorinated water conducted over a 3 month period (Millership and Chattopadhyay, 1984), examined a total of 286 samples from taps and storage tanks in nine London and Essex boroughs and from nine local hospitals. Samples of non chlorinated waters from rivers and lakes both local and in the uplands of Wales were taken for comparison. The isolation rate of *Aeromonas hydrophila* in summer was 25% and in winter was 7%. The respective rates for unchlorinated waters was 82% and 75%. Growth in water distribution systems may be associated with the accumulation of a biofilm on internal surfaces. The growth of biofilms depends on temperature, the availability of organic matter and the degree of stagnation. Biofilms can accumulate in the presence of 0.8 mg/l chlorine and there can be considerable accumulations at concentrations of less than 0.2 mg/l.

Aeromonads have been shown to be able to colonize slow sand filters (von Wolzogen Kuhr, 1932, and van der Kooij, 1988). Treated waters, with or without disinfection. can contain small numbers of *Aeromonas* and regrowth is possible.

3.2.10 References

Altwegg M, Martinetti Lucchini G, Luthy-Hottenstein J, Rohrbach M. Aeromonas-associated gastroenteritis after consumption of contaminated shrimp [letter]. Eur J Clin Microbiol Infect Dis 1991;10(1):44-5.

Burke V, Robinson J, Gracey M, Peterson D, Partridge K. Isolation of *Aeromonas hydrophila* from a metropolitan water supply: seasonal correlation with clinical isolates. Appl Environ Microbiol 1984;48(2):361-6.

Cunliffe DA, Adcock P. Isolation of *Aeromonas* spp. from water by using anaerobic incubation. Appl Environ Microbiol 1989;55(9):2138-40.

Davis PJ, Stirling AJ. *Aeromonas hydrophila* wound infection associated with water immersion: an unusual football injury. Injury 1993;24(9):633-4.

Davis WA, 2d, Kane JG, Garagusi VF. Human aeromonas infections: a review of the literature and a case report of endocarditis. [Review]. Medicine (Baltimore) 1978;57(3):267-77.

Deodhar LP, Saraswathi K, Varudkar A. *Aeromonas* spp. and their association with human diarrheal disease. J Clin Microbiol 1991;29(5):853-6.

Dickson WA, Boothman P, Hare K. An unusual source of hospital wound infection. Br Med J (Clin Res Ed) 1984;2899(6460):1727-1728.

Feaster FT, Nisbet RM, Barber JC. *Aeromonas hydrophila* corneal ulcer. Am J Ophthalmol 1978;85(1):114-7.

Fliermans CB, Gorden RW, Hazen TC, Esch GW. Aeromonas distribution and survival in a thermally altered lake. Appl and Env Microbiol 1977;33(1):114-122.

Genoni L, Domenighetti G. [Near-drowning in an adult: favorable course after a 20-minute submersion]. [German]. Schweiz Med Wochenschr 1982;112(24):867-70.

Goncalves JR, Brum G, Fernandes A, Biscaia I, Correia MJ, Bastardo J. *Aeromonas hydrophila* fulminant pneumonia in a fit young man. Thorax 1992;47(6):482-3.

Gracey M, Burke V, Robinson J. Aeromonas-associated gastroenteritis. Lancet 1982;2(8311):1304-6

Havelaar AH, Schets FM, van Silfhout A, Jansen WH, Wieten G, van der, Kooij D. Typing of Aeromonas strains from patients with diarrhoea and from drinking water. J Appl Bacteriol 1992;72(5):435-44.

Heckerling PS, Stine TM, Pottage JC, Jr., Levin S, Harris AA. *Aeromonas hydrophila* myonecrosis and gas gangrene in a nonimmunocompromised host. Arch Intern Med 1983;143(10):2005-7.

Holmberg SD, Schell WL, Fanning GR, Wachsmuth IK, Hickman-Brenner FW, Blake PA, Brenner DJ, Farmer JJ, 3d. Aeromonas intestinal infections in the United States. Ann Intern Med 1986;105(5):683-9.

Joseph SW, Daily OP, Hunt WS, Seidler RJ, Allen DA, Colwell RR. Aeromonas primary wound infection of a diver in polluted waters. J Clin Microbiol 1979;10(1):46-9.

Kaper JB, Lockman H, Colwell RR, Joseph SW. *Aeromonas hydrophila*: ecology and toxigenicity of isolates from an estuary. J Appl Bacteriol 1981;50(2):359-77.

Krovacek K, Faris A, Baloda SB, Lindberg T, Peterz M, Mansson I. Isolation and virulence profiles of *Aeromonas* spp. from different municipal drinking water supplies in Sweden. Food Microbiol 1992;9:215-222.

Krovacek K, Faris A, Baloda SB, Peterz M, Lindberg T, Mansson I. Prevalence and characterization of *Aeromonas* spp. isolated from foods in Uppsala, Sweden. Food Microbiol 1992;9:29-36.

Le Chevalier MW, Evans TM, Seidler RJ, Daily OP, Merrell BR, Rollins DM, Joseph SW. *Aeromonas sobria* in chlorinated drinking water supplies. Microb Ecol 1982;8:325-333.

Ljungh A, Popoff M, Wadstrom T. *Aeromonas hydrophila* in acute diarrheal disease: detection of enterotoxin and biotyping of strains. J Clin Microbiol 1977;6(2):96-100.

Mercer NS, Beere DM, Bornemisza AJ, Thomas P. Medical leeches as sources of wound infection. Br Med J (Clin Res Ed) 1987; 294(6577):937

Millership SE, Chattopadhyay B. Methods for the isolation of *Aeromonas hydrophila* and Plesiomonas shigelloides from faeces. J Hyg (Lond) 1984;92(2):145-52.

Monfort P, Baleux B. Dynamics of *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas* Caviae in a sewage treatment pond. Appl and Environ Microbiol 1990;56(7):1999-2006.

Morgan DR, Johnson PC, DuPont HL, Satterwhite TK, Wood LV. Lack of correlation between known virulence properties of *Aeromonas hydrophila* and enteropathogenicity for humans. Infect Immun 1985;50(1 :62-5.

Picard B, Arlet G, Goullet P. Water origin of *Aeromonas hydrophila* hospital infections (letter) (French). Presse Med 1983;12(11):700.

Picard B, Goullet P. Seasonal prevalence of nosocomial *Aeromonas hydrophila* infection related to aeromonas in hospital water. J Hosp Infect 1987;10(2):152-5.

Pitarangsi C, Echeverria P, Whitmire R, Tirapat C, Formal S, Dammin, GJ, Tingtalapong M. Enteropathogenicity of *Aeromonas hydrophila* and Plesiomonas shigelloides: prevalence among individuals with and without diarrhea in Thailand. Infect Immun 1982;35(2):666-73.

Poirier T, Laurens E, Viaud JY, Poujol B, Lorre G. [Nosocomial *Aeromonas hydrophila* pneumonia complicating toxic coma]. [French]. Ann Fr Anesth Reanim 1993;12(1):72-4.

Ramsay AM, Rosenbaum BJ, Yarbrough CL, Hotz JA. *Aeromonas hydrophila* sepsis in a patient undergoing hemodialysis therapy. JAMA 1978;239(2):128-9.

Reines HD, Cook FV. Pneumonia and bacteremia due to *Aeromonas hydrophila*. Chest 1981;80(3):264-7.

Rhodes MW, Kator H. Seasonal occurrence of mesophilic *Aeromonas* spp. as a function of biotype and water quality in temperate freshwater lakes. Wat Res 1994;28(11):2241-2251.

Rippey SR, Cabelli VJ. Membrane filter procedure for enumeration of *Aeromonas hydrophila* in fresh waters. Appl Environ Microbiol 1979;38(1):108-13.

Simango C, Dindiwe J, Rukure G. Bacterial contamination of food and household stored drinking water in a farmworker community in Zimbabwe. Cent Afr J Med 1992;38(4):143-9.

Snower DP, Ruef C, Kuritza AP, Edberg SC. *Aeromonas hydrophila* infection associated with the use of medicinal leeches. J Clin Microbiol 1989;27(6):1421-2.

Turnbull PC, Lee JV, Miliotis MD, Van de Walle S, Koornhof HJ, Jeffery L, Bryant TN. Enterotoxin production in relation to taxonomic grouping and source of isolation of *Aeromonas* species. J Clin Microbiol 1984;19(2):175-80.

Van der Kooij d. Properties of Aeromonads and their occurrence and hygienic significance in drinking water. Zbl Bakt Hyg B 1988;187:1-17.

Von Wolzogen Kuhr CAH. Uber eine Garungsmikrobe in Fakalien von Muckenlarven. Zbl Bakt II Abt 85 1932 223-250.

Warburton DW, McCormick JK, Bowen B. Survival and recovery of *Aeromonas hydrophila* in water: development of methodology for testing bottled water in Canada. Can J Microbiol 1994;40(2):145-8.

3.3 Yersinia as an emerging pathogen

3.3.1 Introduction

Yersinia is a genus with eleven recognised species, some of which cause diseases in man. The diseases caused by *Yersinia* spp., other than *Y.pestis*, range from subclinical and mild diarrhoeal infections to severe infection including septicaemia (Corbel, 1990). The group comprising *Y.pseudotuberculosis, Y.enterocolitica, Y.intermedia, Y.kristensenii,* and *Y.frederiksenii* have been associated with diarrhoea, mesenteric lymphadenitis, and occasionally disseminated infection. Secondary immunological complications include reactive arthritis and erythema nodosum. Some patients with appendicitis type abdominal pain have been shown to have yersinia antibodies..

There are diagnostic problems associated with *Yersinia* spp in human disease. Tests for *Y.enterocolitica* infection will usually be carried out when a patient presents with mesenteric adenitis, erythema nodosum or reactive arthritis. Mesenteric adenitis presents with similar symptoms to appendicitis, and is often accompanied by loose stools. Patients with diarrhoea are not always routinely investigated for *Y.enterocolitica*, and different levels of suspicion, and therefore different levels of ascertainment, exist in different laboratories

Yersinia spp have been isolated from faeces using a sensitive enrichment culture in around 3% of patients with diarrhoea, but the main pathogenic O types are not usually isolated. It is not clear if the isolates are responsible for causing diarrhoea or are an incidental finding.

3.3.2 Human pathogenicity

Y.pseudotuberculosis is less commonly associated with enteritis than *Y.enterocolitica.* and produces symptoms other than diarrhoea and vomiting. It presents as septicaemia or, more commonly, acute or subacute inflammation of the mesenteric lymph nodes (pseudoappendicitis syndrome).

3.3.3 Human infectivity

The infectious dose of *Yersinia* spp. for man is not known. It is likely to be related to the virulence factors of the isolate, the individuals previous exposure to virulent and avirulent isolates, and possibly other genetic and environmental factors.

3.3.4 Direct culture

The 18th edition of Standard Methods for the Examination of Water and Wastewater (1992) cites a membrane filtration method (Bartley *et al,* 1982). Most reports predate the commercial availability of Cefsulodin Irgasan Novobiocin (CIN) agar (Schiemann, 1979,1982) which is currently recognised as the medium of choice for isolation of *Yersinia* spp.

Concentration by membrane filtration or centrifugation is advisable before isolation is attempted. Precipitation with ferric chloride and kaolin prior to centrifugation has also been used to concentrate bacteria from surface waters (Fukushima *et al,* 1984).

Fukushima (1992) found that alkali treatment, with potassium hydroxide (see below), of centrifuged deposits of water samples immediately before direct plating resulted in up to 10-fold greater recovery than using enrichment procedures. High levels of competing organisms can mask small numbers of pathogenic Yersinia.

3.3.5 Enrichment culture

Few enrichment procedures have been evaluated for use with water samples. It has been shown that both cold enrichment and pre-enrichment result in a decreased isolation of pathogenic strains and an increased isolation of non-pathogenic strains of Yersinia (Wauters *et al,*1988). Incubation at a higher temperature may therefore be more appropriate for isolation of pathogenic strains.

Phosphate buffered saline (PBS: Gonul and Karapinar, 1991), PBS supplemented with 1% sorbitol and 0.15% bile salts (Marinelli *et al, 1985)*) or 0.25% mannitol and 0.25% peptone (Fukushima *et al,* 1984) have all been used for enrichment of water samples, usually with incubation at 4°C. It has been shown that a nitrogen source such as peptone is particularly important for the recovery of Yersinia from nitrogen-poor samples such as water (Weagant and Kaysner, 1983). Higher incubation temperatures and media rich in peptone but without carbohydrates are now used more frequently than PBS.

BS 5763: Part 16: 1995 (ISO 10273: 1994) describes the detection of pathogenic *Y.enterocolitica* from food and directs the use of two enrichment protocols, a multivalent method which should recover all strains of *Yersinia* and a more selective method developed by Wauters *et al,*(1988) specifically for the isolation of *Y.enterocolitica* serotype O:3. The regime specified in the multivalent method is a 1/10 enrichment in peptone sorbitol bile salts broth (0.5% peptone, 1% sorbitol, 0.15% bile salts, adjusted to pH 7.6) with incubation at 22-25°C for

2-3 days with shaking or 5 days without shaking. This medium has been shown to be effective for isolation from water (Weagant and Kaysner, 1983; Marinelli *et al*, 1985) as well as from foods.

Anaerobic incubation of peptone-based enrichment broths has been used to recover yersinia from streams and lakes (Harvey *et al*,1976) and from drinking water and sewage sludge (Langeland, 1983). Anaerobic incubation helped to prevent overgrowth by the strict aerobes.

The second isolation method described in BS 5763: part 16: 1995 specifies a 1/100 enrichment in Irgasan Ticarcillin Potassium Chlorate (ITC) broth and incubation at 25°C for 48 hours. The basal medium is a modification of Rappaport broth, and contains irgasan 1 mg/l, ticarcillin 1 mg/l and potassium chlorate 0.1%. Most Enterobacteriaceae possess an A-nitratase capable of splitting chlorate, forming toxic by-products which then prevent their growth. Yersiniae possess a B-nitratase without this activity. A-nitratase is only inducible under reduced oxygen pressure; the medium must therefore be dispensed with a small surface area/volume ratio in order to achieve relative anaerobiosis. This protocol was developed by Wauters (1988) specifically for the isolation of *Y.enterocolitica* serotype O:3 from pork. Other pathogenic serotypes have also been isolated using ITC broth (Kwaga *et al*, 1990), but serotype O:8 will not grow in Rappaport media (Schiemann, 1989). The use of ITC medium for isolation of Yersinia from water has not been reported.

3.3.6 Isolation media

The isolation agar of choice is Cefsulodin Irgasan Novobiocin (CIN) agar (Schiemann 1979, 1982), which is capable of growing almost all strains of *Yersinia enterocolitica* and related species. Plates are incubated either at 30°C for 20-24 hours or 25°C for 48 hours. Colonies of *Yersinia* are distinguished by their ability to ferment mannitol and by colony appearance and size. The medium is commercially available.

3.3.7 Summary of appropriate methods

Direct culture

In most circumstances, concentration of the water sample by membrane filtration or centrifugation is desirable. Direct plate culture of the concentrated sample and plating after KOH treatment of the sample concentrate is recommended in addition to enrichment.

Enrichment

In order to optimize the recovery of all serovars, the use of two enrichment media is necessary:

[a] Either peptone sorbitol bile salts broth or tris-buffered peptone water (pH 8.0) are suitable. The optimum temperature of incubation to enhance pathogen isolation is probably 20-22°C. An alkali treatment of the enrichment culture before plating should always be included and may be sufficient without direct plating of the enrichment culture.

[b] Currently the most suitable selective enrichment medium for detection of pathogenic strains is ITC broth. The recommended incubation temperature is 25°C.

Isolation

Although CIN agar is the medium of choice for most strains of yersinia, the growth of *Y.pseudotuberculosis* and certain strains of *Y.enterocolitica* is inhibited on this medium. In order to recover these strains, it is recommended that cefsulodin is omitted from the formulation. CIN agar and its derivatives are also suitable for use after KOH treatments of sample concentrates or enrichments.

SSDC has been found effective for the isolation of some common pathogenic strains of Yersinia whilst inhibiting the growth of some environmental strains. Its use in conjunction with CIN agar or CIN derivative is advocated to enhance the detection of pathogenic strains in the presence of environmental strains which grow readily. SSDC agar is not suitable for recovery of *Yersinia* strains after KOH treatment.

3.3.8 Incidence of confirmed illness

Between 1990 and 1994 there were 1655 reported cases of infection with *Y.enterocolitica* in England and Wales; with 266 *Y.frederiksenii*, 20 *Y.intermedia*, 3 *Y.kristensenii* and 45 *Y.pseudotuberculosis*. Eleven percent of all yersinia infections were non enteric. *Yersinia* spp were isolated from 133 (3.5%) patients faecal samples (Greenwood & Hooper, 1987). Clinical significance could be attributed to the presence of *Yersinia* spp in 90% of patients aged over 15 years old and to more than 50% of patients in younger age groups.

Certain serotypes are considered pathogenic to humans due to the possession of virulence factors and their association with infection. *Y.enterocolitica* serotypes O:3, O:4,32, O:5,27,

O:6,30, O:6,31, O:8, O:9 and O:21 are thought to cause diarrhoea and mesenteric adenitis and occasional systemic infections. The other types can commonly be isolated from patients with diarrhoea but their role in causing diarrhoea is unclear. The severity of disease is affected by the serotype of the infecting organism. Some serotypes and biotypes, not normally considered to be pathogenic, may occasionally be associated with infection in susceptible hosts.

3.3.9 Outbreaks of infection due to *Yersinia sp.*

Human infection caused by yersinia has been associated with the drinking water supply system, well water and surface water (Lassen, 1972; Eden *et al*, 1977; Thompson & Gravel, 1986), untreated spring water (MMWR, 1982) and water from streams (Kohl *et al*, 1976; Schiemann, 1978). *Y.pseudotuberculosis* infection has been associated with untreated drinking water (Sato & Komazawa, 1991), well water (Aldova *et al*, 1979) and surface water (Fukushima *et al*, 1988 and Keet, 1974).

In an epidemic of gastrointestinal illness an estimated 750 people who were in a ski resort in Montana were ill with nausea, vomiting, diarrhoea and abdominal cramps (Eden *et al*, 1977; Highsmith *et al*, 1977). Eighty six employees of the resort (76%) had experienced a gastrointestinal illness. *Y.enterocolitica* was isolated from the water from two wells and *Y. enterocolitica* (69 and 85 colonies from 2 litres of water) was isolated from both wells. *Yersinia* spp. were not isolated from any faecal samples from the patients, and this outbreak could have been caused by other organisms.

3.3.10 Environmental occurrence

Yersinia spp are found in natural bodies of water and can be associated with farms and meat processing plants. There is evidence that they can grow in water but they are sensitive to chlorination. They are uncommon in distribution systems but can occasionally be cultured. Waterborne outbreaks of yersiniosis have occurred.

3.3.11 References

Aldova E, Brezinova A, Sobotkova J. A finding of Yersinia pseudotuberculosis in well water. Zentralbl Bakteriol [B] 1979;169(3-4):265-70.

Aulisio,C.C.G., Mehlman,I.J. and Sanders,A.C. Alkali method for rapid recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from foods. *Applied and Environmental Microbiology* 1980; 39: 135-140.

Bercovier,H., Brault,J., Cohen,S.. Melis,R., Lambert, T., and Mollaret, H.H. A new isolation medium for the recovery of *Yersinia enterocolitica* from environmental sources. *Current Microbiology* 1984; 10: 121-124.

British Standards Institute. BS 5763: Part 16: 1995 (ISO 10273: 1994). Methods for microbiological examination of food and animal feeding stuffs. Part 16. Detection of presumptive pathogenic *Yersinia enterocolitica.*

Chao,W-L., Ding, R-J., and Chen, R-S. Survival of *Yersinia enterocolitica* in the environment. *Canadian Journal of Microbiology* 1988; 34: 753-756.

Corbel MJ, Brewer RA, Hunter D. Characterisation of Yersinia enterocolitica strains associated with ovine abortion. Vet Rec 1990;127(21):526-7.

Curtis,L.M. and Blackman, C de W. Evaluation of four cultural methods for the detection of *Yersinia* in foods. *Journal of Applied Microbiology* 1995; 79 (1): supplement; xvi.

Eden KV, Rosenberg ML, Stoopler M, Wood BT, Highsmith AK, Skaliy P, Wells JG, Feeley JC. Waterborne gastrointestinal illness at a ski resort. --Isolation of Yersinia enterocolitica from drinking water--. Public Health Rep 1977;92(3):245-50.

Fukushima H, Gomyoda M, Shiozawa K, Kaneko S, Tsubokura M. Yersinia pseudotuberculosis infection contracted through water contaminated by a wild animal. J Clin Microbiol 1988;26(3):584-5.

Fukushima,H. and Gomyoda,M. Growth of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* biotype 3B serotype O3 inhibited on Cefsulodin-Irgasan-Novobiocin agar. *Journal of Clinical Microbiology* 1986; 24: 116-120.

Fukushima,H. Direct isolation of *Yersinia pseudotuberculosis* from fresh water in Japan. *Applied and Environmental Microbiology* 1992; 58: 2688-2690.

Fukushima,H. New selective agar medium for isolation of virulent Yersinia enterocolitica. *Journal of Clinical Microbiology* 1987; 25: 1068-1073.

Fukushima,H., Gomyoda,M., Shiozawa,K., Kaneko,S. and Tsubokura,M. *Yersinia pseudotuberculosis* infection contracted through water contaminated by a wild animal. *Journal of Clinical Microbiology* 1988; 26:584-585.

Fukushima,H., Saito,K., Tsubokura,M. and Otsuki,K. *Yersinia* spp. in surface water in Matsue, Japan. *Zentralblatt fur Bakteriologie,Microbiologie und Hygiene -1- Abt-Originale B, Hygiene* 1984; 179 (3): 235-247.

Gonul,S.A. and Karapinar,M. The microbiological quality of drinking water supplies in Izmir city: the incidence of *Yersinia enterocolitica. International Journal of Food Microbiology* 1991; 13: 69-74.

Greenberg,A.E., Clesceri,L.S. and Eaton,A.D. editors. Standard Methods for the Examination of Water and Wastewater. 18th edition. American Public Health Association, 1992.

Greenwood M & Hooper WL. Human carriage of Yersinia spp. J Med Microbiol 1987;23:345-348.

Greenwood,M.H. and Hooper,W.L. Improved methods for the isolation of *Yersinia* species from milk and foods.*Food Microbiology* 1989; 6: 99-104.

Greenwood,M.H. Comparison of enrichment at 9°C and 21°C for recovery of *Yersinia* species from food and milk. *Food Microbiology* 1993; 10: 23-30.

Greenwood,M.H., Hooper,W.L. and Rodhouse,J.C. The source of *Yersinia* spp. in pasteurized milk: an investigation in a dairy.*Epidemiology and Infection* 1990; 104: 351-360.

Harvey,S., Greenwood,J.R., Pickett,M.J. and Mah,R.A. Recovery of *Yersinia enterocolitica* from streams and lakes in California. *Applied and Environmental Microbiology*; 32: 352-354.

Highsmith AK, Feeley JC, Skaliy P, Wells JG, Wood BT. Isolation of Yersinia enterocolitica from well water and growth in distilled water. Appl Environ Microbiol 1977;34(6):745-50.

Kapperud,G., Vardund,T., Skjerve,E., Hornes,E. and Michaelson,T.E. Detection of pathogenic *Yersinia enterocolitica* in foods and water by immunomagnetic separation, nested polymerase chain reactions, and colorimetric detection of amplified DNA. *Applied and Environmental Microbiology* 1993; 59: 2938-2944.

Keet EE. Yersinia enterocolitica septicemia. Source of infection and incubation period identified. N Y State J Med 1974;74:2226-2230.

Kohl S, Jacobson JA, Nahmias A. Yersinia enterocolitica infections in children. J Pediatr 1976;89(1):77-79.

Kwaga,J., Iversen,J.O. and Saunders,J.R. Comparison of two enrichment protocols for the detection of *Yersinia* in slaughtered pigs and pork products. *Journal of Food Protection* 1990; 53 (12): 1047-1049.

Langeland,G. *Yersinia enterocolitica* and *Yersinia enterocolitica*-like bacteria in drinking water and sewage sludge. *Acta Pathologica Microbiologica Immunologica Scandinavia*, Section B, 1983; 91: 179-185.

Lassen J. Yersinia enterocolitica in drinking water. Scand J Infect Dis 1972;4:125-127.

Marinelli,G., D'Innocenzo,C., Fabiani,L. and Leoni,V.Application of a simplified method for the recovery of *Yersinia enterocolitica* from surface waters. *Applied and Environmental Microbiology* 1985; 49:1348-1349.

MMWR Sept,1982.

Sato K, Komazawa M. Yersinia pseudotuberculosis infection in children due to untreated drinking water. Contrib Microbiol Immunol 1991;12:5-10.

Schiemann DA. Association of Yersinia enterocolitica with the manufacture of cheese and occurrence in pasteurized milk. Appl Environ Microbiol 1978;36(2):274-7.

Schiemann, D.A. Synthesis of a selective agar medium for *Yersinia enterocolitica*. *Canadian Journal of Microbiology* 1979; 25: 1298-1304.

Schiemann,D.A. and Olson,S.A. Antagonism by Gram-negative bacteria to growth of *Yersinia enterocolitica* in mixed cultures. *Applied and Environmental Microbiology* 1984; 48: 539-544.

Schiemann,D.A. Development of a two-step enrichment procedure for recovery of virulent strains of *Yersinia enterocolitica* from food. *Applied and Environmental Microbiology* 1982; 43: 14-27.

Schiemann,D.A. *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. In: Doyle,M.P. editor. Foodborne Bacterial Pathogens. New York, Marcel Dekker, 1989: 601-672.

The Microbiology of Water 1994. Part 1-Drinking Water. Report on Public Health and Medical Subjects No 71. Methods for the Examination of Waters and Associated Materials. HMSO.

Thompson JS, Gravel MJ. Family outbreak of gastroenteritis due to Yersinia enterocolitica serotype 0:3 from well water. Can J Microbiol 1986;32(8):700-1.

Toora,S., Budu-Amoako,E., Ablett,R.F. and Smith,J. Evaluation of different antimicrobial agents used as selective supplements for isolation and recovery of *Yersinia enterocolitica*. *Journal of Applied Microbiology* 1994; 77: 67-72.

Walker SJ, Gilmour A. A comparison of media and methods for the recovery of *Yersinia enterocolitica* and *Yersinia enterocolitica*-like bacteria from milk containing simulated raw milk microfloras. *Journal of Applied Bacteriology* 1986; 60: 175-184.

Wauters,G., Goossens,V., Janssens,M. and Vandepitte,J. New enrichment method for isolation of pathogenic *Yersinia enterocolitica* serogroup O:3 from pork. *Applied and Environmental Microbiology* 1988; 54: 851-854.

Weagant,S.D. and Kaysner,C.A. Modified enrichment broth for isolation of *Yersinia enterocolitica* from nonfood sources. *Applied and Environmental Microbiology* 1983; 45: 468-471.

Section 4

VIRUSES IMPLICATED AS POTENTIAL EMERGING PATHOGENS

Ms Jane Sellwood
Water Virology Unit, Reading Public Health Laboratory
Royal Berkshire Hospital
Reading RG1 5AN

in collaboration with

Dr Owen Caul
Bristol Public Health Laboratory
Bristol Royal Infirmary
Bristol BS8 8HW

4.1 Introduction

Cell culture techniques based on those used in the clinical laboratory have been used for 20 years to detect many groups of enteric viruses, such as enterovirus, culturable adenovirus and reovirus in water. Use of the electron microscope (EM) for examination of faecal samples has identified other virus groups which cause gastroenteritis but which could not be grown readily in cell culture. These include rotavirus, adenovirus 40/41, astrovirus and the calicivirus/SRSV group. The review by Madeley (1994) contains both a pictorial study and a discussion on each of these groups. Environmental material is unsuitable for examination by electron microscopy.

Only in recent years has the introduction of novel cell culture lines and the development of molecular techniques allowed studies to determine the presence of these fastidious or non-culturable viruses in the environment. There is no evidence for a change in prevalence of infection of these virus groups in the community although our understanding of true incidence in association with disease and their presence in water is only now developing.

4.2 The issue of emerging viral pathogens

In the virological context, emerging pathogens are thus understood to be those agents which cause disease on a scale yet to be confirmed through improvements in detection methods linked to better reporting and recording procedures.

The Norwalk virus, one serotype of the Small Round Structured Viruses (SRSV) has now been classified as part of the calicivirus family. The SRSVs, of which there are many strains, are recognised as the major viral cause of adult gastroenteritis outbreaks in the UK and

throughout the world. This role has been clear for 10 years as has the association with waterborne outbreaks (Short 1988). However only recently has the complexity of strain types been recognised (Green *et al,*1995, Lewis *et al,*1995) and the ability to detect them in shellfish and water been developed (Lees *et al,*1995). It is therefore appropriate to regard them as emerging pathogens. Classic human caliciviruses, morphologically distinct from the SRSVs have also been recognised as associated with cases of diarrhoea although the prevalence is much lower than that of SRSVs.

Adenoviruses serotype 40/41 and the astrovirus group, may also be regarded as emerging pathogens for similar reasons. Both can now be cultured using novel cell culture lines but also rely on molecular techniques for detection in water. The adenovirus serotypes 40/41 (also called adenovirus F) and astrovirus (Kurtz 1994) cause gastroenteritis mainly in children. The level of disease is less than that caused by SRSVs.

Rotavirus can not be regarded as an emerging pathogen as its major role as a cause of gastroenteritis in children has been well understood for 20 years. The epidemiology and range of strains of the virus is also well recognised. Detection of infectious rotavirus from water is difficult but can be achieved using a novel cell culture line or molecular techniques. Waterborne outbreaks are rare and unlikely as the majority of adults have protective levels of immunity.

Hepatitis A virus (HAV) is an enteric virus which primarily affects the liver. It can not be regarded as an emerging pathogen as its approximately 10 year epidemic cycle is well recognised. The number of infections have reduced significantly in the past 50 years (Gay *et al,*1994) as a result of improved personal hygiene and an effective sewerage system. Waterborne outbreaks have occurred in different parts of the world and these are discussed later in this Section.

Direct contact with sewage by occupational exposure may increase the risk of infection if the illness is endemic in the community. Transmission is by the faecal/oral route and is especially efficient amongst young children. Shellfish grown in water with high levels of faecal contamination where HAV is endemic can also be a vehicle of virus spread. An effective vaccine is available and should be used when travelling to areas of high incidence around the world.

Hepatitis E virus is also an enteric virus again primarily affecting the liver. It is newly described (Skidmore 1995) and has been associated with large outbreaks of waterborne

disease (Skidmore *et al,* 1992) in areas of the Far East and India. Only imported cases have been reported in the UK. This newly identified pathogen would need to be re-assessed as a potential cause of waterborne disease if the infection became established in the UK.

The other groups of viruses which inhabit the gut are discussed in a later section but none can be regarded as emerging pathogens.

SRSVs, adenovirus 40/41, astroviruses and human caliciviruses, all of which cause gastroenteritis, are therefore the viruses discussed in detail within this report.

Some of the recently described bloodborne viral pathogens such as HIV, Hepatitis B and Hepatitis C virus have caused concern as potential hazards in sewage. The number of carriers in the community is relatively few and hence the level of blood input to the sewerage system is small. As these viruses are cell associated and labile outside the body they will survive for only short periods in blood contaminated material (Moore 1993). They will quickly denature in sewage and pose no risk in treated drinking water. The Proceedings of a Symposium "Survival of HIV in Environmental Waters" (Farzadegan 1993) reviews current research. These viruses are not within the remit of this report.

4.3 Pathogenesis of Viral Gastroenteritis

Knowledge of the mechanisms of intestinal damage from viral infection is limited for rotavirus, adenovirus 40/41 and astrovirus but more extensive for the SRSVs (Kapikian 1994). Virus replication occurs mainly along the upper bowel in the columnar epithelial cells on the villus tip. This results in cell death, villous atrophy, fluid inbalance and a reduction of brush border enzyme production. Recovery involves the restoration of the columnar epithelial cells from the undamaged crypt. More detailed descriptions may be found in Mandell *et al,*(1995).

It is not yet clear how much, if any cross protection against future disease is afforded by infection with one SRSV strain from another. Astrovirus serotypes may give some cross protection resulting in long lasting immunity (Kurtz 1994).

4.4 Laboratory Diagnosis of Clinical Disease

Examination of faeces within 48 hours of the onset of symptoms of gastroenteritis by electron microscopy is the most common diagnostic method at the present time. It relies on the recognition of morphological features of the virus either viewed directly or from photographs.

Adenoviruses are relatively large and have a distinctive symmetrical structure compared to the astroviruses and SRSV/caliciviruses. Astrovirus have a six pointed star shape in a proportion of particles. SRSVs have a more amorphous surface than the classic human caliciviruses. Skill and experience is required to confidently differentiate between these smaller particles. The chapter by Madeley in Principles & Practice of Clinical Virology (1994) contains a pictorial study of the virus groups; the classification scheme by Caul & Appleton (1982) is widely used.

For the smaller virus particles more than a million particles/g must be present for detection using electron microscopy which usually means the sample must be taken within 48h of onset of symptoms. Use of antibody coated grids for electron microscopy increases the sensitivity, but for SRSVs only human convalescent antibody is available as no animal model exists.

Adenovirus 40/41 can now be detected using enzyme based chemical assays (ELISA) which are being evaluated in comparison with EM. An astrovirus ELISA is under development and it is also possible to grow this virus using CaCo2 cell culture followed by immunofluorescence stain to identify virus replication. These methods have not yet been used on a large scale.

Recent developments with molecular techniques include the sequencing of the genomes of astrovirus (Willcocks *et al*,1995) and strains of SRSV (Lambden *et al*,1993). This should enable the production of genetically engineered reagents. The Polymerase Chain Reaction (PCR) technique replicates specific sections of genome with subsequent detection of those products. This technique has been developed to detect SRSV (Khan *et al*,1994) in faecal samples for the diagnosis of clinical disease. Evaluation with EM studies has shown PCR to be more sensitive (Green *et al*,1995) and more specific. SRSVs may be detectable by PCR for longer periods after the onset of symptoms. The PCR based assays should improve the quality of data on pathogens associated with gastroenteritis.

4.5 Incidence of confirmed disease

The number of cases of gastroenteritis associated with each virus group is shown in Figs 4.1-4.4 and rates per 100,000 in Table 4.1. SRSVs are the most numerous and found in children and adults, particularly the elderly whereas the other groups comprise mainly children's specimens (Table 4.2). This may reflect the number of serotypes within a group and the level of long lasting immunity after infection. The number of positives in children under 5 years

should be viewed in the context of 20,000 rotavirus infections diagnosed each year making it as the most prevalent endemic viral cause of gastroenteritis. Increased numbers of reports of all the virus groups may occur during the winter months.

Figure 4.1

Laboratory Reports of Astrovirus England and Wales 1985-95

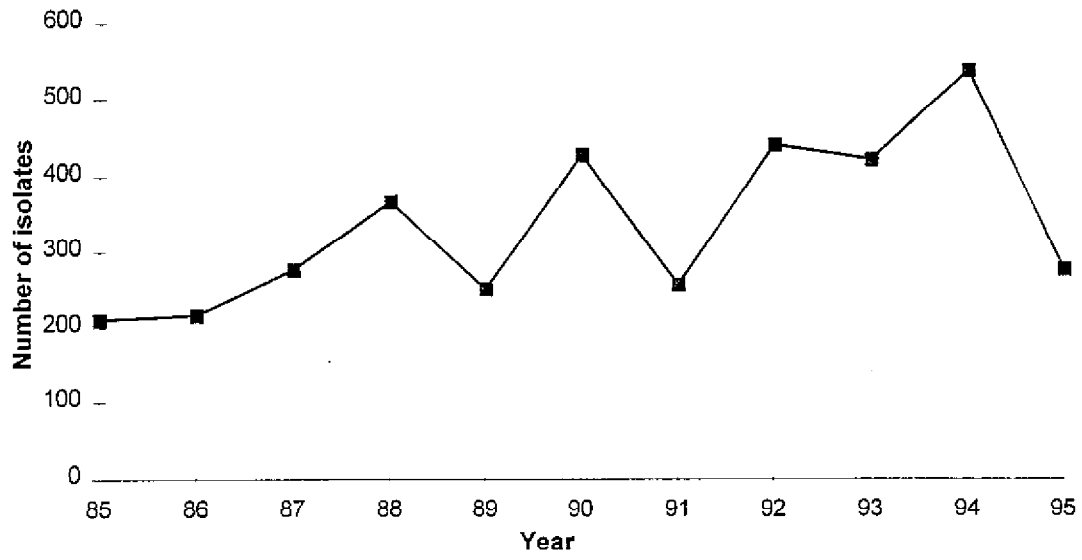


Figure 4.2

Laboratory reports of SRSV England and Wales 1980-1995

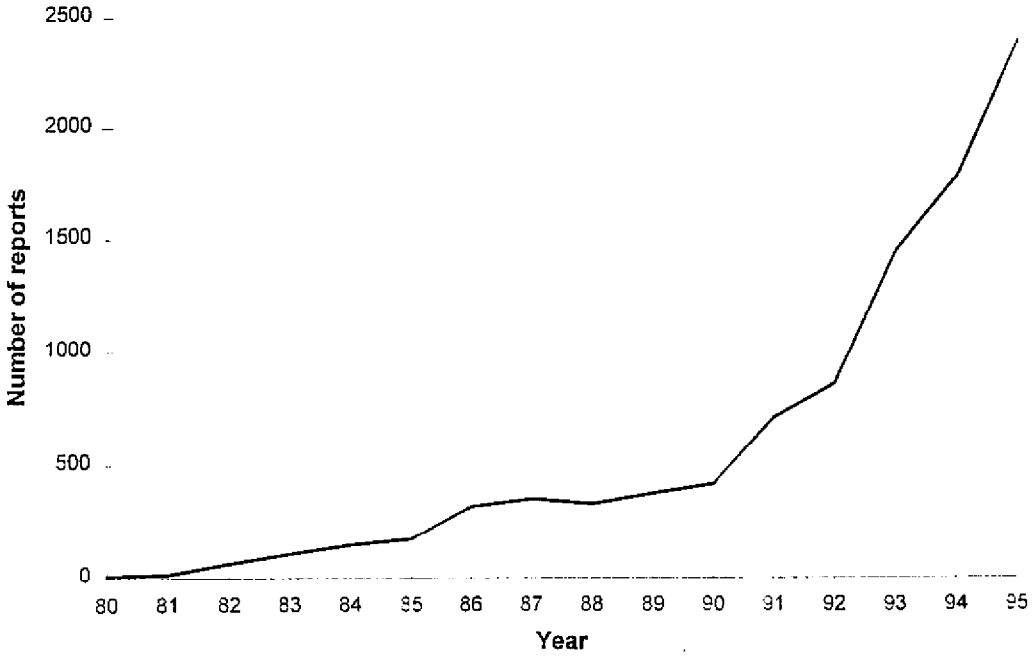


Figure 4.3

Laboratory reports of Adenovirus 40 and 41
England and Wales 1989-95

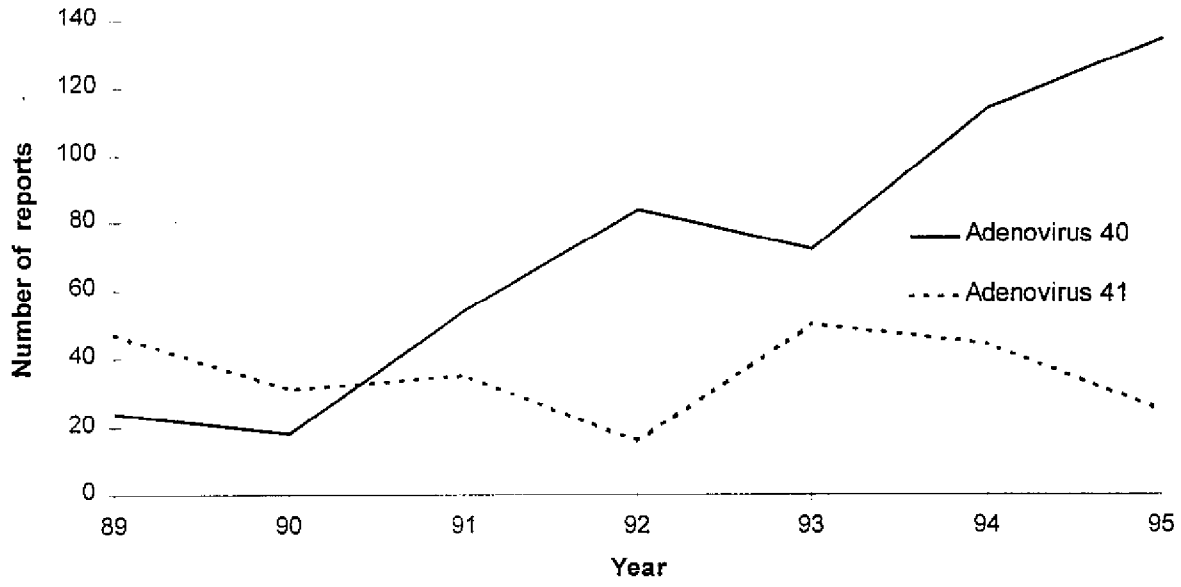
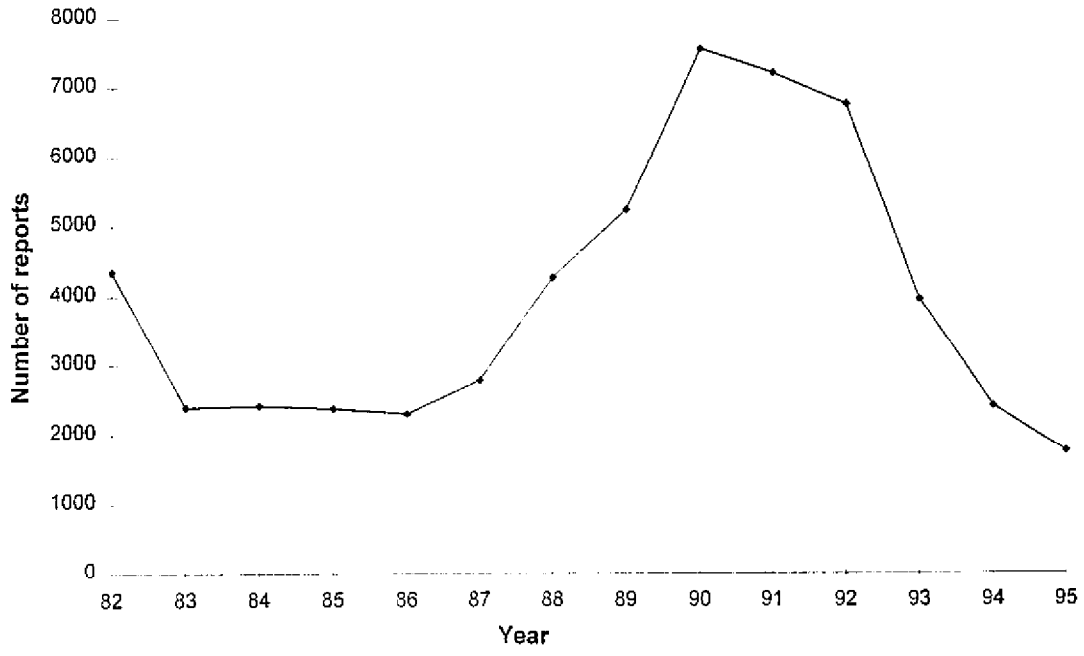


Figure 4.4

Laboratory reports of Hepatitis A England and Wales 1982-1995



When considering the prevalence of these viruses the number of outbreaks involved is important (Table 4.3 a-c). The SRSVs are the main cause of viral gastroenteritis outbreaks.

These occur in residential homes, hospital wards, families and after social gatherings (Viral Gastroenteritis Sub-committee 1993). The number of cases with a positive laboratory result are a fraction of the number of clinical cases involved in outbreaks as usually few specimens are sent for diagnosis and the detection rate by EM can be 10%. Nearly half of the putative viral outbreaks in the community do not have a pathogen detected by current methods.

The PHLS Viral Gastroenteritis Sub-Committee has initiated an enhanced surveillance mechanism through the laboratory EM Network. Baseline data on specimen numbers and results, and for outbreak information are being collected. Preliminary data indicate a significant improvement on the number of outbreak reports, usually involving SRSVs (Communicable Disease Report Weekly 1995).

There are a range of SRSV strains (eg Norwalk, Southampton, Hawaii, Mexico) which may circulate in the community and which will vary in dominance (Green *et al*, 1995).

From the small number of viruses detected astrovirus serotype 1 is the most prevalent (19 cases in 1995). In 1994 serotype 4 was the next most common (4 cases), in 1995 it was type 2 (8 cases) (Lee & Kurtz 1994, Kurtz pers comm). Sporadic adult cases and outbreaks in adults have also been occasionally reported (Oishi *et al*, 1994).

4.6 Viruses of the water cycle

Enteric viruses are shed in large numbers in faecal material and thence into sewage where the viruses are protected by organic debris. The virus content of sewage will therefore reflect those viruses present in the human population. Many virus groups inhabit the gut but not all cause sufficient local damage to produce clinical disease (Madeley 1995). Those recognised as causative agents of gastroenteritis are :

rotavirus (mainly Group A)
SRSVs (many strains identified)
calicivirus
astrovirus (7 serotypes)
adenovirus 40/41 (group F)

Viruses which may cause other types of illness are:

adenovirus (culturable serotype)

enterovirus

(poliovirus, coxsackievirus A and B, echovirus)

enterovirus 68 - 71

coronavirus

HAV

HEV

Viruses which, at present, are not associated with disease or the association is unclear are:

reovirus

small, round featureless viruses (including parvoviruses)

Ground or surface derived water is used to produce drinking water by a series of appropriate treatments. Groundwater is abstracted from underground aquifers and usually contains few microbial contaminants. Only one report of enteroviruses being detected in a chalk aquifer in the UK is documented (Slade 1985). Private water supplies, usually based on bore holes, may be at greater risk of sewage contamination than deeper commercial structures. Surface water is derived from reservoirs and rivers which may, in the latter case, contain varying portions of sewage effluent.

Sewage treatment (activated sludge or biological filtration and anaerobic digestion) will reduce virus numbers in the liquid portion by predation of higher organisms and by sedimentation of suspended solids. The viruses identified from raw inlet by culturable methods include serotypes of poliovirus, echovirus, coxsackievirus, adenovirus, reovirus (Sellwood *et al*, 1981). A similar range is also found in the final effluent although in smaller numbers (Payment *et al*, 1986). Increasingly tertiary treatment is being applied to final sewage effluent. Irradiation with UV light or chlorination will inactivate virus particles not protected by debris. Treated effluent is then discharged to the receiving water.

The virus content of river water decreases as the distance downstream from a sewage treatment works outfall increases (Morris & Sharp 1984). Surveys of river water in the UK have identified the range of virus types, numbers and seasonality (Edwards & Wyn-Jones 1982, Hughes *et al*. 1992). Viruses will survive longer when associated with organic material (Goyal 1984).

The treatment of surface water to produce drinking water treatment can entail rapid sand filtration, settling, coagulation and flocculation to remove organic material. Disinfection with chlorine compounds is the final treatment before distribution.Removal of organic material will include debris associated viruses then any remaining viruses will be inactivated by the chlorine disinfection process (Grabow *et al,*1983, Payment & Armon 1989).

As viruses cannot replicate outside a suitable host, no problem of "re-growth" exists in the water distribution system or elsewhere in the environment.

Bivalve shellfish are filter feeders of organic matter and will therefore utilise sewage for food. Human consumption of mussels, clams, and oysters has resulted in outbreaks of gastroenteritis due to SRSVs (VGE 1993) and infectious hepatitis due to Hepatitis A virus (Appleton 1990) thereby demonstrating the presence of those viruses in sewage contaminated water. Other enteric viruses may also be present but will not cause disease because of consumer immunity. It is only recently that the viruses have been detected directly in shellfish meat by molecular methods (Lees *et al,*1995).

Viruses should not be of increased concern in water if there is an increase of global temperature. Increased sunlight levels would increase UV levels on water surfaces and higher temperatures would increase general microbial activity; both effects would be likely to reduce the virus content of water (PHL Think Tank 1991).

4.7 Methods for concentrating virus in water

For finished potable water which will contain little particulate material and the unlikely presence of virus, 100-1000L samples are needed. This necessitates on-site filtering and automatic acid dosing. Of the few published reports from the UK none have detected enteroviruses (Morris & Sharp 1985). Payment *et al,*(1985) have reported the reduction in virus content as water passes through the different processes of a drinking water treatment works. Report 71 (SCA 1994) and Methods for Virus Detection (SCA 1995) do not recommend routine virus analysis of potable water but list circumstances when tests may be worthwhile. Multiple samples of 10L may be an alternative to large volumes.

Investigation of sea and river water for the presence of enteroviruses is the most common virological assay undertaken in the UK. The standard method is to pass 10L through either cellulose nitrate disc membranes or fibreglass cartridges filters (SCA 1995). The viruses are adsorbed on to the filter matrix under acidic conditions as the water is discarded to waste.

Virus is then eluted from the matrix using a proteinaceous liquid (skimmed milk or beef extract) at high pH.

Second stage concentration involves skimmed milk or beef extract acidification until the protein, including the viruses come out of suspension and form a floc. Centrifugation deposits the floc which is re-suspended in approximately 10mL buffer and assayed for virus content. This method has been developed for enterovirus recovery as they are resistant to extremes of pH.

Fibreglass cartridges are also in widespread use in the US and Canada for water volumes of 10L - 1000L but in France and Spain columns of glass powder or glass wool are preferred. The ready-to-use filters are more convenient to use for multiple samples. Tangential flow ultrafiltration cartridges are more expensive and slower than simpler membranes but recovery rates are higher. These are favoured in Australia and South Africa.

Skimmed milk or beef extract is commonly used for the second concentration stage as it is inexpensive and effective. Small volume ultrafiltration with or without centrifugation is also efficient (Divizia *et al*, 1989). Polyethylene glycol (PEG) (Yang & Xu 1993) and magnetic beads (Monceyron *et al*, 1994) have been also been used for this stage. These methods may also be used as well as ultracentrifugation when volumes of less than 1L of more polluted water, sewage, effluent, sludge, sediment or soil are to be processed.

The PHLS Water Virology External Quality Scheme has been distributing samples for over three years involving all laboratories in the UK undertaking enterovirus recovery from various water types. As the number of laboratories is less than 10, statistical analysis of the results is not appropriate. It is however evident that recovery of virus varies in efficiency between laboratories and between specimens within a laboratory. No difference of efficiency is seen between cartridge or membrane filtration systems.

A laboratory with particularly good consistent results has recovery rates of 60-70% over seven distributions of various enterovirus types. The laboratory employs precise "Standard Operating Procedures" and stringent Internal Quality Controls. This robust attitude indicates that good laboratory practice can produce an effective enterovirus detection system.

Many of the studies on seeded water samples in the literature report a recovery rate for enteroviruses (usually the model for developmental studies) of 30-50% based on glassfibre cartridges (Morris & Waite 1980). Marginally better are recovery rates reported for ultrafiltration (Grabow 1990) and glass wool (Menut *et al*, 1993). The range of virus types

reported concentrated from 10L samples of water by the various methods is shown in Table 4.5. These include the few studies available on the emerging viruses in large volumes of water though most of these are seeding experiments.

Table 4.4

CONCENTRATION METHODS FOR VIRUSES IN WATER

(10L sample)

Enteroviruses

cartridge filtration (Morris & Sharp 1984)

membrane filtration (Morris & Waite 1980)

glass wool (Vilagines *et al*, 1993)

ultrafiltration (Grohmann *et al*. 1993)

PEG 6000 - 10mL river water (Guyader *et al*, 1995)

Hepatitis A

cartridge filtration (Bloch *et al*. 1990)

(Sobsey *et al*, 1985)

membrane filtration (Sellwood & Appleton unpublished data - seeded)

glass wool (Gajardo *et al*, 1991)

ultrafiltration (Divizia *et al*. 1993)

vortex flow filtration (Tsai *et al*. 1993 - seeded)

SRSV

membrane filtration (Wyn-Jones pers comm)

(Sellwood unpublished data - seeded)

glass wool (Wolfaardt *et al*. 1995 - seeded)

Adenovirus 40/41

ultrafiltration (Genthe *et al*. 1995)

membrane filtration (Enriquez & Gerba 1995 - seeded)

Adenovirus culturable

ultrafiltration (Grohmann *et al,*1993)

Astrovirus

glass wool (Marx *et al,*1995)

4.8 Detection of Virus

In the UK enteroviruses are detected using a suspended BGM cell plaque assay which particularly favours the isolation of poliovirus and coxsackievirus B. The Water Virology EQA Scheme results demonstrate that consistent counts of virus are obtained by all the laboratories when testing virus preparations directly. Cell culture, usually BGM cells is used throughout the world for the detection of the enteroviruses, adenoviruses and reoviruses.

Now new cell culture systems are used to detect the fastidious viruses. The PLC/PRF/5 cell line will support adenovirus 40/41 (Grabow *et al,* 1993) and astroviruses (Marx *et al,*1995), FRhK-4 cells support HAV (Sobsey *et al,*1985); CaCO2 cells are sensitive to astrovirus (Willcocks 1990) and other enteric viruses. Virus replication is detected by immunofluorescent staining or plaque assays: No cell culture system exists for the replication of SRSVs or human calicivirus. All cell culture assays ensure that only viable infectious virus is detected in contrast to molecular techniques which detect small sections of virus genome.

It is against the cell culture standard, often a poliovirus model that molecular methods are evaluated. Initially molecular methods utilised gene probe technology but this has been superseded by PCR technology. The latter is capable of detecting very small numbers of virus particles in the original sample. Clewley (1995) edits a detailed review of PCR and viral diagnosis including methodology for RNA viruses, the structure of most enteric viruses and DNA for adenoviruses.

Briefly the method entails using two sets of short nucleotide sequences (primers) which will match and pair with sections of the virus genome under investigation. For RNA viruses the genome must first be coverted to complementary DNA by using the enzyme reverse transcriptase. The primers can then be mixed with and attached to the genome so enabling the genome length between them to be copied many times using DNA polymerase (hence RT-PCR). Agar gel electrophoresis is used to visualise and identify the copies of genome produced. This methodology depends on knowledge of the nucleotide sequence of the virus

genome from which a conserved segment is chosen so that it is likely to be in all strains of a virus group. The structure of some SRSV strains has only recently been elucidated (Lambden 1994) enabling PCR assays to be developed.

Inhibitors of PCR in both water and the beef extract used in the concentration process have affected the development of reliable assays. The use of guanidinium thiocyanate and silica as described by Boom *et al,*(1990) for the extraction of viral RNA has significantly reduced this problem. Careful use of controls throughout the assay is essential to prevent both false negative and false positive results. The PCR test entails very small quantity samples (0.25mL) so that the testing of 10mL of environmental concentrate can be problematic.

Low levels of virus antigen can be detected by PCR but do not correlate with detectable infectious virus as demonstrated by cell culture assay (Ma *et al,*1995, Straub *et al.*1995, Deng *et al,*1994). Other studies have addressed the issue of the length of time nucleic acid may be detectable when not protected by protein or after contact with chlorine compounds (Maier *et al,*1995, Battigelli & Sobsey 1995). It seems likely nucleic acid can be detected for a short time after the disintegration of the viable virion but will then be degraded by environmental enzymes (Tsai *et al,*1995)

Table 4.5

DETECTION OF VIRUSES BY PCR

Enterovirus
- river water (Puig *et al,*1994, Schwab *et al,*1995)
- seawater (Wyn-Jones *et al,*1995)
- sediment (Guyader *et al,*1994)
- shellfish (Guyader *et al,*1994, Lees *et al,*1994)

Hepatitis A
- river water (Divizia *et al,*1993)
- sediment (Guyader *et al,*1994)
- sewage/sludge (Graff *et al,*1993)
- shellfish (Guyader *et al,*1994, Goswami *et al,*1993)

SRSV
- seawater (Wyn-Jones in press)
- shellfish (Lees *et al,*1994)

Adenovirus 40/41
- river water (Puig *et al,*1994)
- sewage (Puig *et al,*1994)

Astrovirus
river water (Marx *et al*, 1995)

Hepatitis E
sewage (Jothikumar *et al*, 1993)

PCR is now being incorporated into a range of novel assays for specific viral studies which have potential for adaption to adenovirus, astrovirus and SRSV projects. PCR has been used in association with cell culture to detect viable progeny virus after short incubation period for rotavirus (Grohmann pers comm) and enterovirus (Egger *et al*, 1995). It is sensitive, rapid and reduces interference with environmental complexes. Antigen-capture PCR has been used by Flehmig's group (Graff *et al*, 1993) for HAV particles by coating PCR reaction tubes with antibody. Magnetic beads can also be used as the solid phase (Muir *et al*, 1993, Monceyron *et al*, 1993, Gilgen *et al*, 1995). Poliovirus vaccine strains from the environment have been studied using restriction fragment length polymorphism (RFLP) and PCR to determine changes in genomic structure (Sellwood *et al*, 1995). Mixtures of primers have been used to detect up to three viruses, poliovirus, HAV and rotavirus, in a single reaction (Tsai *et al*, 1994).

Much effort has been made to develop PCR for the detection of SRSVs by using seeded sewage and water (Wolfaardt *et al*, 1995) and shellfish (Atmar *et al*, 1995, Romalde *et al*, 1994, Gouvea *et al*, 1994). Reports of detection in naturally contaminated environmental samples (Table 6) are less frequent. The report by Puig *et al*, on adenoviruses utilised primers which identified all adenoviruses including 40/41. Gene probes were used to detect only adenovirus 40/41 by Genthe *et al*, (1995) with results indicating over half the river water samples were positive. This method will need to be evaluated for sensitivity and specificity. A brief report by Myint *et al*, (1994) claims large numbers of astrovirus detected in seawater but no details of results or method were given. Methods for the detection in environmental samples of classic human calicivirus have not been documented.

4.9 Waterborne outbreaks

Waterborne outbreaks have been caused in the US and Australia by SRSVs where ice had been made from sewage polluted well water (Cannon *et al*, 1991); where sewage had polluted the water tanks at a mobile home park (McAnulty *et al*, 1993); where sewage had seeped into

the borehole water supply of a tourist resort hotel (Lawson *et al,*1991), and where poor quality water had contaminated a municipal water supply (Kaplan *et al,*1982).

SRSVs were not confirmed as the pathogen but are the most likely cause of a large outbreak of gastroenteritis in 1980 in Bramham, Yorkshire when 3000 people were affected. Sewage leaked into a broken drinking water pipe (Short 1988). A similar incident occurred in Naas, Ireland in 1991 (Fogarty *et al,*1995) when sewage contaminated a borehole supply to nearly 6000 residents causing widespread symptoms of gastroenteritis. Nearly 50 cases of gastroenteritis occurred in 1992 after water from the River Thames being used for irrigation entered the distribution system as a result of backflow from a farm installation (Gutteridge & Haworth 1994).

Seven possible outbreaks of viral gastroenteritis between 1987-1990 are documented by Stanwell-Smith (1990). Of these one was the Bramham incident mentioned above and another was in Northumberland where river water was accidently pumped into a village water supply. Only one probable outbreak has been documented by CDSC since 1990: sewage pollution of a water supply in 1994 resulted in four cases of gastroenteritis from which astrovirus was detected in one stool sample.

Failures of the chlorination process at drinking water treatment plants have been reported to the Drinking Water Inspectorate (1992) and to Department of Public Health (Mayon-White *et al,*1992). Despite large numbers of consumers drinking the water and some publicity, no increase in illness rates were identified.

CDCS reviewed surveillance of waterborne disease in 1994 with suggested classification of events (Nazareth *et al.*1994). No outbreaks of Hepatitis A virus associated with water have been reported in the UK. Thornton *et al.*(1995) specifically investigated this aspect in the Naas incident but found no evidence of increased illness levels.

4.10 FUTURE RESEARCH NEEDS

Adequate diagnostic facilities are essential for assessing the significance of these emerging enteric pathogens. Support for the PHLS Network of electron microscope units should continue. As PCR is fully evaluated for SRSV detection it may replace some of the EM burden of diagnosis but EM remains the only 'catch-all' test system through which new pathogens may be identified.

Immunochemical assays such as ELISA are under development for adenovirus and astrovirus by commercial companies. These will need thorough evaluation in diagnostic laboratories.

The first stage of virus recovery from environmental samples, particularly river water as the source of drinking water is the concentration stage. Research is needed for methods to concentrate SRSV. adenovirus, and astrovirus as few studies have been reported. The well standardised UK filtration methods may be adequate but alternatives such as glass wool and ultrafiltration should be evaluated.

Cell culture based detection systems for adenovirus and astrovirus show promise but need full evaluation with river water samples. Cell culture combined with ELISA detection has been shown to be useful in rotavirus detection (Sellwood & Wyn-Jones 1995) so may be adapted to other virus culture systems.

Development of a PCR for SRSV in water is a primary goal for environmental research. Three main aspects need addressing:

1/ primer development so detection will cover as many strains of SRSV as possible

2/ reduction of inhibition of PCR by materials in water

3/ a method to assay 5-10mL of the virus concentrate

Surveys of river water for these viruses will be needed when the detection systems are available. These should take into account the proportion of sewage effluent in the river, the seasons, the level of disease in the community and the presence of bacterial indicators and other enteric viruses.

The development of assay systems for drinking water is not practical.

4.11 Conclusions

By using a broad interpretation of the term 'emerging pathogen' three enteric virus groups may be included: caliciviruses (ie SRSV and classic calicivirus), adenovirus 40/41 and astroviruses. Only the SRSVs seem to be significant pathogens for adults as infection in children by the other types confers long lasting immunity. Recent advances in the clinical diagnosis of these infections are very encouraging. Results from the newer ELISA tests for adenovirus and astrovirus and PCR for SRSVs will soon lead to increased diagnosis and eventually to a better understanding of the relationship between serotypes.

These new techniques are now being adapted for use with environmental samples. The methods are still in the developmental stage so must be evaluated fully for accuracy, practicality and usefulness. Testing of drinking water is unlikely to be worthwhile but surveys of source water particularly river water should demonstrate the presence of the viruses in the environment. An assessment of the potential risks may then be made.

However epidemiological evidence of waterborne viral gastroenteritis does not depend on detailed virological knowledge. There is evidence for only a small number of outbreaks over the last 20 years and no virological reason why this should increase in the future. Continuing the effective surveillance programme for waterborne outbreaks will be required to provide data on this aspect.

The likelihood of waterborne viral disease is less than protozoan as enteric viruses are sensitive to the standard chlorination at the drinking water treatment works and in the distribution system. Evidence suggests sporadic failures in this process do not result in viral disease.

Gross contamination of drinking water by sewage or river water has the potential to cause disease especially gastroenteritis. The pathogen involved would depend on which one was most prevalent in the community and therefore the sewage at that particular time. A serotype of SRSV would be the most likely but as the level of virus varies in the community, so would the risk of outbreak. Good management of the sewerage and drinking water distribution infrastructure is essential to reduce the risk to a minimum.

4.12 References

Appleton H. Foodborne illness: Foodborne viruses. Lancet; **336**: 1362-1364,1990.

Atmar RL, Neill FH, Romalde JL *et al,*. Detection of Norwalk virus and hepatitis A virus in shellfish tissues with PCR. App & Environ Microbiol; **61**: 3014-3018,1995.

Battigelli DA, Sobsey MD. Inactivation of Norwalk virus (NV), poliovirus type 1 and coliphage MS-2 by free chlorine. Abstracts of the Proceedings of the American Society for Microbiology; 454,1995.

Bloch AB, Stramer SL, Smith JD *et al,*. Recovery of hepatitis A virus from a water supply responsible for a common source outbreak of Hepatitis A. Amer J of Public Health; **80**: 428-430,1990.

Boom R, Sol CJA, Salimans MMM *et al,*. Rapid and simple method for purification of nucleic acids. J Clin Microbiol; **28**: 495-503,1990.

Cannon RO, Poliner JR, Hirschhorn RB *et al,*. A multistate outbreak of Norwalk virus gastroenteritis associated with consumption of commerical ice. J Infect Dis; **164**: 860-3.1991.

Clewley JP. The Polymerase Chain Reaction (PCR) for human viral diagnosis. CRC 1995.

Communicable Disease Report (CDR). SRSVs: numbers are increasing. CDR; **5** no23 1995.

Deng MY. Day SP, Cliver DO. Detection of hepatitis A virus in environmental samples by antigen-capture PCR. App & Environ Microbiol; **60**: 1927-1933,1994.

Divizia M, Santi AL, Pana A. Ultrafiltration: an efficient second step for hepatitis A virus and poliovirus concentration. J Virol Meth; **23**: 55-62,1989.

Divizia M, Morace G, Gabriel R *et al,*. Application of the PCR technique to the detection of hepatitis A virus in the environment. Wat Sci Tech; **27**: 223-225,1993.

Edwards E, Wyn-Jones AP. Incidence of human enteroviruses in water and wastewater systems associated with the River Wear. Viruses & Wastewater Treatment Symposium. University of Surrey 109-112,1981.

Egger D, Pasamontes L, Ostermayer M *et al,*. Reverse transcription multiplex PCR for differentiation between polio- and enteroviruses from clinical and environmental samples. J Clin Microbiol; **33**: 1442-1447,1995.

Enriquez CE, Gerba CP. Concentration of enteric adenovirus 40 from tap, sea and waste water. Wat Res; **29**: 2554-2560,1995.

Farzadegan H. Proceedings of a Symposium: survival of HIV in environmental waters. John Hopkins University 1993.

Fogarty J, Thornton L, Hayes C *et al,*. Illness in a community associated with an episode of water contamination with sewage. Epidemiol Infect; **114**: 289-295,1995.

Gajardo r, Diez JM, Jofre J *et al,*. Adsorptionn-elution with negatively and positively-charged glass powder for the concentration of hepatitis A virus from water. J Virolog Meth; **31**: 345-352,1991.

Gay NJ, Morgan-Capner P, Wright J *et al,*. Age-specific antibody prevalence to hepatitis A in England: implications for disease control. Epidemiol Infect; **113**: 113-120,1994.

Genthe B, Gericke M, Bateman B *et al,*. Detection of enteric adenoviruses in South African waters using gene probes. Wat Sci Tech; **31**: 345-350,1995.

Gilgen M, Wegmuller B, Burkhalter P *et al,*. Reverse transcription PCR to detect enteroviruses in surface water. App & Environ Microbiol; **61**: 1226-1231,1995.

Goswami BB, Koch WH, Cebula TA. Detection of hepatitis A virus in Mercenaria mercenaria by coupled reverse transcription and polymerase chain reaction. App & Environ Microbiol; **59**: 2765-2770,1993.

Gouvea V, Santos N, Timenetsky MC *et al,*. Identification of Norwalk virus in artificially seeded shellfish and selected foods. J Virolog Meth; **48**: 177-187,1994.

Goyal SM. Preservation of environmental samples. In: Gerba & Goyal ed. Methods in Environmental Virology. New York Marcel Dekker. 49-57,1982.

Grabow WOK, Gauss-Muller V, Prozesky OW *et al.*. Inactivation of hepatitis A virus and indicator organisms in water by free chlorine residuals. App & Environ Microbiol; **46**: 619-624,1983.

Grabow WOK. Microbiology of drinking water treatment: reclaimed wastewater. In: McFeters GA ed. Drinking Water Microbiology. Springer-Verlag. 185-203,1990.

Grabow WOK, Puttergill DL, Bosch A. Plaque assay for adenovirus type 41 using the PLC/PRF/5 liver cell line. Wat Sci Tech; **27**: 321-327,1993.

Graff J, Ticehurst J, Flehmig B. Detection of hepatitis A virus in sewage sludge by antigen capture polymerase reaction. App & Environ Microbiol; **59**: 3165-3170,1993.

Green J, Hale AD, Brown DWG. Recent developments in the detection and characterisation of small round structured viruses. PHLS Microbiol Digest; **12**: 219-222,1995.

Grohmann GS, Ashbolt NJ, Genova MS *et al,*. Detection of viruses in coastal and river water systems in Sydney, Australia. Wat Sci Tech; **27**: 457-461,1993.

Gutteridge W, Haworth EA. An outbreak of gastrointestinal illness associated with contamination of the mains supply by river water. CDR **4** R50-51,1994.

Guyader FL, Dubois E, Menard D *et al,*. Detection of hepatitis A virus, rotavirus, and enterovirus in naturally contaminated shellfish and sediment by reverse transcrition-seminested PCR. App & Environ Microbiol; **60**: 3665-3671,1994.

Hughes MS, Coyle PV, Connolly JH. Enteroviruses in recreational waters of Northern Ireland. Epidemiol Infect; **108**: 529-536,1992.

Jothikumar N, Aparna K, Kamatchiammal S *et al,*. Detection of hepatitis E virus in raw and treated wastewater withthe polymerase chain reaction. App & Environ Microbiol; **59**: 2558-2562,1993.

Kapikian AZ. Norwalk and Norwalk-like viruses. In: Kapikian AZ, ed. Viral infections of the gastrointestinal tract. 2nd ed. New York: Marcel Dekker 471-518,1994.

Kaplan JE, Goodman RA, Schonberger LB *et al,*. Gastroenteritis due to Norwalk virus: an outbreak associated with a municipal water system. J Infect Dis; **146**: 190-193,1982.

Khan AS, Moe CL, Glass RI *et al,*. Norwalk virus-associated gastroenteritis traced to ice consumption aboard a cruise ship in Hawaii: comparison and application of molecular method-based assays. J Clin Microbiol; **32**: 318-322,1994.

Kurtz JB. Astroviruses. In: Kapikian AZ, ed. Viaral infections of the gastrointestinal tract. 2nd ed. New York: Marcel Dekker 569-580,1994.

Lawson HW, Braun MM, Glass RIM *et al,*. Waterborne outbreak of Norwalk virus gastroenteritis at a southwest US resort: role of geological formations in contamination of well water. Lancet; **337**: 1200-04,1991.

Lee TW, Kurtz JB. Prevalence of human astrovirus serotypes in the Oxford region 1976-92, with evidence for two new serotypes. Epidemiol Infect: **112**: 187-193,1994.

Lees DN, Henshilwood K, Dore WJ. Development of a method for detection of enteroviruses in shellfish by PCR with poliovirus as a model. App & Environ Microbiol; **60**: 2999-3005,1994.

Lees DN, Henshilwood K, Green J *et al.*. Detection of small round structured viruses in shellfish by reverse transcription-PCR. App & Environ Microbiol; **61**: 4418-4424,1995.

Lewis D, Ando T, Humphrey CD *et al,*. Use of solid-phase immune electron microscopy for classification of Norwalk-like viruses into six antigenic groups from 10 outbreaks of gastroenteritis in the United States. J Clin Microbiol; **33**: 501-504,1995.

Ma JF, Straub TM, Pepper IL *et al,*. Cell culture and PCR determination of poliovirus inactivation by disinfectants. App & Environ Microbiol: **60**: 4203-4206,1994.

Madeley CR. Viruses associated with acute diarrhoeal disease. In: Zuckerman AJ *et al*,ed. Principals and Practice of Clinical Virology. 3rd ed. John Wiley & Sons, 189-277,1994.

Mandell GL, Bennett JE, Dolin R. ed. Principles and Practice of Infectious Diseases. 4th ed. Churchill Livingstone. 1995.

Marx Fe, Taylor MB, Grabow WOK. Optimization of a PCR method for the detection of astrovirus type 1 in environmental samples. Wat Sci Tech; **31**: 359-362,1995.

Mayon-White R, Del Fante P, Murphy M *et al,*. A water contamination incident. CDR; **2**: R69-70, 1992.

McAnulty JM, Rubin GL, Carvan CT *et al,*. An outbreak of Norwalk-like gastroenteritis associated with contaminated drinking water at a caravan park. Austral J Public Health; **17**: 36-41,1993.

Menut C, Beril C, Schwartzbrod L. Poliovirus recovery from tap water after concentration over glass powder and glass wool. Wat Sci Tech; **27**: 291-294,1993.

Monceyron C, Grinde B. Detection of hepatitis A virus in clinical and environmental samples by immunomagnetic separation and PCR. J Virolog Meth; **46**: 157-166,1994.

Moore BE. Survival of Human Immunodeficiency Virus (HIV), HIV-infected lymphocytes, and poliovirus in water. App & Environ .
Microbiol; **59**: 1437-1443,1993.

Morris R, Waite WM. Evaluation of procedures for the recovery of viruses from water. 1. Concentration procedures. Wat Res; **14**: 791-793,1980.

Morris R, Sharp DN. Enteric virus levels in wastewater effluents and surface waters in the Severn Trent Water Authority 1979-1981. Wat Res; **18**: 935-939,1984.

Morris R, Sharp DN. Failure to detect cytopathic enteroviruses in drinking water. Wat Sci Tech; **17**: 105-109.1985.

Muir P, Nicholson F, Jhetam M *et al,*. Rapid diagnosis of enterovirus infection by magnetic bead extraction and polymerase chain reaction detection of enterovirus RNA in clinical specimens. J Clin Microbiol; **31**: 31-38,1993.

Myint S, Manley R, Cubitt D. Viruses in bathing waters. Lancet; **343**: 1640-1641.1994.

Nazareth B, Stanwell-Smith RE, Rowland MGM *et al.*. Surveillance of waterborne disease in England and Wales. CDR; **4**: R93-95,1994.

Oishi I, Yamazaki K, Kimoto T *et al,*. A large outbreak of acute gastroenteritis associated with astrovirus among students and teachers in Osaka, Japan. J Infect Dis; **170**: 439-443,1994.

Payment P, Trudel M, Plante R. Elimination of viruses and indicator bacteria at each step of treatment during preparation of drinking water at seven water treatment plants. App & Environ Microbiol; **49**: 1418-1428,1985.

Payment P, Fortin S, Trudel M. Elimination of human enteric viruses during conventional waste water treatment by activated sludge. Can J Microbiol; **32**: 922-925,1986.

Payment P, Armon R. Virus removal by drinking water treatment processes. CRC Critical Reviews in Environmental Control; **19**: 15-31,1989.

PHLS Think Tank. Report on the potential effects of global warming and population increase on the epidemiology of infectious disease (Draft). 1991.

Puig M, Jofre J, Lucena F *et al.*. Detection of adenovirusesand enteroviruses in polluted waters by nested development PCR amplification. App & Environ Microbiol; **60**: 2963-2970,1994.

Romalde JL, Estes MK, Szucs G *et al.*. In situ detection of hepatitis A virus in cell cultures and shellfish tissues. App & Environ Microbiol; **60**: 1921-1926,1994.

SCA. Methods for the isolation and identification of human enteric viruses from waters and associated materials. HMSO. 1995.

Schwab KL, De Leon R, Shieh YSC *et al.*. Antibody capture and guanidinium extraction for the detection of enteric viruses in surface water samples. Abstracts of the Proceedings of the American Society for Microbiology; 400,1995.

Sellwood J, Dadswell JV, Slade JS. Viruses in sewage as an indicator of their presence in the community. J Hyg Camb; **86**: 217-225,1980.

Sellwood J, Litton PA, McDermott J *et al.*. Studies on wild and vaccine strains of poliovirus isolated from water and sewage. Wat Sci Tech; **31**: 317-321,1995.

Sellwood J, Wyn-Jones AP. A novel method for the detection of infectious rotavirus from water. Wat Sci Tech; **31**: 367-370.1995.

Short CS. The Bramham incident. 1980: an outbreak of waterborne infection. J Inst Water Manag; **2**: 383-390,1988.

Skidmore SJ, Yarbough PO, Gabor KA *et al.*. Hepatitis E virus: the cause of a waterborne hepatitis outbreak. J Med Virol: 37: 58-60,1992.

Skidmore SJ. Hepatitis E. BMJ; **310**: 414-415,1995.

Slade JS. Viruses and bacteria in a chalk well. Wat Sci Tech; **17**: 111-125,1985.

Sobsey MD, Oglesbee SE, Wait DA. Evaluation of methods for concentrating hepatitis A virus from drinking water. App & Environ Microbiol; **50**: 1457-1463,1985.

Straub TM, Pepper IL, Gerba CP. Comparison of PCR and cell culture for detection of enteroviruses in sludge-amended field soils and determination of their transport. App & Environ Microbiol; **61**: 2066-2068,1995.

Thornton L, Fogarty J, Hayes C *et al,*. The risk of hepatitis A from sewage contamination of a water supply. CDR; **5**: R1-4,1995.

Tsai YL, Sobsey MD, Sangermano LR *et al,*. simple method of concentrating enteroviruses and hepatitis A virus from sewage and ocean water for rapid detection by reverse transcriptase-PCR. App & Environ Microbiol; **59**: 3488-3491,1993.

Tsai YL, Tran B, Palmer CJ. Analysis of viral RNA persistence in seawater by reverse transcriptase-PCR. App & Environ Microbiol; **61**: 363-366,1995.

Vilagines P, Sarrette B, Husson G. Glass wool for virus concentration at ambient water pH level. Wat Sci Tech; **27**: 299-306,1993.

Viral Gastroenteritis Sub-Committee (VGE) of the PHLS Virology Committee. Outbreaks of gastroenteritis associated with SRSVs. PHLS Microbiol Digest; **10**: 2-8,1993.

Willcocks MM, Kurtz JB, Lee TW *et al,*. Prevalence of human astrovirus serotype 4: capsid protein sequence and comparison with other strains. Epidemiol Infect; **114**: 385-391,1995.

Woolfaardt M, Moe CL, Grabow WOK. Detection of small round structured viruses in clinical and environmental samples by PCR. Wat Sci Tech; **31**: 375-382,1995.

Yang F, Xu X. A new method of RNA preparation for detection of hepatitis A virus in environmental samples by the polymerase chain reaction. J Virolog Meth; **43**: 77-84,1993.

Section 5

PROTOZOA IMPLICATED AS POTENTIAL EMERGING PATHOGENS

Dr David Casemore
Head, PHLS Cryptosporidium Reference Unit
Rhyl Public Health Laboratory
Glan Clwyd District General Hospital
Rhyl, Clwyd LL18 5UJ

Among the protozoa, there are two which may be considered as potential emerging pathogens, the microsporidia (comprising at least eight species in five genera infecting man) and *Cyclospora*. The microsporidia are associated mainly with AIDS patients; waterborne transmission has not yet been demonstrated but this possibility must be viewed with concern. *Cyclospora* is primarily but not exclusively associated with travel to underdeveloped areas. A few indigenous infections have been reported in the USA and the UK and transmission is most likely to occur by means of contaminated food or water. In addition, two others species, *Toxoplasma* and the closely related *Neospora*, have been reported as having a link with waterborne transmission although the significance of this in the UK is currently uncertain.

5.1 Literature Review: Microsporidia

5.1.1. Introduction

The microsporidia (a non-taxonomic term) are obligate intracellular spore-forming protozoa which have emerged only recently as human pathogens. They are eukaryotes but lack mitochondria, peroxisomes, and Golgi apparatus and have small rRNA of prokaryotic size. They are unable to grow or multiply in the environment. They have a life cycle consisting of merogonic (proliferative) and sporogonic (spore forming) stages. Diagnosis generally depends upon detection of the endogenous (tissue) stages, or of spores in faeces, body fluids or tissues, depending on the site of multiplication. The spores of species infecting man are generally comparable in size with bacterial spores. They differ, however, structurally and functionally from those of bacteria in that they arise from the sporogonic phase of the life cycle as a reproductive stage. They represent the transmissive and infective stage of the parasite rather than a resting stage or response to adverse conditions which is the function of bacterial spores. As with bacterial spores, however, they do permit survival in the environment. The spores of microsporidia have a resistant wall or exospore, mainly protein, and an endospore containing

chitin. Microsporidial spores contain a unique extrusion apparatus through which the spore's cytoplasmic material (sporoplasm) is "inoculated" into a suitable host cell. The tubular coils of the extrusion apparatus provide a diagnostic feature when examined by electron microscopy (Bulla, Cheng, 1976; Canning, Hollister, 1992; Webber at al 1994). By light microscopy the spores can be detected by various means such as chitin stains but these are non-specific and require considerable expertise for interpretation.

Microsporidia are widely distributed in nature and have been recognized as pathogens in insects, fish, birds and mammals for many years but only recognized in human infections in recent years (Canning, Lom 1986; Canning, Hollister, 1992; Wittner at al 1993; Webber *et al.*1994; Sun 1994; Bryan 1995; Heyworth 1996). In man they are primarily opportunist pathogens, ie requiring sub-optimal immune function in the host to cause disease. It is not yet known whether immunocompetent subjects may be infected subclinically with the potential for this to develop into clinical disease if immune function is compromised. They may be zoonotic (transmissible between animals and man) in origin although this is by no means certain and may vary with the species (Bryan 1995). They have become more prominent since the advent of AIDS, reflecting both the opportunistic nature of the infection and the more intensive investigation of such patients (Dobbins, Weinstein, 1985; Nichols 1986; Shadduck 1989; Schattenkerk *et al.*1991; Canning *et al.*1992; Webber *et al*, 1992; Field *et al.*1993; Pol *et al.*1993; Curry, Canning 1993; Lom 1993; Molina *et al.*1993; Topazian, Bia 1994). However, microsporidiosis has also been described in a few immunocompetent patients (Wittner *et al.*1993; Sandfort *et al.*1994; Webber at al 1994; Bryan 1995). Some of these infections were in immunologically privileged sites such as the eye, while others may have been in temporarily immunosuppressed states and current evidence would support an opportunistic role for these parasites. Sub-clinical infections may be wide spread (Canning, Hollister 1992). Increasing awareness and the increase in availability of expertise may increase the clinical spectrum associated with and the frequency of, detection of these infections.

TAXONOMY - Microsporidia are obligate intracellular parasites of the kingdom Protozoa and of the phylum Microspora; two suborders, Pansporoblastina and Apansporoblastina, have been proposed but consensus on taxonomy has not yet been settled and is likely to change as more information becomes available (Bulla, Cheng, 1976; Canning, Hollister, 1992; Wittner at al 1993; Webber *et al.*1994; Sun 1994; Bryan 1995; Heyworth 1996). They are found in most

invertebrates and all classes of vertebrates with more than 100 genera and about 1000 species. The species reported in man include the following which represent five genera:

Enterocytozoon bieneusi is the species most commonly found infecting humans, and has also been found in some non-immunocompromised patients. It is associated with enteric tract infection, diarrhoea, malabsorption, wasting, biliary tract infection, respiratory tract infection, etc. *E.bieneusi* may be a specific human pathogen rather than a zoonosis.

Septata intestinalis may be an *Encephalitozoon* species by molecular biological comparison (Hartskeerl *et al,*1995). It is associated with gastrointestinal infection with diarrhoea, and may become disseminated, involving the kidneys and the biliary tract. It is often found coinfecting with *E.bieneusi.*

Encephalitozoon cuniculi may occur as a zoonosis although there are molecular biological differences between human and some animal isolates (Hollister *et al,*1995). It has been associated with fulminant hepatitis, peritonitis, and disseminated infection. The kidney may also become infected with the disruption of tubule cells. Spores are passed in the urine and contaminate the environment.

Encephalitozoon hellem is associated with ocular infections, and sometimes with disseminated and respiratory infection.

Encephalitozoon species have been found in some ocular and sinus and nasal infections where there was insufficient information to permit definitive identification.

Nosema corneum (Synonymous with proposed new name *Vittaforma corneas n.comb*, Silveira *et al,*1995). *Nosema connori. Nosema ocularum.* Nosema species have been implicated in ocular infections in immunologically competent patients and with disseminated infection in a patient with thymic aplasia.

Pleistophora (Some may be reclassified as Trachypleistophora) may be fishborne and thus presumed to be from uncooked fish but could be waterborne. Associated clinically with myositis.

Microsporidium ceylonensis, Microsporidium africanum. These species have been reported associated with ocular infections.

Unclassified Species. There are a few reports of cases of microsporidial infection where there was insufficient detail to permit identification.

5.1.2 Previous outbreaks

There are no reports of outbreaks of human infection. However, currently there is probably insufficient knowledge of the natural history of the microsporidia, or of expertise nor sufficiently common screening to permit recognition of outbreaks. Developments in molecular biology may in due course permit epidemiological typing to be carried out on isolates.

5.1.3 Prevention & Control

Little is known about reservoirs or routes of transmission or of susceptibility to disinfectants, etc (see also below (Environmental Resistance). Large numbers (=>10^{10}/g) of spores may be excreted, in faeces and sometimes in other body fluids (eg urine) by infected hosts. They are thus transmissible by the environmental route. The small size of spores (ca 1-4.5μm) makes penetration of larger pore size (>1μm) filters likely. Enteric precautions are needed to prevent secondary (person-to-person) transmission by the faecal oral route. Some species may be spread by other means including aerosols.

5.1.4 Pathogenicity

Individuals most affected are those in the late stages of AIDS (Stage C, CD4 count <100). The parasitic infection may be generalised, localised to the enteric tract or restricted to particular sites (eg ocular infections). In enteric infections they can be found in duodenal biopsies, small intestinal enterocytes, and the biliary tree. They may sometimes also be found in the lamina propria etc (ie deeper tissues) without apparent effect. They are also found in pulmonary, ocular, muscular, renal, nasopharyngeal, and CNS tissues. Often clinically inapparent in the

non-immunocompromised. Infected intestine may show non-specific changes to villous architecture and there is some limited evidence for malabsorption (Dobbins, Weinstein, 1985; Shadduck 1989; Cali *et al,*1991; Canning, Hollister 1992; Cali *et al,*1993; Chupp *et al,*1993; Curry, Canning 1993; Lom 1993: Molina *et al,*1993; Pol *et al,*1993; Wittner *et al,*1993; Topazian, Bia 1994; Webber *et al.*1994; Bryan 1995; Heyworth 1996).

There is little or no evidence concerning pathogenetic mechanisms or of relative pathogenicity of different isolates.

5.1.5 Infectivity

Little if anything is known about infectivity but the infective dose, in common with other protozoa, is probably low, depending on species and also dependent on the level of host immune competence.

5.1.6 Treatment

There has been only limited success with attempts at treatment. Albendazole appears to be effective for *S.intestinalis,* and possibly for *E.bieneusi* though less well (Blanshard *et al,*1993; Sobottka *et al,*1995). Treatment with metronidazole or octreotide (non-specific or symptomatic) may possibly be effective in some cases. Some eye lesions respond to itraconazole or propamidine isethionate (Metcalfe *et al.*1992).

5.1.7 Laboratory Diagnosis

Clinical diagnosis requires expertise which is lacking in most laboratories. Microsporidial spores are difficult to detect in infected samples from patients using the methods currently available. Methods for environmental detection are rudimentary and not yet specific for human pathogens. Various methods of detection, usually of the spores, but also sometimes of the endogenous (tissue) stages, have been described (Webber *et al.*1992, 1994), including the following:

Light microscopy (for faeces, urine, histological tissues, biopsies, etc) by traditional histological stains, sometimes modified for the purpose, such as haematoxylin and eosin, Gram's stain, etc (Rijpstra *et al,*1988; Field *et al.*1993; Schwartz *et al,*1994); modified trichrome (Webber *et al,*1992; Ryan *et al,*1993; Garcia *et al,*1994); modified Giemsa (van Gool *et al.*1990); fluoro-

chromes which non-specifically stain the chitin in the spore wall, eg Uvitex 2B; Calcofluor White (Vavra *et al,*1993a,b); indirect immunofluorescence IFAT (Zierdt *et al,*1993; Aldras *et al,*1994; Garcia *et al,*1994).

Electron microscopy has been widely used and is the "gold standard" for definitive identification (Dobbins & Weinstein 1985; Canning & Hollister 1992; Canning *et al,*1992; Schwartz *et al,*1994; Webber *et al,*1994).

Tissue culture Some species may be isolated in tissue culture such as human embryonic lung cells, Madin Darby Canine Kidney (Shadduck *et al,*1990; Didier *et al,*1991; Visvesvara *et al,*1991; Schwartz *et al,*1994; Visvesvara *et al,*1995; Hollister *et al,*1995).

Molecular Methods such as PCR, RFLP, SDS PAGE, Western Blot, have been applied to distinguishing and typing of isolates (Hollister *et al,*1993; Weiss *et al,*1994; Zhu *et al,*1994; De Groote *et al,*1995; Hollister *et al,*1995).

Serological methods Some limited sero-prevalence studies have been reported (Canning & Holister 1993; Whittner 1993; Schwartz *et al,*1994; Webber *et al,*1994) with widely varying results but suggesting widespread exposure. There are uncertainties with regard to the specificity of the tests used. Serological methods are not yet generally useful for diagnostic purposes.

Environmental Detection

Continuous flow centrifugation has been used to recover mosquito microsporidia from ditch water (Avery & Undeen 1987).

Flow cytometry Flow cytometric analysis has been used to detect and distinguish microsporidial spores in water, derived from fish, using bis-benzimide and propidium iodide (Amigo *et al,*1994).

5.1.8 Incidence

In general, little is known as many reports are of single cases or small series. Reports suggest rates of 10-30% among HIV positive patients. There are only 8 - 10 reports of infection in non-HIV patients. There is some evidence of acquisition of infection during travel to underdeveloped countries and this parasite may represent a form of travellers' diarrhoea (Canning & Hollister 1993; Mollina *et al,*1993; Whittner 1993; Schwartz *et al,*1994; Sandfort *et al,*1994; Webber *et al,*1994; Bernard *et al,*1995; Bryan 1995; Degirolami *et al,*1995; Ombrouck *et al,*1995).

5.1.9 Outbreaks

No outbreaks of human infection have been reported to date but this probably reflects the limitations of the methods, the lack of diagnostic expertise, and the absence of typing schemes to identify outbreak strains.

5.1.10 Secondary Transmission

The source of the majority of infections is unknown but faecal spores are generally small, some as small as 1-2μ, and they are excreted in very large numbers (=>10^{10} per ml/gram of stool). Infection may be transmitted by person-to-person transmission, or zoonotically. Environmental transmission is probable (Webber *et al,*1994; Bernard *et al.*, 1995; Bryan 1995). Some human clinical infections may result from endogenous reactivation when immune function is compromised. Few transmission studies have been reported. Infection has been successfully transmitted to athymic but not euthymic mice; other models have included rats, rabbits, and monkeys (Shadduck *et al,*1989; Lom 1983; Didier *et al,*1994).

5.1.11 Resistance to Water Treatment

Little is known definitively but spores are generally hardy. In an *in vitro* study using *Encephalitozoon cuniculi*, 9 of 11 disinfectants tested were effective (of which the only one relevant to water treatment was 0.1% chloramine) and killed after 30 mins exposure at 22°C (Waller 1979). Exposure to UV at 121μW/cm^2 reduced infectivity of a mosquito microsporidian by more than 80% after 1min and 99.9% after 8 mins (Kramer 1976).

5.1.12 Environmental Resistance

Microsporidia are generally believed to be hardy, although little is known definitively but resistance may vary with the species (Weber *et al,*1994; Bryan, 1995). In tests using *E. cuniculi*, spores survived for one day at -20°C but other tests showed survival at -70°C; spores survived 98 days at 4°C in tissue culture medium, 6 days at 22°C, 2 days at 37°C; 2.5% survived 30mins at 56°C while boiling for 5 minutes killed all spores tested (Waller 1979). Spores may survive for 4 weeks in a dry environment: sunlight is known to inactivate some spores while exposure to UV at 121μW/cm^2 reduced infectivity of a mosquito microsporidian by more than 80% after 1min and 99.9% after 8 mins (Kramer 1976; Vavra 1976; Kelly & Anthony 1979; Waller 1979).

Spores from some species (eg *Nosema bombycis*) are known to have remained viable for up to 10 years in distilled water (Bulla, *et al*, 1976/7).

5.1.13 Environmental Occurrence

Little is known about these species in the environment. Spores are known to occur in animals (including dogs, etc), insect and fish excreta, but the significance of these for man in unknown (Webber at al 1994; Bryan 1995). Flow cytometry has been used to detect some non-human (fish) species (Amigo *et al*, 1994), while continuous flow centrifugation followed by microscopy was used with insect inoculation (xeno-diagnosis) for recovery from water of species derived from insects.

5.1.14 Risk Assessment

As so little is known of reservoirs and routes of transmission, infective dose size, or such characteristics as pathogenicity and virulence of isolates, it is difficult to assess the risk.

5.1.15 Research Needs

Much work is needed on the definitive identification of species, comparison of isolates at the molecular level, identification of sources and reservoirs of infection, etc. Studies need to be undertaken on the effect of water treatment systems on spore viability.

5.1.16 Summary

Microsporidia are emerging human pathogens of particular concern in the context of AIDS and other seriously immunocompromised patients but can also be found in immunologically deficient sites, such as the eye, in normal subjects. The spore stage of at least some species are likely to be present in the environment and, given their generally small size, could penetrate water treatment systems. Little if anything is known of their resistance to disinfectants used in water treatment.

5.1.17 References

Aldras AM, Orenstein JM, Kotler DP, Shadduck JA, Didier ES. Detection of microsporidia by indirect immunofluorescence antibody test using polyclonal and monoclonal antibodies. *Journal of Clinical Microbiology* 1994;**32**:608-612.

Amigo JM, Gracia M-P, Comas J, Salvado H, Vivares CP. Comparative study of microsporidian spores by flow cytometric analysis. *J Euk Microbiol* 1994;**41**:210-214.

Avery SW, Undeen AH. The isolation of microsporidia and other pathogens from concentrated ditch water. *Journal of the American Mosquito Control Association* 1987;3:54-58.

Bernard E, Carles M, Pradier C, Boissy C, Roger PM, Hebuterne X, Mondain V, Michiels JF, Le Fichoux Y, Dellamonica P. Persistent diarrhea in HIV infected patients: role of *Enterocytozoon bieneusi*. *Presse Medicale* 1995;**24**:671-674.

Blanshard C, Ellis DS, Dowell SP, Tovey G, Gazzard BG. Electron microscopic changes in *Enterocytozoon bieneusi* following treatment with albendazole. *J Clin Pathol* 1993;**46**:898-902.

Bulla LA, Cheng TC. Comparative Pathobiology. Plenum Press. New York. 1976;**1**:3363-791.

Cali AC, Meisler DM, Lowder CY, Lembach R, Ayers L, Takvorian PM, Rutherford I, Longworth DL, Mc Mahon J, Bryan RT. Corneal microsporidiosis: Characterization and identification. *J Protozool* 1991;**38**:215S.

Cali A, Kotler DP, Orenstein JM. *Septata intestinalis* N.G.N. Sp., an intestinal microsporidian associated with chronic diarrhea and dissemination in AIDS patients. *J Euk Microbiol* 1993;**40**:101-112.

Canning EU, Curry A, Lacey CJN, Fenwick JD. Ultrastructure of *Encephalitozoon* sp. Infecting the conjunctival, corneal and nasal epithelia of a patient with AIDS. *Europ J Protistol* 1992;**28**:226-237.

Canning EU, Hollister WS. Human infections with microsporidia. *Reviews in Medical Microbiology* 1992;**3**:35-42.

Chupp GL, Alroy J, Adelman LS, Breen JC, Skolnik PR. Myositis due to *Pleistophora* (Microsporidia) in a patient with AIDS. *Clinical Infectious Diseases* 1993;**16**:15-21.

Curry A, Canning EU. Human microsporidiosis. *Journal of Infection* 1993;**27**:229-236.

D'Antonio RG, *et al.* Waterborne outbreak of Cryptosporiosis in normal hosts Annals Int. Med. 1985; **103** 886-88.

De Groote MA, Visvesvara G, Wilson ML, Pieniazek NJ, Slemenda SB, DaSilva AJ, Leitch GJ, Bryan RT, Reves R. Polymerase chain reaction and culture confirmation of disseminated *Encephalitozoon cuniculi* in a patient with AIDS: Successful therapy with albendazole. *The Journal of Infectious Diseases* 1995;**171**:1375-8.

Didier ES, Didier PJ, Friedberg DN, Stenson SM, Orenstein JM, Yee RW, Tio FO, Davis RM, Vossbrinck C, Millichamp N, Shadduck JA. Isolation and characterization of a new human microsporidian, *Encephalitozoon hellem* (n. sp.), from three AIDS patients with Keratoconjunctivitis. *The Journal of Infectious Diseases* 1991;**163**:617-621.

Didier ES, Varner PW, Didier PJ, Aldras AM, Millichamp NJ, Murphey-Corb M, Bohm R, Shadduck JA. Experimental microsporidiosis in immunocompetent and immunodeficient mice and monkeys. *Folia Parasitologica* 1994;**41**:1-11.

Dobbins WO, Weinstein WM. Electron Microscopy of the intestine and rectum in acquired immunodeficiency syndrome. *Gastroenterology* 1985;88:738-49.

Field AS, Hing MC, Milliken ST, Marriott DJ. Microsporidia in the small intestine of HIV-infected patients. *The Medical Journal of Australia* 1993;**158**:390-394.

Field AS, Marriott DJ, Hing MC. The Warthin-Starry stain in the diagnosis of small intestinal microsporidiosis in HIV-infected patients. *Folia Parasitologica* 1993;**40**:261-266.

Garcia LS, Shimizu RY, Bruckner DA. Detection of microsporidial spores in fecal specimens from patients diagnosed with cryptosporidiosis. *Journal of Clinical Microbiology* 1994;**32**:1739-1741.

Gool TV, Hollister WS, Schattenkerk JE, Van Den Bergh Weerman MA, Terpstra WJ, Van Ketel, RJ, Reiss P, Canning EU. Diagnosis of *Enterocytozoon bieneusi* microsporidiosis in AIDS patients by recovery of spores from faeces. *Lancet* 1990;**336**:697-698.

Hartskeerl RA, Van Gool T, Schuitema AR, Didier ES, Terpstra WJ. Genetic and immunological characterisation of the microsporidian Septata intestinalis Cali, Kotler and Orenstein. Reclassification to Encephalitozoon intestinalis, 1993. *Parasitology* 1995;**110**:277-285.

Heyworth MF. Giardiasis, Balantidiasis, Isosporiasis and Microsporidiosis. In: Oxford Textbook of Medicine, 3rd edition. Eds D Weatherall, JGG Ledingham, DA Warrell; OUP. 1996. 883-886.

Hollister WS, Canning EU, Colbourn NI. A species of *Encephalitozoon* isolated from an AIDS patient: criteria for species differentiation. *Folia Parasitologica* 1993;**40**:293-295.

Hollister WS, Canning EU, Colbourn NI, Aarons EJ. *Encephalitozoon cuniculi* isolated from the urine of an AIDS patient, which differs from canine and murine isolates. *J Euk Microbiol* 1995;**42**:367-372.

Kelly JF, Anthony DW. Susceptibility of spores of the microsporidian *Nosema* algerae to sunlight and germicidal ultraviolet radiation. *Journal of Invertebrate Pathology* 1979;**34**:164-169.

Kramer JP. The Extra-Corporeal Ecology of Microsporidia. In: Comparative Pathobiology. Eds LA Bulla, TC Cheng. 1976;**1**:127-135.

Lacey CJN, Clarke AMT, Fraser P, Metcalfe T, Bonsor G, Curry A. Chronic microsporidian infection of the nasal mucosae, sinuses and conjuctivae in HIV disease. *Genitourin Med* 1992;**68**:179-181.

Lom J. Introductory remarks on microsporidia in the AIDS era. *Folia Parasitologica* 1993;**40**:255-256.

Metcalfe TW, Doran RML, Rowlands PL, Curry A, Lacey CJN. Microsporidial keratoconjunctivitis in a patient with AIDS. *British Journal of Ophthalmology* 1992;**76**:177-178.

Bryan RT. Microsporidia. In: Infectious Diseases and their etiologic agents. Ed Mandell *et al*,1995;2513-2524.

Molina J-M, Sarfati C, Beauvais B, Lemann M, Lesourd A, Ferchal F, Casin I, Lagrange P, Modigliani R, Derouin F, Modai J. Intestinal microsporidiosis in human immunodeficiency virus - infected patients with chronic unexplained diarrhea: Prevalence and clinical and biologic features. *Journal of Inf Dis* 1993;**167**:217-21.

Nichols G. Microsporidiosis in AIDS. *CDR 86/21* 1986;3-4.

Ombrouck C, Romestand B, da Costa JM, Desportes-Livage I, Datry A, Coste F, Bouix G, Gentilini M. Use of cross-reactive antigens of the microsporidian Glugea atherinae for the possible detection of *Enterocytozoon bieneusi* by western blot. *American Journal of Tropical Medicine & Hygiene* 1995;**52**:89-93.

Pol S, Romana CA, Richard S, Amouyal P, Desportes-Livage I, Carnot F, Pays J-F, Berthelot P. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *The New England Journal of Medicine* 1993;**328**:95-99.

Ryan NJ, Sutherland G, Coughlan K, Globan M, Doultree J, Marshall J, Baird RW, Pedersen J, Dwyer B. A new trichrome-blue stain for detection of microsporidial species in urine, stool, and nasopharyngeal specimens. *Journal of Clinical Microbiology* 1993;**31**:3264-3269.

Rijpstra AC, Canning EU, Van Ketel RJ, Eeftinck Schattenkerk JKM, Laarman JJ. Use of light microscopy to diagnose small-intestinal microsporidiosis in patients with AIDS. *Journal of Infectious Diseases* 1988;**157**:827-831.

Sandfort J, Hannemann A, Gelderblom H, Stark K, Owen RL, Ruf B. *Enterocytozoon bieneusi* infection in an immunocompetent patient who had acute diarrhea and who was not infected with the human immunodeficiency virus. *Clinical Infectious Diseases* 1994;**19**:514-6.

Schwartz DA, Bryan RT, Weber R, Visvesvara GS. Microsporidiosis in HIV positive patients: current methods for diagnosis using biopsy, cytologic, ultrastructural, immunological, and tissue culture techniques. *Folia Parasitologica* 1994;**41**:101-109.

Schattenkerk J, Van Gool T, Van Ketel R. Microsporidia: A New Gastrointestinal AIDS Pathogen? Clinical significance of small intestinal microsporidiosis in HIV-1-Infected individuals. *Lancet* 1991;**337**:895-898.

Shadduck JA. Human Microsporidiosis and AIDS. *Reviews of Infectious Diseases* 1989;**11**:203-207.

Shadduck JA, Meccoli RA, Davis R, Font RL. Isolation of a microsporidian from a human patient. *The Journal of Infectious Diseases* 1990;**162**:773-776.

Silveira H, Canning EU. Vittaforma corneae n.comb. for the human microsporidium Nosema corneum Shadduck, Meccoli, Davis & Font, 1990, based on its ultrastructure in the liver of experimentally infected athymic mice. *Journal of Eukaryotic Microbiology* 1995;**42**:158-165.

Sobottka I, Albrecht H, Schafer H, Schottelius J, Visvesvara GS, Laufs R, Schwartz DA. Disseminated *Encephalitozoon (Septata) intestinalis* infection in a patient with AIDS: Novel diagnostic approaches and autopsy-confirmed parasitological cure following treatment with albendazole. *Journal of Clinical Microbiology* 1995;**33**:2948-2952.

Sun T. Current Topics in Protozoal Diseases. Microsporidiosis. *American Journal of Clinical Pathology* 1994;**102**:18-20.

Topazian M, Bia FJ. New Parasites on the Block: Emerging Intestinal Protozoa. *The Gastroenterologist* 1994;**2**:147-159.

Vavra J, Dahbiova R, Hollister WS, Canning EU. Staining of microsporidian spores by optical brighteners with remarks on the use of brighteners for the diagnosis of AIDS associated human microsporidioses. *Folia Parasitologica* 1993;**40**:267-272.

Vavra J, Maddox JV. Storage of microsporidian spores. In: Comparative Pathobiology. Eds LA Bulla, TC Cheng. 1976;**1**:281-320.

Vavra J, Nohynkova E, Machala L, Spala J. An extremely rapid method for detection of microsporidia in biopsy materials from AIDS patients. *Folia Parasitologica* 1993;**40**:273-274.

Visvesvara GS, Leitch GJ, Moura H, Wallace S, Weber R, Bryan RT. Culture, Electron Microscopy, and immunoblot studies on a microsporidian parasite isolated from the urine of a patient with AIDS. *J Protozool* 1991;**38**:105S

Visvesvara GS, Leitch GJ, Peiniazek NJ, Da Silva AJ, Wallace S, Slemenda SB, Weber R, Schwartz DA, Gorelkin L, Wilcox CM, Bryan RT. Short-term in vitro culture and molecular analysis of the microsporidian, *Enterocytozoon bieneusi*. *J Euk Microbiol* 1995;**42**:506-510.

Waller T. Sensitivity of *Encephalitozoon cuniculi* to various temperatures, disinfectants and drugs. *Lab Animal* 1979;**13**:227-30.

Weber R, Bryan RT, Owen RL, Wilcox CM, Gorelkin L, Visvesvara GS, and the Enteric Opportunistic Infections Working Group. Improved light-microscopical detection of

microsporidia spores in stool and duodenal aspirates. *The New England Journal of Medicine* 1992;**326**:161-166.

Weber R, Bryan RT, Schwartz DA, Owen RL. Human Microsporidial Infections. *Clinical Microbiology Reviews* 1994;7:426-461.

Weber R, Sauer B, Spycher MA, Deplazes P, Keller R, Ammann R, Briner J, Luthy R. Detection of *Septata intestinalis* in stool specimens and coprodiagnostic monitoring of successful treatment with albendazole. *Clinical Infectious Diseases* 1994;**19**:342-5.

Weiss LM, Shu X, Cali A, Tanowitz HB, Wittner M. Utility of microsporidian rRNA in diagnosis and phylogeny: a review. *Folia Parasitologica* 1994;**41**:81-90.

Weber R, Kuster H, Keller R, Bachi T, Spycher MA, Briner J, Russi E, Luthy R. Pulmonary and intestinal microsporidiosis in a patient with the acquired immunodeficiency syndrome. *American Review of Respiratory Disease* 1992;**146**:1603-1605.

Wittner M, Tanowitz HB, Weiss LM. Microsporidiosis. In: Parasitic Infections in AIDS patients. *Infectious Disease Clinics of North America* 1993;7:577-581.

Zierdt CH, Gill VJ, Zierdt WS. Detection of microsporidian spores in clinical samples by indirect fluorescent-antibody assay using whole-cell antisera to *Encephalitozoon cuniculi* and *Encephalitozoon hellem*. *Journal of Clinical Microbiology* 1993;**31**:3071-3074.

Zhu X, Wittner M, Tanowitz HB, Cali A, Weiss LM. Ribosomal RNA sequences of *Enterocytozoon bieneusi, Septata intestinalis* and *Ameson michaelis*: Phylogenetic construction and structural correspondence. *J Euk Microbiol* 1994;**41**:204-209.

5.2 Cyclospora: Literature Review

5.2.1. Biology/Introduction

Cyclospora are coccidian protozoa transmitted by means of oocysts which are excreted in an unsporulated form. When fully sporulated, oocysts have two sporozoites in each of two sporocysts. The oocysts require an extrinsic period (ie, outside the host) of about a week, depending on conditions, for sporulation to occur. The *Cyclospora* species described in man has not previously been reported in other hosts. Cyclospora was first described in man by Ashford (1979) in stools from patients in New Guinea although he was unable to definitively identify it other than as a coccidian. The oocysts appear as smooth, round bodies containing an inner morular (ie, mulberry- like) structure; the oocyst wall fluoresces blue under UV light. By acid-fast staining, the bodies are larger than cryptosporidium (8-10μm cf 5μm) with more variable staining. They have been recognised in faecal specimens from patients with diarrhoea, examined by modified Ziehl-Neelsen stain for the detection of cryptosporidium, since the mid-1980s. They have been described variously as cryptosporidium-like bodies, fungal spores, and cyanobacteria(blue-green algae)-like (Long *et al,*1990; Hart *et al,*1990; Long *et al,*1991; Kocka *et al,*1991; Anon 1991; Shlim *et al,*1991; Pollock *et al,*1992). More recently, they have been definitively identified as oocysts of a protozoan parasite belonging to the genus Cyclospora and named *Cyclospora cayetanensis* (Bendall *et al,*1993; Ortega *et al,*1993; Anon 1993; Ortega *et al,*1994; Casemore 1994; Chiodini 1994). They have been detected worldwide, most often in travellers in or returning from, developing countries (Long *et al,*1990; Long *et al,*1991; Shlim *et al,*1991; Wurtz *et al,*1993; Hodge *et al,*1993; Rijpstra *et al,*1993; Albert *et al,*1994; Berlin *et al,*1994; Butcher *et al,*1994; Hodge *et al,*1995); in indigenous patients (Ortega *et al,*1993; Deluol *et al,*1994; Albert *et al,*1994; Berlin *et al,*1994; Hodge *et al,*1995) and also in AIDS patients (Hart *et al,*1990; Wurtz *et al,*1993; Pape *et al,*1994).

5.2.2 Outbreaks

Outbreaks have been reported in a hospital in Chicago in 1990 (Kocka *et al,*1991), and in Nepal in 1989 and 1990 (Kocka *et al.*, 1991; Shlim *et al.*, 1991) and in 1994 (Rabald *et al,*1994). Of the outbreaks reported, all but the last described the agent concerned as algal-like. The outbreak in Chicago involved hospital staff; specimens were examined from 17 house physicians and three other employees who had explosive watery diarrhoea, anorexia, severe abdominal cramps,

nausea, and in some cases, vomiting. Diarrhoea usually lasted three to four days, which was followed by a cycle of relapses and remission for up to four weeks. Epidemiological investigation implicated the water supply. Another two cases, unconnected with the affected hospital were identified when the local laboratory instituted surveillance for Cyclospora in all stools submitted to the laboratory.

Studies in Nepal in 1989 revealed a cluster of more than 50 persons, mainly expatriate visitors, who were infected with Cyclospora. The cases occurred during May to November, five of whom became ill 2-11 days following arrival. Ages ranged from 1 to 67 years; symptoms were similar to those described above. and lasted for 4 to 107 days (mean 43 days).

No further cases were discovered until the second outbreak which occurred in May to October 1990, in patients (again mainly expatriates) using the same clinic in Kathmandu. Oocysts were detected in stools of 85 patients. Of 72 patients interviewed, about 95% had watery, non bloody diarrhoea which in many cases was accompanied by other symptoms such as fatigue and anorexia. During the same period. stools were examined from 184 local people, of whom six (3%) were also positive for Cyclospora. Analysis of water and food samples yielded one positive sample (lettuce). The cases in both outbreaks began to appear before the beginning of the monsoon period.

The outbreak in 1994 occurred in Pokhara, Nepal, and involved British soldiers and their dependants, of whom six had diarrhoea associated with Cyclospora infection. Epidemiological evidence suggested an association with water and Cyclospora was found in a water sample. Two outbreaks are believed to have occurred in the USA, in New York and Florida (Charles Stirling, pers comm) but no details have yet been reported.

Efforts were made to obtain samples during a visit to Nepal by a member of staff of the London School of hygiene and Tropical Medicine (Dr Whitworth pers. comm). Samples taken included various water sources (water storage tanks, water containers, streams, etc, using the SCA method) and livestock and wildlife in Nepal. None of the samples revealed oocysts typical of *Cyclospora sp.*

5.2.3 Prevention and Control

Although infection is believed to be derived from other infected humans, the requirement for an extrinsic period of sporulation implies that the majority of infections occur by an indirect route which is likely to involve food and water. There is some evidence, including the peak incidence in the rainy season, for water-borne transmission (Hodge *et al*, 1993; Rabold *et al*, 1994; Hodge *et al*, 1995). Use of boiled water and where possible, protection of water supplies, is therefore necessary; together with hygienic measures to prevent contamination of fruit, salads, etc.

5.2.4 Pathogenicity

The parasite is believed to develop fully intracellularly in the enterocytes of the small bowel (Bendall *et al*, 1993). Little is known, however, of the full life cycle or of pathogenesis; such studies have not yet been reported but recent studies in the USA are believed to confirm involvement of epithelial cells in the jejunum. To-date, an animal model has not been identified (CR Sterling, personal communication).

5.2.5 Infectivity

Nothing is yet known of the likely infective dose for man but it is probably low as with other enteric protozoa.

5.2.6 Treatment

Cotrimoxazole (160/800mg, 2-4 times per day for 7-10 days or 5/25mg/kg) appears to be effective (Madico *et al*, 1993; Hoge *et al*, 1995).

5.2.7 Lab Diagnosis

The oocysts of the parasite can be detected in clinical samples by phase-contrast or DIC microscopy of wet-mount preparations of faecal material and by modified Ziehl-Neelsen staining. Such methods are often restricted to samples from patients giving a history of foreign travel and indigenous cases would therefore tend to be missed. Attempts to detect them in environmental samples using the SCA methodology have so far been unsuccessful. Oocysts measure 8-10μm, with a smooth hyaline outer wall with an internal morular structure of small (1-2μm) membrane bound granules. By modified Ziehl-Neelsen stain (Casemore 1991) the oocysts stain variably acid-fast with a mottled surface pattern of staining; many oocysts do not

take up the stain and remain refractile. Monoclonal antibodies for IFAT are not yet available. Work is being undertaken to this end by the University of Arizona (C R Sterling personal communication).

5.2.8 Incidence

Cases have been detected in indigenous subjects and in travellers staying in or returning from Nepal, SE Asia, South and Central America and a number of holiday areas (Turkey, Dominican Republic), etc (Anon 1991; Casemore 1994; Chiodini 1994). Cases are not commonly associated with the African continent.

A few sporadic cases and one outbreak (Chicago) have been reported from patients in the USA and UK who have not travelled abroad. The source of these infections is not known.

5.2.9 Secondary Transmission

The requirement for extrinsic (environmental) sporulation implies that direct secondary transmission is likely to be an uncommon mode of transmission. Secondary transmission is most likely to occur via ingestion of contaminated food or water.

5.2.10 Resistance to water treatment

Little is known, given the absence of an animal or tissue culture model, but it is likely to be high, as with other protozoa.

5.2.11 Environmental resistance

Not known but is likely to be high, especially in water or moist conditions.

5.2.12 Environmental occurrence

Not known; limited attempts to recover oocysts from the environment in Nepal (SCA water method and examination of livestock and wildlife excreta) were unrewarding (Whitworth, unpublished communication).

5.2.13 Risk assessment

In the absence of data on infectivity and the natural history of the parasite, including host range, it is not possible to define parameters for a meaningful risk assessment. The risk of spread from imported infections within the UK is probably low.

5.2.14 Summary

Cyclospora infection has emerged in recent years, mainly as a cause of travellers diarrhoea. A few cases in the UK and in America without a history of travel may indicate more widespread indigenous infection which will not be detected if screening for the parasite is limited to those with a history of recent travel to underdeveloped countries.

5.2.15 References

Albert MJ, Kabir I, Azim T, Hossain A, Ansaruzzaman M, Unicomb L. Diarrhea associated with *Cyclospora* sp. in Bangladesh. *Diagn Microbiol Infect Dis* 1994;**19**:47-49.

Anon. World Health Organisation, Geneva. Diarrhoeal diseases. *Weekly Epidemiological Record* 1991;**66**:241-48.

Anon. Diarrhoea associated with *Cyclospora species* PHLS Communicable Disease Report 1993;**3**:103.

Ashford RW. Occurrence of an undescribed coccidian in man in Papua New Guinea. *Annals Trop Med Parasitol* 1979;**73**:497-500.

Ashford RW, Warhurst DC, Reid GDF. Human infection with cyanobacterium-like bodies. (Letter) *The Lancet* 1993;**341**:1034.

Bendall RP, Lucas S, Moody A, Tovey G, Chiodini PL. Diarrhoea associated with cyanobacterium-like bodies: a new coccidian enteritis of man. *The Lancet* 1993;**341**:590-592.

Berlin OGW, Novak SM, Porschen RK, Long EG, Stelma GN, Schaeffer FW. Recovery of *Cyclospora* organisms from patients with prolonged diarrhea. *Clin Inf Dis* 1994;**18**:606-09.

Butcher A R, Lumb R, Coulter E, Nielsen DJ. Coccidian/cyanobacterium-like body associated diarrhea in an Australian traveller returning from overseas. *Pathology* 1994;**26**:59-61.

Casemore DP. ACP Broadsheet 128: Laboratory methods for diagnosing cryptosporidiosis. *J Clin Pathol* 1991;**44**:445-51.

Casemore DP. Cyclospora - another "new" pathogen. *J Gen Microbiol* 1994;**41**:217-219.

Chiodini PL. A "new" parasite: human infection with *Cyclospora cayetanensis. Transactions of the Roy Soc of Trop Med and Hyg* 1994;**88**:369-371.

Deluol A-M, Junod C, Poirot J-L, Heyer F, N'Go Y, Cosnes J. Travellers diarrhea associated with *Cyclospora* sp. *J Euk Microbiol* 1994;**41**:32S.

Hart AS, Ridinger MT, Soundarajan R, Peters CS, Swiatlo AL, Kocka FE. Novel organism associated with chronic diarrhoea in AIDS. *Lancet* 1990:**335**:169-70.

Hoge CW, Shlim DR, Echeverria P. Cyanobacterium-like cyclospora species. *The New Engl Journal of Med* (Letter) 1993:1504-05.

Hoge CW, Shlim DR, Rajah R, Triplett J, Shear M, Rabold JG, Echeverria P. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travellers and foreign residents in Nepal. *Lancet* 1993;**341**:1175-79.

Hoge CW, Shlim DR, Ghimire M, Rabold JG, Pandey P, Walch A,

Rajah, R, Gaudio P, Echeverria P. Placebo-controlled trial of co-trimoxazole for cyclospora infections among travellers and foreign residents in Nepal. *Lancet* 1995;**345**:691-693.

Hoge CW, Echeverria P, Ramachandran R, Jacobs J, Malthouse S, Chapman E, Jimenez LM, Shlim DR. Prevalence of *Cyclospora* species and other enteric pathogens among children less than 5 years of age in Nepal. *Journal of Clinical Microbiology* 1995;**33**:3058-60.

Korka, F. Peters, C. Dacumos E, *et al.*. Outbreaks of Diarrheal illness Associated with Cyanobacteria (Blue-Green Algae)-like bodies - Chicago and Nepal, 1989 and 1990. *MMWR* 1991;**40**:325-327.

Long EG, Ebrahimzadeh A, White EH, Swisher B, Callaway, CS. Alga associated with diarrhea in patients with acquired immunodeficiency syndrome and in travellers. *Journal of Clinical Microbiology* 1990;**28**:1101-04.

Long EG, White EH, Carmichael WW, Quinlisk PM, Raja R, Swisher BL, Daugharty H, Cohen MT. Morphologic and staining characteristics of a cyanobacterium-like organism associated with diarrhea. *Journal of Inf Dis* 1991;**164**:199-202.

Madico G, Gilman RH, Miranda E, Cabrera L, Sterling CR. Treatment of cyclospora infections with co-trimoxazole. *Lancet* 1993;**342**:122-23.

Ortega YR, Sterling CR, Gilman RH, Cama VA, Diaz F. *Cyclospora* Species - A new protozoan pathogen of humans. *New England Journal of Medicine* 1993;**328**:1308-12.

Ortega YR, Gilman RH, Sterling CR. A new coccidian parasite (*Apicomplexa:eimeriidae)* from humans. *J Parasitol* 1994;**80**:625-629.

Rabold JG, Hoge CW, Shlim DR, Kefford C, Rajah R, Echeverria P. Cyclospora outbreak associated with chlorinated drinking water. *Lancet* 1994;**344**:1360-61.

Rijpstra AC, Laarman JJ. Reported findings of unidentified small Isospora-like coccidia in faecal specimens from travellers returning to The Netherlands. *Trop and Geograph Med* 1993;**45**:280-282.

Sears CL. Cyclospora Species. In: Principles and Practice of Infectious Diseases. Eds Mandell GL, Bennett JE, Dolin R. New York. 1995:2512-13.

Shlim DR, Cohen MT, Eaton M, Rajah R, Long EG, Ungar BLP. An alga-like organism associated with an outbreak of prolonged diarrhea among foreigners in Nepal. *Am J Trop Med Hyg* 1991;**45**:383-389.

Soave R, Dubey JP, Ramos LJ, Tummings M. A new intestinal pathogen? *Clinical Research* 1986;**34**:No.2.

Soave R, Johnson WD. *Cyclospora*:conquest of an emerging pathogen. *Lancet* 1995;**345**:667-68.

Wurtz RM, Kocka FF, Peters CS, Weldon-Linne CM, Kuritza A, Yungbluth P. Clinical characteristics of seven cases of diarrhea associated with a novel acid-fast organism in the stool. *Clin Inf Dis* 1993;**16**:136-8.

5.3 Other Protozoa of Interest

The following descriptions are of protozoa which have been shown to be transmissible by the water route but the significance of these in the UK is currently unknown.

5.3.1. Toxoplasma

There are two reports of Toxoplasma being transmitted by the water route by means of oocysts (Benenson *et al*, 1983;Anon 1995). Toxoplasma requires two hosts for development, the oocysts (10-12μm) arising from the sexual stage of development in members of the cat family and which require a period of extrinsic sporulation in the environment. The tissue cysts which develop in the infected secondary host (eg man) gives rise to toxoplasmosis which can be benign or cause a glandular fever-like illness, but may cause serious illness (including malformation) in the foetus if the mother is infected; it is also known to cause serious problems in AIDS patients but some of this may be the result of reactivation of earlier infection. Infection may arise from ingestion of oocysts from cat faeces contaminating fingers, oocyst contaminated food or water; infection may also arise from ingestion of raw meat containing tissue stages (Dubey. 1988; Casemore 1990). The first of the above mentioned reports describes infection in US service men arising from consumption of raw water contaminated with faeces from a jungle cat. The second incident was a community-wide outbreak, with 110 identified cases, involving a municipal drinking water supply in British Columbia, Canada. The infection was believed to have been derived through contamination of a reservoir by feral cats or possibly wild cats.

Nothing is known of this route of transmission in the UK.

References

Anon. Outbreak of toxoplasmosis associated with municipal drinking water - British Columbia. *Canada Communicable Disease Report* 1995;21-18:F--3

Benenson MW, Takafuji ET, Lemon SM, Greenup RL, Sulzer AJ. Oocyst-transmitted toxoplasmosis associated with ingestion of contaminated water. N Eng J Med 1983;**307**:666-9.

Casemore DP. Foodborne protozoal infection. *The Lancet* 1990;336:1427-32.

Dubey JP. Toxoplasmosis of animals and man. CRC Press, Boca Raton, 1988.

5.3.2 Neospora

Neospora is a parasite, apparently related to Toxoplasma, for which it is easily mistaken. It is associated with dogs and cattle in which host it causes abortion (Dubey 1992; Dubey & Lindsay 1993; Trees *et al*,1993. Nothing is currently known about human infection with this parasite. The definitive (oocyst producing) host is unknown and the full life cycle, including identification of the oocyst stage has not yet been described. There is some evidence of transmission to cattle in the UK by the (raw) water route (JP Duff, personal communication). The parasite is currently an emerging problem for veterinary workers. Further work is needed to investigate whether there is evidence of this infection in the human population.

References

Dubey JP. A review of *Neospora caninum* and *Neospora*-like infections in animals. *J Protozool Res* 1992;**2**:40-52.

Dubey JP & Lindsay DS. Neosporosis. *Parasitology Today* 1993;**9**:452-8.

Trees AJ, Guy BJ, Balfour AH, Dubey JP. Prevalence of antibodies to *Neospora caninum* in a population of urban dogs in England. *Vet Record* 1993;**132**:125-6.

Dannatt L, Guy F, Trees AJ. Abortion due to *Neospora* species in a dairy herd. *Vet Record* 1995;137:566-7.

Section 6

FREE-LIVING AMOEBAE THAT HAVE BEEN IMPLICATED AS POTENTIAL EMERGING PATHOGENS

Dr Simon Kilvington
Public Health Laboratory, Royal United Hospital, Combe Park, Bath BA1 3NG

6.1 Introduction

Free-living amoebae (FLA) are unicellular protozoa common to most soil and aquatic environments (Page, 1988). Of the many hundreds of FLA species, only *Acanthamoeba* spp., *Balamuthia mandrillaris* and *Naegleria fowleri* are known to infect humans, sometimes fatally. *Acanthamoeba* spp. are the most significant pathogens of man causing a potentially blinding infection of the cornea (keratitis) mainly in contact lens wearers (Stehr-Green *et al.*, 1989; Kilvington & White, 1994). In the United Kingdom, the incidence of *Acanthamoeba* keratitis has risen significantly in recent years with approximately 60 cases reported in 1995 compared to 3 in 1989. *B. mandrillaris* is a newly recognised human pathogenic FLA first described in 1990 (Visvesvara *et al.*, 1993). The organism causes an invariably fatal encephalitis (infection of the brain) in the severely immunocompromised host. Approximately 60 cases have been diagnosed world-wide although to date there have been none in the United Kingdom. *N. fowleri* causes fatal primary amoebic meningoencephalitis (PAM), an infection of the brain and meninges, in previously healthy persons (Cain *et al.* 1981; John, 1982). Some 250 cases have occurred world-wide including one confirmed infection in England (Kilvington *et al.* 1991).

Accordingly, of the known pathogenic FLA, only *Acanthamoeba* can be described as a new emerging pathogen in this country. Evidence will be presented to show that tap water outlets in the homes of contact lens wearers can harbour the organism and this is the likely source of infection in such cases. Whilst no cases of *B. mandrillaris* infection have been reported in the United Kingdom, the organism is a newly recognised pathogen and cases may have passed unrecognised. *B. mandrillaris* has yet to be isolated from the environment and tap water as a source of infection has yet to be excluded. *N. fowleri* is only found in thermal water and most cases of human infection are associated with swimming in contaminated water. The exception being cases from South Western Australia where infections have been traced to the water supply (Robinson, *et al*,1996)

6.1.1. References

Cain, A.R.R., Wiley, P.F., Brownwell, B. and Warhurst, D.C. Primary amoebic meningoencephalitis. *Archives of Diseases in Childhood.* 56: 140-143 1981.

John, D.T. Primary amoebic meningoencephalitis and the biology *of Naegleria fowleri. Annual Review of Microbiology.* 36: 101-103, 1982.

Kilvington, S., Mann, P.G. and Warhurst, D.C. Pathogenic *Naegleria* amoebae in the waters of Bath: a fatality and its consequence. *Hot Springs of Bath.* Kellaway, G.A. (ed.). 89-96. 1991.

Kilvington, S. and White, D. G. *Acanthamoeba*: biology, ecology and human disease. *Reviews in Medical Microbiology.* 5: 12-20. 1994.

Page, F.C. A new key to freshwater and soil gymnamoebae. The Freshwater Biological Association, The Ferry House, Ambleside, Cumbria, England. 1988.

Robinson, B., Dobson, P., Christy, P. and Hayes, S. Spatial and seasonal distributions of *Naegleria fowleri* in Australian water supplies. 7th International Conference On Small Freeliving Amoebae. Women's And Children's Hospital, Adelaide, South Australia. 7-12 January, 1996.

Stehr-Green, J.K., Bailey, T.M. and Visvesvara, G.S. The epidemiology of *Acanthamoeba* keratitis in the United States. *American Journal of Ophthalmology.* 107: 331-336, 1989.

Visvesvara, G.S., Schuster, F.L. and Martinez, J. *Balamuthia mandrillaris*, N. G., N. Sp., agent of meningoencephalitis in humans and other animals. *Journal of Eukaryotic Microbiology.* 40, 504-514, 1993.

6.2 *Acanthamoeba*

Acanthamoeba spp. are characterised by a feeding and dividing trophozoite and dormant cyst stage (Page, 1988). The resistance of *Acanthamoeba* cysts to extremes of temperature, desiccation and disinfection accounts for the almost ubiquitous distribution of the organism in the environment (Page, 1988). *Acanthamoeba* has been isolated from soil, natural aquatic habitats, chlorinated bathing pools, domestic tap water and the atmosphere (Kilvington & White, 1994).

The taxonomic classification of the *Acanthamoeba* is derived from observations of the trophozoite and cyst morphology (Page, 1967 & 1988). The most detailed study to date being that of Pussard and Pons (1977) who concluded that 18 species existed which could be assigned to three distinct morphological groups labelled I-III. Detailed descriptions of the

morphological groups and the species within are given by Pussard and Pons (1977) and Page (1967 & 1988). Briefly, in group I the cysts are large ranging from 18-30 μm in diameter. The inner endocyst and outer ectocyst are widely separated and arms or rays are formed where the two meet. At the ends of the arms pores, or ostioles, occur that are closed by an operculum. In group II the cysts are smaller being approximately 12-18 μm and the endocyst and ectocyst are more closely associated. The endocyst can vary from stellate to polygonal and the ectocyst may appear wrinkled. A single ostiole is present and the operculum is in a depression formed by the infolding of the ectocyst. Members of group III have the smallest cysts with mean values of 11-18 μm. The ectocyst and endocyst are close together, giving the cysts a smooth and rounded appearance.

The general appearance of the trophozoites and cysts readily defines the genus *Acanthamoeba* and allows species to be separated into one of the three morphological groups. On this basis, *A. castellanii*, *A.hatchetti*, *A. lugdunensis*, *A. polyphaga* and *A. rhysodes* of morphological group II, and *A. culbertsoni* of group III have been identified as causing keratitis. However, the large variation in cyst morphology that can occur even within clonal populations makes the differentiation of species within the groups a difficult and subjective process. The problem in identifying *Acanthamoeba* species by morphological criteria has prompted the examination of biochemical and molecular techniques for this purpose. The detection of mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs) has demonstrated both inter and intra-specific differences between strains assigned to separate species (Kilvington *et al*, 1992; Kilvington & Beeching, 1996). Such studies have highlighted the current inadequacies of the present taxonomic classification of the *Acanthamoeba*. Furthermore, while improvements in diagnosis and treatment have greatly improved the prognosis for acanthamoeba keratitis (Larkin *et al*, 1992; Elder *et al*, 1994) the identification of species causing this increasingly reported disease is unclear.

Acanthamoeba cause two distinct forms of disease in man, granulomatous amoebic encephalitis (GAE) and acanthamoeba keratitis (infection of the cornea). GAE is a rare and invariably fatal infection of the central nervous system (Martinez, 1991). Since the disease was first recognised in 1971, approximately 100 cases have been reported world-wide. Patients are usually immunosupressed either from chemotherapy, alcohol abuse or other chronic

disease (Martinez, 1991). GAE as the primary cause of death in patients with acquired immunodeficiency syndrome (AIDS) has also been reported (Visvesvara & Stehr-Green, 1990; Martinez, 1991). A single case of GAE has been reported in the United Kingdom in a patient receiving immunosuppresive therapy for Hodgkin's Disease (Jager & Stamm, 1972). The route of infection in GAE is unclear. It has been suggested that invasion of the brain results from haematogenous spread from a primary infection elsewhere in the body (Warhurst, 1985).

In contrast to GAE, acanthamoeba keratitis effects previously healthy persons and occurs with far greater frequency. The disease is one of the most challenging ocular infections to manage successfully. Untreated infections can result in permanently impaired vision or blindness. The first case of acanthamoeba keratitis was diagnosed in 1973 in a Texan farmer with a history of ocular trauma from straw fragments and rinsing the affected eye with tap water (Jones *et al.*, 1975). Two further cases from the United Kingdom were described around the same time (Nagington *et al.* 1974). Occasional reports of the disease followed and acanthamoeba keratitis was considered an extremely rare opportunistic infection arising from accidental injury to the eye (Ma *et al.* 1981). However, from 1985 a significant increase in the incidence of the disease was recognised particularly amongst contact lens wearers (Moore *et al.*, 1985; Moore *et al.* 1987). Stehr-Green and colleagues (1989) examined the number of reported cases of acanthamoeba keratitis in the USA from 1973 until the first-half of 1988 and identified 208 cases, 157 (75%) of which occurred from 1985. Of 189 cases where details were available, 160 (85%) wore contact lenses, predominantly daily-wear or extended-wear soft lenses.

A major risk factor identified in the study was the use of home-made saline rinsing solutions in the care of the contact lenses. Salt tablets and distilled water were widely sold in the USA as an alternative to the more costly sterile aerosol saline solutions for the care of lenses. As a consequence *Acanthamoeba* were either present in the distilled water bottles or subsequently contaminated the prepared saline solution, possibly from exposure to the atmosphere. *Acanthamoeba* cysts have been isolated from the air (Kingston & Warhurst, 1969). A marked reduction in the incidence of acanthamoeba keratitis has occurred since the sale of salt tablets and distilled water for contact lens care was been banned following the study (personal communication Dr Govinda Visvesvara, Centers for Disease Control, Atlanta, USA).

6.2.1. Acanthamoeba keratitis in the United Kingdom

A similar survey has not been undertaken in the United Kingdom. However, the incidence of acanthamoeba keratitis in this country has risen dramatically in recent years as judged by the number of isolates referred to the Public Health Laboratory Service (PHLS) reference unit at Bath, as shown in the accompanying graph. These figures undoubtedly under represent the true incidence of the disease as it is known that at least ten cases have occurred in Scotland and three in Northern Ireland. In addition, many larger centres for microbiology and ophthalmology now feel confident enough not to seek assistance in the isolation or identification of the organism. As a result some cases go unreported. The number of cases rose from 3 in 1989 to 64 in 1994 and 60 in 1995. In accord with the findings from the USA, the numbers of cases in which the patients were contact lens wearers ranged between 80% to 90%.

The figures presented in the graph show that the incidence of acanthamoeba keratitis in the United Kingdom has risen steadily. This cannot be attributed solely to increased awareness of the disease among clinicians and microbiologists. Approximately 50% of all keratitis isolates received at the Bath PHLS come from Moorfields Eye Hospital, London. This centre of excellence for the diagnosis and treatment of acanthamoeba keratitis has been actively seeking the disease since the mid 1980's. They now diagnose approximately forty new cases per year compared with ten in 1990.

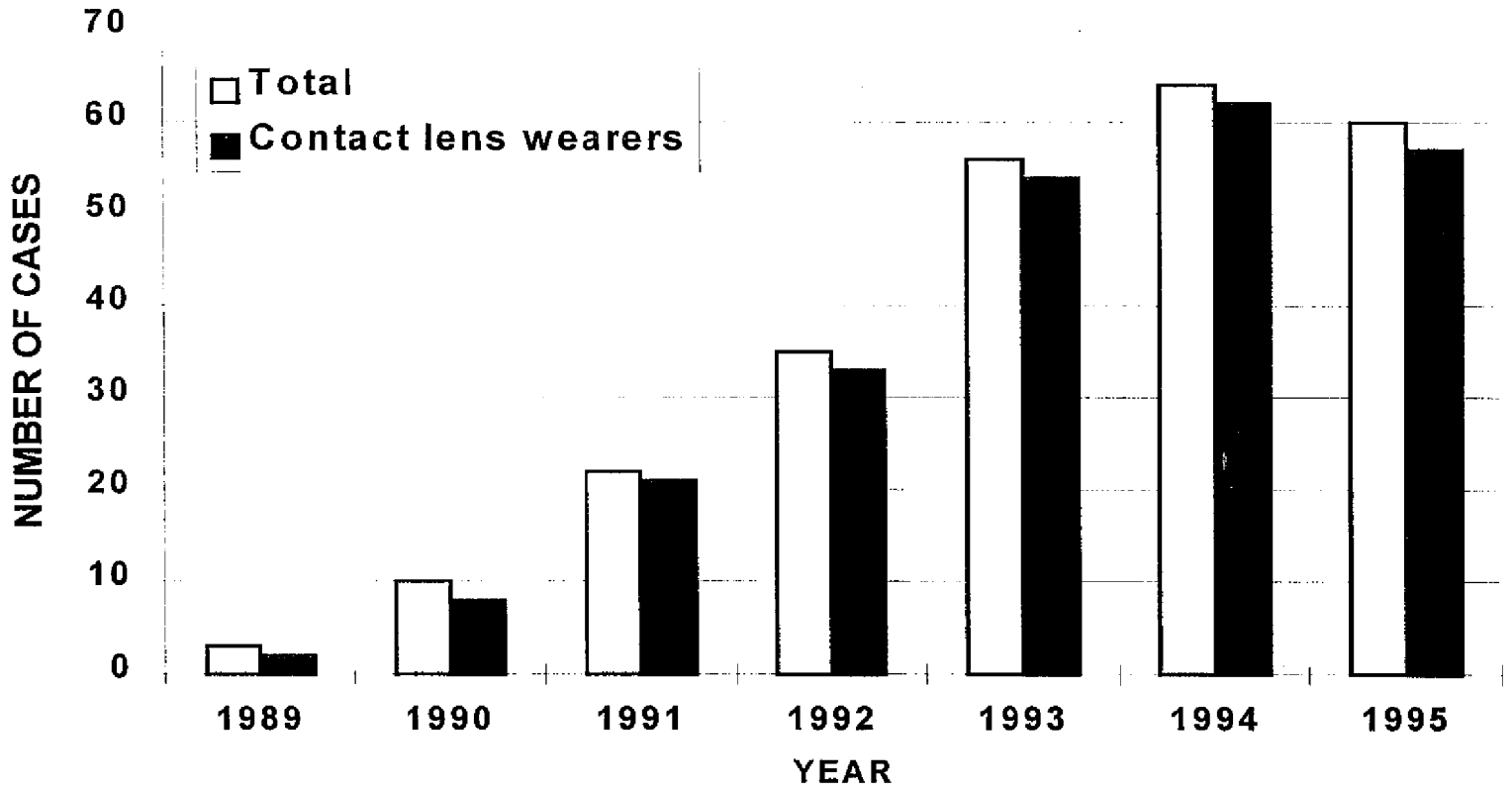


Figure 1. Incidence of acanthamoeba keratitis in the United Kingdom

6.2.1.1. Risk to contact lens wearers of acanthamoeba keratitis

The mechanism by which acanthamoeba keratitis arises in contact lens wearers has yet to be fully elucidated. The wearing of contact lenses produce alterations in the integrity of the corneal epithelium and this may facilitate *Acanthamoeba* attachment and invasion. Alternatively, minor trauma to surface epithelium as a result of debris between the contact lens and cornea may result in infection (Kilvington & White, 1994). The mechanism by which *Acanthamoeba* produce disease once they have invaded the cornea is also unknown. Although pathogenic *Acanthamoeba* will kill mice when inoculated intranasally or intracerebrally, no animal model has successfully reproduced the keratitis seen in human infections (Larkin & Easty, 1990). *Acanthamoeba* destruction of tissue culture cells has been considered to be by direct phagocytic action although recent studies have characterised an extracellular neuraminidase released by pathogenic strains which may be relevant to the damage of corneal epithelium which is rich in sialic acid (Pellegrin *et al,* 1991).

What is more fully understood is the process by which *Acanthamoeba* gain access to the eye in contact lens wearers. Several studies have shown that the storage cases of asymptomatic contact lens wearers can be grossly contaminated with bacteria, fungi and protozoa (Dart, 1990; Grey *et al,* 1995). Microbiological examination of one hundred and two contact lens storage containers from asymptomatic wearers showed 43% to contain greater than a million bacteria per ml of fluid and seven contained *Acanthamoeba* (Larkin *et al,* 1990). Other studies have confirmed the wide spread gross microbial contamination of storage cases with an *Acanthamoeba* presence of approximately 10%. *Acanthamoeba* have been shown to adhere to a variety of contact lens types, with attachment greatest to higher water content lenses (John *et al,* 1989; Kilvington & Larkin, 1990). Indeed, observing *Acanthamoeba* adhering to the contact lens surface can provide a rapid, provisional diagnosis in suspected keratitis patients (Kilvington & Larkin, 1990: Johns *et al,* 1991). Thus, *Acanthamoeba* contaminate the contact lens storage case, feed and multiply on the associated microbes, adhere to the contact lens and are then inoculated on to the cornea.

6.2.1.2 Role of domestic tap water in acanthamoeba keratitis

Acanthamoeba cysts are highly resistant. They can withstand drying, 50 ppm free chlorine (public bathing pool concentrations are usually 1-2 ppm chlorine and drinking water <1 ppm) and moist heat at 56°C (Kilvington & Price, 1990). This accounts for the presence of the organism in virtually all natural and man-made aquatic habitats.

The Bath PHLS amoeba unit has focused attention on the role of domestic tap water as a source of acanthamoeba keratitis. Although the presence of *Acanthamoeba* in tap water of contact lens keratitis patients has been reported (Seal *et al,* 1992), the common occurrence of the organism in the environment could only circumstantially implicate this as a possible source of infection. The first clear indication of tap water as the source of acanthamoeba keratitis in the United Kingdom was made by workers in 1990 (Kilvington *et al.*., 1990). Using DNA fingerprinting (Kilvington, *et al,* 1991), *Acanthamoeba* isolates from a patient's cornea, contact lens storage case and kitchen cold water tap (used to prepare a home-made saline rinsing solution for the lenses) were found to be identical and implicated the tap water supply as the source of infection.

DNA fingerprinting analysis has been used also to identify bathroom cold tap water as the source of contact lens related acanthamoeba keratitis in a patient from Belfast, Northern Ireland (manuscript in preparation). Although the patient used sterile aerosol saline, she admitted to occasionally rinsing her lenses in tap water.

More recently, the PHLS unit has been involved in a retrospective survey of domestic tap water in the homes of acanthamoeba keratitis patients diagnosed at Moorfields Eye Hospital in 1995. Free-living amoebae were isolated from tap water in the homes of 19/22 patients studied. In this patient community, only the kitchen cold taps are supplied with fresh mains water, all other taps are fed from attic holding tanks. The number of isolates and amount of amoebae grown from these two sources differed significantly: 12 isolates from the kitchen cold taps vs. 26 isolates from bathroom cold taps. *Acanthamoeba* contamination was identified in 6 separate households of which 4

were found to be identical in DNA type to the patient corneal isolate. In all cases the *Acanthamoeba* were isolated from cold water taps supplied by the attic holding tanks. The presence of cold water storage tanks for the supply of bathroom water is traditional in most of the United Kingdom. UK by-law requirements for the protection of stored water have been progressively enhanced. Since 1987 by-laws have required that household cold water storage tanks are fitted with a close fitting lid which is securely fixed and excludes light. They also require that any vents are fitted with an appropriate mesh screen. Good practice is to ensure that any accumulation of sludge in the bottom (particularly where water is drained from the side of the tank) is removed periodically. There is no guarantee that tanks are maintained in this way in summer months the water roof tanks can become sufficiently warm to provide an ideal environment for microbes, including *Acanthamoeba*, to multiply.

It should be noted that this was a retrospective study and the presence of *Acanthamoeba* in the tap water outlets of other patients may have been transient and thus not present at the time of sampling. In addition, large numbers of other faster growing amoebae present in the samples may have suppressed the *Acanthamoeba* so resulting in an underestimation of the true level of contamination. A further study is needed to examine longitudinally the domestic tap water of all new cases of acanthamoeba keratitis as soon as a clinical diagnosis is made. Such a study should examine water from the mains and attic tanks.

6.2.2. Isolation and identification methods for *Acanthamoeba*

Unlike other known protozoal pathogens of man, *Acanthamoeba* can be easily cultured from environmental samples, including drinking water, by inoculating material on to non-nutrient agar plates seeded with a lawn of *Escherichia coli* (Kilvington *et al.* 1990). Methods for the concentration of water samples by filtration or centrifugation for the isolation of amoebae are detailed in Anon (1990). *Acanthamoeba* are easily recognised by the morphological characteristics of the trophozoite and cyst form. This also enables separation into one of the three morphological groups discussed in the beginning of section 6.2. Identification of

species requires molecular techniques such as mtDNA RFLP analysis that are not available in most routine microbiology laboratories. However, any environmental *Acanthamoeba* strain capable of growth at 37°C should be considered pathogenic.

Acanthamoeba keratitis is diagnosed by culturing scrapings from the cornea on non-nutrient agar plates seeded *E. coli*. Antibodies to *Acanthamoeba* are not produced in keratitis cases and cannot therefore be used for the diagnosis of the disease.
The development of an isolation medium that inhibits the faster growing non-pathogenic amoebae, thus allowing for the selective growth of *Acanthamoeba* would greatly facilitate additional studies on the role of domestic tap water in acanthamoeba keratitis. So far efforts to develop such a medium or to identify selective inhibitors have been unsuccessful.

6.2.3. Conclusions

i. Acanthamoeba keratitis is a potentially blinding infection most commonly occurring in contact lens wearers.

ii. The incidence of the disease in the United Kingdom has risen significantly in recent years. The number of reported cases for 1995 far exceeds the combined total for the rest of Europe.

iii. The reasons for this are unclear but research at the Bath PHLS and Moorfield's Eye Hospital indicate that bathroom cold water supplied from water holding tanks in the attic are a source of the infection.

6.2.4. Summary: acanthamoeba keratitis

Organism:	*Acanthamoeba* spp.
Biology:	Feeding and replicating trophozoite that forms a dormant, resistant cyst.

Resistance:	Cysts highly resistant to desiccation, disinfection (50 ppm chlorine) and extremes of temperature (freezing and 56°C).
Ecology:	*Acanthamoeba* is common in most soil and water habitats. Studies to date have implicated bathroom cold tap water supplied from roof storage tanks as the source of infection in some cases of keratitis.
Disease:	Acanthamoeba keratitis.
Diagnosis:	Culture of corneal scrapings on non-nutrient agar seeded with *E. coli*.
Treatment:	Intensive topical application of 0.02% polyhexamethylene biguanide usually in combination with 0.1% propamidine isethionate. Corneal grafting usually required.
Incidence:	Number of diagnosed cases has risen from three in 1989 to at least sixty in 1995. Contact lens wearers account for 80-90% of reported cases.
Isolation and identification from the environment:	Adequate methods available for isolation from water and soil. Development of a selective growth medium would aid surveys. Improved methods for species identification required.
Emerging pathogen status:	Significant.

6.2.5. Recommendations

i. Continued studies are required to determine the incidence and source of *Acanthamoeba* in domestic tap water.

ii. DNA typing of acanthamoeba keratitis isolates should be routinely undertaken for epidemiological surveillance.

iii. Tap water investigations at the homes of all new keratitis patients should be performed and isolates compared by DNA analysis to confirm source of infection.

The development of a selective medium for the isolation of *Acanthamoeba* from the environment would greatly aid such studies.

6.2.6 References

Anon. Isolation and identification of *Giardia* cysts and *Cryptosporidia* oocysts and free-living pathogenic amoebae in water 1989. HMSO, London.1990.

Dart, J. Contamination of contact lens storage cases. *British Journal of Ophthalmology*, 74: 129. 1990.

Elder, M.J., Kilvington, S. and Dart, J.K.G. A clinicopathological study of in-vitro sensitivity testing and *Acanthamoeba* keratitis. *Investigative Ophthalmology and Visual Sci*ence. 35:1059-1064, 1994.

Gray, T. B. Cursons T. M. Sherwan J. F. and Rose P. R. *Acanthamoeba*, bacterial, and fungal contamination of contact lens storage cases. *British Journal of Ophthalmology*, 79: 601-605, 1995.

Jager, B. V. and Stamm W. P. Brain abscess caused by free-living amoeba probably of the genus *Hartmannella* in a patient with Hodgkin's disease. *Lancet*, ii: 1343-1345, 1972.

John, T., Desai, D. and Sahm, D. Adherence of *Acanthamoeba castellanii* cysts and trophozoites to unworn soft contact lenses. *American Journal of Ophthalmology*. 108: 658-664, 1989.

Johns, K. J., Head, S.E., Robinson, R.D., Williams, T.E. and O'Day, D.M. Examination of the contact lens with light microscopy: an aid to diagnosis of *Acanthamoeba* keratitis. *Reviews of Infectious Diseases*. 3: 425, 1991.

Jones, D.B., Visvesvara, G.S. and Robinson, N. M. *Acanthamoeba polyphaga* keratitis and Acanthamoeba uveitis associated with a fatal meningoencephalitis. *Transactions of the Ophthalmological Societies UK*. 95: 221-232, 1975.

Kilvington, S. Moist-heat disinfection of pathogenic *Acanthamoeba* cysts. *Letters in Applied Microbiology*. 9: 187-189, 1989.

Kilvington, S. and Larkin, D.F.P. *Acanthamoeba* adherence to contact lenses and removal by cleaning agents. *Eye*. 4: 589-593, 1990.

Kilvington, S. and Price, J. Survival of *Legionella pneumophila* with *Acanthamoeba polyphaga* cysts following chlorine exposure. *Journal of Applied Bacteriology*. 68: 519-525, 1990.

Kilvington, S. and White, D. G. *Acanthamoeba*: biology, ecology and human disease. *Reviews in Medical Microbiology*. 5: 12-20. 1994.

Kilvington, S., Beeching, J.R. and White, D.G. Differentiation of *Acanthamoeba* strains from infected corneas and the environment by using restriction endonuclease digestion of whole-cell DNA. *Journal of Clinical Microbiology*. 29: 310-314, 1991

Kilvington, S., Larkin, D.F.P., White, D.G. and Beeching, J.R. Laboratory investigation of *Acanthamoeba* keratitis. *Journal of Clinical Microbiology*. 28: 2722-2725, 1990.

Kilvington, S. and Beeching, J.R. Taxonomic investigation of the genus *Acanthamoeba* using mitochondrial DNA restriction fragment length polymorphisms. *The Journal of Eukaryotic Microbiology* (in press).

Kingston, D. and Warhurst, D.C. Isolation of amoebae from the air. *Journal of Medical Microbiology*. 2: 27-36, 1969.

Larkin, D.F.P., Kilvington, S. and Easty, D.L. Contamination of contact lens storage cases by *Acanthamoeba* and bacteria. *British Journal of Ophthalmology*. 74:133-135, 1990.

Larkin, D.F.P. and Easty, D.L. Experimental *Acanthamoeba* keratitis: I. Preliminary findings. *British Journal of Ophthalmology*. 74: 551-555, 1990.

Larkin, D.F.P., Kilvington, S. and Dart, J.K.G. Treatment of *Acanthamoeba* keratitis with polyhexamethylene biguanide. *Ophthalmology*. 99: 185-191, 1992.

Ma, P., Willaert, E., Juechter, K.B. and Stevens, A.R. A case of keratitis due to *Acanthamoeba* in New York, and features of 10 cases. *Journal of Infect Disease*. 143: 662-667, 1981.

Martinez, A.J. Infections of the central nervous system due to *Acanthamoeba*. *Reviews of Infectious Diseases*. 13: S399-402, 1991.

Moore, M.B., McCulley, J.P., Luckenbach, M., Gelender, H., Newton, C., McDonald, M.B and Visvesvara, G.S. *Acanthamoeba* keratitis associated with soft contact lenses. *American Journal of Ophthalmology*. 100: 396-403, 1985.

Moore, M.B., McCulley, J.P., Newton, C., Cobo, L.M., Foulks, G.N., O'Day, D.M., Johns, K.J., Driebe, W.T., Wilson, L.A., Epstein, R.J. and Doughman, D.J. *Acanthamoeba* keratitis: a growing problem in soft and hard contact lens wearers. *Ophthalmology*. 94: 1654-61, 1987.

Nagington, J., Watson, P.G., Playfair, T.J., McGill, J., Jones, B.R. and Steele, A.D. Amoebic infections of the eye. *Lancet*. ii: 1537-1540, 1974.

Page, F.C. Re-definition of the genus *Acanthamoeba* with descriptions of three species. *Journal of Protozoology*. 14: 709-724, 1967.

Page, F.C. A new key to freshwater and soil gymnamoebae. The Freshwater Biological Association, The Ferry House, Ambleside, Cumbria, England. 1988.

Pellegrin, J-l. J., Ortega-Barria, E., Barza, M., Baum, J. and Pereira, M.E.A. Neuraminidase activity in *Acanthamoeba* species trophozoites and cysts. *Investigative Ophthalmology and Visual Science.* 32: 3061-3066, 1991.

Pussard, M. and Pons, R. Morphologie de la paroi kystique et taxonomie du genre *Acanthamoeba* (Protozoa, Amoebida). *Protistologica.* 13: 557-598. 1977.

Seal, D., Stapleton, F. and Dart, J. Possible environmental sources of *Acanthamoeba* spp. in contact lens wearers. *British Journal of Ophthalmology.* 76: 424-427. 1992.

Stehr-Green, J.K., Bailey, T.M. and Visvesvara, G.S. The epidemiology of *Acanthamoeba* keratitis in the United States. *American Journal of Ophthalmology.* 107: 331-336, 1989.

Visvesvara, G.S. and Stehr-Green, J.K. Epidemiology of free-living amoeba infections. *Journal of Protozoology.* 37: 25S-33S, 1990.

Warhurst, D.C. Pathogenic free-living amoebae. *Parasitology Today.* 1: 24-28, 1985.

6.3. *Balamuthia mandrillaris*

It has recently been reported that *Acanthamoeba* spp. and *N. fowleri* are not the only FLA pathogenic to man. In 1990, Visvesvara and colleagues (1990) described cases of fatal encephalitis in man and primates due to a previously undescribed FLA. By morphological appearance the amoebae resembled members of the genus *Leptomyxa,* however on closer examination they were found to be sufficiently distinct to be described as a new genus and species. *Balamuthia mandrillaris* (Visvesvara *et al,* 1993). Using antiserum to the organism they were able to demonstrate that certain cases of GAE attributed to *Acanthamoeba* were in fact caused by *B. mandrillaris.*

B. mandrillaris trophozoites range from 12-60 μm, with a mean of about 30 μm, are pleomorphic and highly branched. The cysts range from 6-30 μm, with a mean of 15 μm, are round or oval and have a tripartite wall consisting of a loose ectocyst enclosing a mesocyst and endocyst. The taxonomic status of the amoebae is uncertain at the present time but appears most similar to members of the genus *Leptomyxa* (Page, 1988; Visvesvara *et al.* 1990).

Like *Acanthamoeba* GAE, *B. mandrillaris* meningoencephalitis is a disease of the immunocompromised host (Visvesvara *et al.* 1990 & 1993). However, two recent cases

from Australia have occurred in young children with no apparent immunosupression (presented at the 7th International Conference On Small Freeliving Amoebae. Women's And Children's Hospital, Adelaide, South Australia. 7-12 January, 1996). The clinical course of the disease in man ranges from 14 days to 6 months with a mean of 75 days. Infection is invariably fatal. Clinical symptoms and histopathological findings are similar to those seen in GAE. At present there are no reports of *in vitro* or *in vivo* antimicrobial activity against this organism. Approximately 60 cases of *B. mandrillaris* meningoencephalitis have been described world-wide with over 50% coming from the USA. Other cases have been identified from Argentina, Australia, Canada, Japan, Mexico and Peru (Visvesvara *et al,* 1990; Martinez & Visvesvara, 1996).

6.3.1. Isolation and identification methods for *B. mandrillaris*

B. mandrillaris has been cultured from only a few cases of infection (Visvesvara *et al.* 1990 & 1993) and most are diagnosed post mortem using specific immunohistological staining of brain material. *B. mandrillaris* produces fatal encephalitis in mice 8-10 days after intraperitoneal inoculation (Visvesvara *et al.*. 1993). Unlike *N. fowleri* and *Acanthamoeba*, the organism does not grow on plain agar seeded with living *E. coli*. It can be cultured on mammalian tissue culture cell lines and once growing can be adapted to a semi-defined broth medium. Difficulties in growing the organism may account for their being no reports of *B. mandrillaris* in water or other environmental samples.

6.3.2. Conclusions

i. *B. mandrillaris* encephalitis is a new disease, the incidence of which is still unknown.

ii. Because the organism can not be grown on standard media used for isolating other pathogenic free-living amoebae, the environmental habitat and probable sources of infection are unknown.

iii. Cases so far identified show a world-wide distribution and is likely that recognized infections will occur in the United Kingdom.

6.3.3. Summary: *B. mandrillaris* encephalitis

Organism:	*Balamuthia mandrillaris.*
Biology:	Feeding and replicating trophozoites that form cysts.
Resistance:	Unknown but the cysts, like those of other FLA, are likely to be resistant to disinfection and desiccation.
Ecology:	Unknown, the organism has yet to be isolated from the environment.
Disease:	Amoebic encephalitis.
Diagnosis:	Immunofluorescent antibody staining of brain biopsy material or culture with mammalian cell lines.
Treatment:	Unknown. No survivors of the disease exist.
Incidence:	Approximately 60 cases world-wide, none from the United Kingdom. The number of cases now being reported is rising due to increased awareness of the organism among clinicians and microbiologists. However, the disease would appear to be rare and confined mainly to the severely immunocompromised host.
Isolation and identification from the environment:	Need to develop methods for isolating organism from the environment.
Emerging pathogen status:	Yes. However, cases of human infection are rare.

6.3.4 Recommendations

i. Strains of *B. mandrillaris* should be obtained so that microbiologists in the United Kingdom can become familiar with the morphological and cultural properties of the amoeba.

ii. Antiserum to *B. mandrillaris* should be produced and made available for use in diagnostic procedures should cases of human infection be suspected in this country.

iii. Research should be undertaken to establish whether techniques can be developed that would enable the isolation of the *B. mandrillaris* from the environment. This would either be through the development of a suitable culture medium or the use of DNA technology that could detect the presence of the organism without the need for culture isolation.

6.3.5 References

1. Visvesvara, G.S. Martinez, A.J., Schuster, F.L., Leitch, G.J., Wallace, S.V., Sawyer T.K. and Anderson, M. Leptomyxid ameba, a new agent of amebic meningoencephalitis in humans and animals. *Journal of Clinical Microbiology*, 28: 2750-2756, 1990.

2. Visvesvara, G.S., Schuster, F.L. and Martinez, J. *Balamuthia mandrillaris*, N. G., N. Sp., agent of meningoencephalitis in humans and other animals. *Journal of Eukaryotic Microbiology*. 40, 504-514, 1993.

3. Visvesvara, G.S., Schuster, F.L. and Martinez, J. Free-living amebas and immunodeficiencies. 7th International Conference On Small Freeliving Amoebae. Women's And Children's Hospital, Adelaide, South Australia. 7-12 January, 1996.

6.4. Naegleria fowleri

N. fowleri causes primary amoebic meningoencephalitis (PAM) in humans (John, 1982). PAM results from the instillation of *N. fowleri* into the nasal passages, usually whilst bathing. Young males are most at risk from infection probably because of their more vigorous bathing habits. From the anterior nares the organism invades the nasal epithelium and migrates along the olfactory lobes, via the cribriform plate, to infect the

brain and meninges. PAM is usually fatal with death occurring in 3-10 days after exposure. Clinical symptoms and microscopic appearance of the cerebro-spinal fluid (CSF) are typical of bacterial meningitis (Cain *et al*, 1981). Differential diagnosis is made by observing motile amoebal trophozoites in a warmed wet mount preparation of freshly taken CSF. Prompt diagnosis and intensive therapy with the anti-fungal agent amphotericin B has resulted in the survival of four PAM cases (Brown, 1991).

N. fowleri is characterised by a life-cycle of trophozoite, cyst and flagellate stage (John, 1982). The trophozoites are approximately 10-20 μm in length and exhibit a limax or slug-like mobility by protrusion of distinct hyaline pseudopodia. The cysts are round, double walled and vary in size from approximately 7 to 18 μm. The inner and outer cyst wall are parallel and occasional pores, through which the trophozoite excysts, join the walls. Flagellates are formed from the trophozoites. These appear pear shaped with typically two flagella of equal length which give rise to a cork-screw pattern of swimming. The flagellates stage is temporary and non-feeding or replicating.

N. fowleri is found in thermal aquatic environments and can tolerate temperatures up to 45°C. Although *N. fowleri* is most likely to be isolated from sites where the temperature is above 30°C, the cysts can survive at 4°C for at least 12 months with retention of virulence by the excysted trophozoites (Warhurst, 1985). *N. fowleri* occurs world-wide and has been isolated from both natural and artificial thermally enriched habitats such as natural hot springs, fresh water lakes, domestic water supplies, chlorinated swimming pools, water cooling towers and effluent from industrial processes.

Since PAM was first recognised in 1965, at least 250 cases of PAM have been reported world-wide. Clustering of cases can occur when a single site is the source of infection. In Usti, Czechoslovakia, 16 cases were associated with a public swimming pool (Cerva & Novak, 1968). The source of the contamination was eventually traced to a cavity behind a false wall used to shorten the pool length. The pool took water from a local river which was the likely source of the organism.

Only in South Western Australia have infections been associated with the reticulated mains supply water. In this region water is supplied to remote localities via over-ground steel pipes. Solar heating of the water in the system enabled *N. fowleri* to proliferate and resulted in approximately 20 cases of PAM. The instillation of chlorifiers at regular intervals along the pipe-lines and regular monitoring of the supply has now eliminated the problem (Robinson *et al*, 1996).

One confirmed case of PAM occurred in Bath Spa, England in 1978. The victim was a young girl who swam in a public bathing pool fed with water from the historic thermal springs that rise naturally in the City. Despite intensive medical therapy, the child died 9 days after admission to hospital (Cain *et al*, 1981). Subsequent analysis confirmed the thermal springs to be the source of the infection.

The *N. fowleri* from the thermal springs represented the only isolates of the organism to be made in the United Kingdom. However, in 1992 *N. fowleri* was isolated from the cooling circuits of two electricity power stations situated on the River Trent in Nottingham (Kilvington & Beeching, 1996). DNA fingerprinting of the isolates showed them to be distinct from those recovered from the Bath hot springs and indicated that the two sites have been colonised independently.

The isolation of *N. fowleri* from water cooling circuits of electricity power stations in Nottingham confirms previous observations from Belgium, Czechoslovakia, France and the USA on the suitability of such sites to harbour *N. fowleri* (De Jonckheere, 1987). There is no evidence of PAM cases among employees at the water cooling circuits of the Nottingham power stations and the risk to workers from *N. fowleri* at such sites is considered minimal. Cases of PAM have been reported from Belgium and Czechoslovakia in persons swimming in warm effluent water from industrial processes (De Jonckheere, 1987).

6.4.1. Isolation and identification methods for *N. fowleri*

As with the *Acanthamoeba*, *N. fowleri* can be easily cultured from the environment on non-nutrient agar plates seeded with a lawn of *E. coli* (Kilvington *et al*, 1991).

Methods for the concentration of water samples by filtration or centrifugation for the isolation of *N. fowleri* are detailed in Anon (1990). Differentiation of *N. fowleri* from other closely related but nonpathogenic species is difficult because of shared morphological, cultural and antigenic properties. These problems have been addressed extensively at the Bath PHL amoeba unit and have led to the development of specific, sensitive and rapid methods for the identification of *N. fowleri* soon after primary isolation from the environment (Kilvington, 1995; Kilvington and Beeching, 1995a & 1995b).

6.4.2. Conclusions

N. fowleri cannot be described as an emerging pathogen. However, studies to date in England indicate that:

i. *N. fowleri* is more widely distributed in the United Kingdom than has previously been supposed.

ii. The thermophilic nature of *N. fowleri* would normally preclude the organism from the drinking water supplies in this country.

iii. Although PAM is a rare disease, the potential for new cases should not be ignored.

iv. Improved and rapid methods for the identification of *N. fowleri* from the environment have now been developed and would greatly facilitate future environmental investigations (Kilvington, 1995; Kilvington & Beeching, 1995a & 1995b).

6.4.3. Summary: *N. fowleri* primary amoebic meningoencephalitis

Organism:	*N. fowleri*
Biology:	Trophozoite that forms a flagellate and cyst.

Resistance:	Unlike most other FLA *N. fowleri* cysts are inactivated by 1-2 ppm chlorine and are susceptible to desiccation.
Ecology:	Found only in thermal aquatic habitats world-wide. *N. fowleri* has been isolated from natural hot springs in Bath and more recently from electricity power stations in Nottingham.
Disease:	Primary amoebic meningoencephalitis.
Diagnosis:	Observing trophozoites in freshly taken CSF and culture of specimen on non-nutrient agar seeded with *E. coli*.
Treatment:	Usually fatal. However, prompt diagnosis and intensive therapy with amphotericin B has resulted in the survival of four PAM cases.
Incidence:	Approximately 250 cases world-wide. One confirmed case in the United Kingdom in 1978.
Isolation and identification from the environment:	Methods available for isolation and identification from water and soil. However, techniques are specialised and may not be available in routine microbiology laboratories.
Emerging pathogen status:	Of no significance in the drinking water supply in this country but likely to be found in industrial process effluents.

6.4.4. Recommendations

i. Further studies are required to determine the presence of *N. fowleri* in other thermally enriched industrial sites in this country to assess the risks to workers and the public.

ii. If necessary, measures should be undertaken to prevent people from bathing in effluent water at such sites.

6.4.5. References

Anon. Isolation and identification of *Giardia* cysts and *Cryptosporidia* oocysts and free-living pathogenic amoebae in water 1989. HMSO, London.1990.

Brown, R.L. Successful treatment of primary amoebic meningoencephalitis. *Archives of Internal Medicine.* 151: 1201-1202, 1991.

Cain, A.R.R., Wiley, P.F., Brownwell, B. and Warhurst, D.C. Primary amoebic meningoencephalitis. *Archives of Diseases in Childhood.* 56: 140-143 1981.

Cerva, L. and Novak, K. Amoebic meningoencephalitis: sixteen fatalities. *Science.*160: 92. 1968.

De Jonckheere, J.F. Epidemiology. *In: Amphizoic Amoebae, Human Pathology.* E.G. Rondanelli (ed.), Piccin Nuova Libraria, 127-147, 1987.

John, D.T. Primary amoebic meningoencephalitis and the biology *of Naegleria fowleri. Annual Review of Microbiology.* 36: 101-103, 1982.

Kilvington, S., Mann, P.G. and Warhurst, D.C. Pathogenic *Naegleria* amoebae in the waters of Bath: a fatality and its consequence. *Hot Springs of Bath.* Kellaway, G.A. (ed.). 89-96, 1991.

Kilvington, S. Identification of *Naegleria fowleri* and other *Naegleria* spp. (free-living amoebae) using cellulose acetate membrane electrophoresis of glucose phosphate isomerase. *FEMS Microbiology Letters.* 133: 219-223. 1995.

Kilvington, S. and Beeching, J.R. Identification and epidemiological typing of *Naegleria fowleri* with DNA probes. *Applied and Environmental Microbiology.* 61: 2071-2078, 1995a.

Kilvington, S. and Beeching, J.R. Development of the polymerase chain reaction for the identification of *Naegleria fowleri* from the environment. *Applied and Environmental Microbiology.* 61: 3764-3767, 1995b.

Kilvington, S. and Beeching, J.R. Detection of a novel restriction fragment length polymorphism type in *Naegleria fowleri* isolates from electricity power stations in England and France. 7th International Conference On Small Freeliving Amoebae. Women's And Children's Hospital, Adelaide, South Australia. 7-12 January, 1996.

Robinson, B., Dobson, P., Christy, P. and Hayes, S. Spatial and seasonal distributions of *Naegleria fowleri* in Australian water supplies. 7th International Conference On Small Freeliving Amoebae. Women's And Children's Hospital, Adelaide, South Australia. 7-12 January, 1996.

Warhurst, D.C. Pathogenic free-living amoebae. *Parasitology Today.* 1: 24-28, 1985.

Section 7

7.1 RISK ASSESSMENT IN THE INDENTIFICATION OF EMERGING PATHOGENS

Dr Hilary Tillett
PHLS, Communicable Disease Surveillance Centre,
London NW9 5EQ

7.1 Risk assessment

The application of risk assessment in studies of disease is a growing discipline. The terminology must be used with caution since many publications use slightly varying definitions and notations. The usefulness in water-borne microbiological disease can be summarised as follows:

If a pathogenic micro organism is present in drinking water then this is a hazard. Risk is the estimation of illness resulting from consumption of that water. Risk assessment is an exercise in expressing the complex process from potential hazard to possible morbidity. It uses statistical and mathematical tools.

Risk assessment ideally requires the identification of the source(s) of the hazard(s) and the conditions under which adverse consequences could occur. In practice, with water-borne disease, models have started with the patterns of organism frequency in pre-treatment source water rather than any earlier stage in the contamination process. These patterns in the source water are described as closely as possible with mathematical functions. in conjunction with other functions best thought to describe the chances of these hazardous organisms reaching the potable water supply. The risk assessment model then aims to quantify the risk of morbidity. These models can be used to study different possible scenarios consequent upon different assumptions within the model. Such "if... then ....." exercises help to establish the factors which are most influential on the estimate of risk and to identify any areas which need further research because the process is too poorly understood. Risk assement models for water microbiology use equations to link such concepts as:-

(1) contamination in untreated water.

(2) effects of water treatment.

(3) volume consumed by each customer of the water supply.

(4) infective dose, susceptibility to and severity of disease.

The researcher using this model can insert actual or suggested values at chosen parts of the equations. By repeatedly running the models for different scenarios the researcher can try to see which are the most important factors affecting the end point i.e.the incidence of disease.

Risk assessment has been used in the USA and Netherlands to study potential disease patterns and to set water treatment standards (Rose *et al*,1991, Rose and Gerba 1991 and Teunis *et al*,1994). Most work has involved cryptosporidia and giardia, but some viruses and bacteria have also been studied.

All published work indicates that key factors are extensive sampling results from untreated source waters, measurements of the effectiveness of treatment procedures in removing live organisms and the minimum infective dose (number of organisms) likely to cause disease in a susceptible individual. These factors should be assumed to be important for "emerging pathogens".

Risk assessment is only useful if some parts of the process are understood, at least approximately. For example, the exercise to set standards for cryptosporidia removal from water used an arbitrary maximum acceptable risk of infection of 0.0001 per person per year (ie one infection per 10,000 population per annum) but applied data from extensive testing of source water and some dose-response data from an experiment. Models based entirely on guess work are of little use and therefore it is not recommended that new modelling be undertaken for "emerging pathogens" at this stage.

7.2 Conclusion

Risk assessment studies with established water-borne pathogens suggest that useful areas of study are:

levels of contamination in untreated water

effectiveness of water treatment procedures

infective dose for human illness.

These should be studied for "emerging pathogens". As yet there is insufficient information available to justify constructing new risk assessment models and there is no reason to suppose that the resulting key factors would be other than those listed.

7.3 References

Rose J B, Haas C N & Regli S (1991) Risk assessment and control of waterborne giardiasis. Am J of Public Health **81**, 709-713.

Rose J B, & Gerba C P (1991) Use of risk assessment for development of microbiol standards. Wat. Sci & Tech **24**, 29-34.

Teunis P F M, Havelaar A H & Medena G J (1994) A literature survey on the assessment of microbiol risk for drinking water. Report number 734301006 of the National Institute of Public Health and Environmental Protection (RIVM), The Netherlands.

SECTION 8

CONCLUSIONS

Dr John de Louvois
Head, PHLS Environmental Surveillance Unit
PHLS Headquaters, London NW9 5DF

The definition of an emerging pathogen used by the United States Department of Health and Human Services which refers to emerging pathogens as those micro-organisms "...whose incidence in humans has increased within the past two decades or threatens to increase in the near future" is not entirely satisfactory and may be misleading with regard to the number and importance of emerging pathogens. The incidence and prevalence of infectious diseases, and micro-organisms responsible for them are in a state of continuing change. These changes may be global, continental, national or local, and are due to a variety of factors, some of which relate to the microbe, some to the host and some to the environment in which the host and the microbe co-exist. The situation is further complicated by the consequences of developing technology and scientific understanding. The end result is one in which a variety of factors contribute to whether the prevalence of a pathogen increases, only one of which may be its real emergence as a cause of increased human illness.

It is well established that some micro-organisms undergo genetic shift or other alteration in their genetic structure which allows them to breach the host resistance generated by their predecessor. The best documented example of this phenomenon concerns the viruses responsible for human influenza, each new strain of which could appropriately be considered as an emerging pathogen. The significance of these individual strains is however short-lived since having run their course in a susceptible population the community will develop long term immunity. The consequence of this is that the number of subsequent infections due to the viral strain concerned would be insignificant.

Similarly, other microbes have a well established cyclical pattern of infection. In the context of this report a good example is Hepatitis A (see section 2) which has a well established pattern of disease prevalence with peaks every 10 years. Thus, from figure 2.8 it may be anticipated that the prevalence of Hepatitis A in England and Wales will rise.

There is no reason at present to believe that the projected rise will be any different in scale from that which occurred between 1986 and 1990. There are a number of reasons why patterns of disease have a cyclical presentation, the most obvious of which is that this pattern relates to the percentage of susceptible individuals within a given population. Prior to the widespread use of vaccination against the childhood infectious diseases cyclical patterns of incidence were common.

Whether or not a micro-organism is identified as an emerging pathogen depends increasingly on the technology available for its initial isolation and subsequent detailed characterisation. Previously emergence related more to the disease than to the micro-organism which caused it. As a result changes in the incidence and prevalence of conditions such as tuberculosis. bacillary dysentery, influenza and cholera were in the main considered to be changes in pattern of infection due to a single causative organism. It is now known that the situation is far more complex than this and that within each taxonomic species there may be a large number of stable variants which show fundamental differences, each from the other. In some instances these differences are so great that the diseases caused by different strains are fundamentally distinct. A topical example of this is shown by the various cytotoxin producing strains of *Escherichia coli* characterised by *E.coli* 0157 VTEC.

Within the overall pattern of variation it is not unusual for the prevalence of individual strains to change with time while the overall rate of infections due to the taxonomic species remains largely unaltered. This is exemplified by the situation with *Salmonella typhimurium* (figure 8.1). It may be seen that over the last twenty years there has been a steady but undramatic increase in the number of infections reported in England and Wales due to this organism. However within this total a number of emerging pathogens have been identified, for example *S. typhimurium* PT193, PT204, and PT104. Prior to 1990 there were 200-500 isolations of *S. typhimurium* PT 104 per year and when this strain did occur it was essentially highly susceptible to antimicrobial drugs. In 1987-88 the first reports appeared of a strain of *S. typhimurium* PT104 which was resistant to a number of antibiotics. This strain rapidly became epidemic in England and Wales and now accounts for more than 50% of all isolates of *S. typhimurium*. Clearly those countries of the world which are unable to

characterise microbial isolates in such detail would be unaware of emerging pathogens of this kind.

The facilities available through the Public Health Laboratory Service probably constitute the most effective protection against the possibility of a new pathogen suddenly emerging within our population. Detailed surveillance and accurate characterisation of microbial isolates ensures that even very subtle changes in the characters of the organisms identified will be rapidly recognised. During the last six to eight years there has been a major problem in England and Wales due to *Salmonella enteritidis* PT4, an organism which has been shown to be associated with eggs and chickens. However, long before the source or the extent of the problem were apparent the PHLS Laboratory of Enteric Pathogens reported, in 1987, that they had received more isolates of this organism than previously and publicised their concern that the organism might create problems in the future, as indeed it did.

Similar situations exist with regards to other organisms for example *Staphylococcus aureus*. This common cause of purulent lesions and wound infections can be divided into a large number of phage types. As a consequence widespread outbreaks of hospital infection due to phage type 80/81 were recognised in the 1960's as originating from a common source and being distinct from other strains of staphylococci. For reasons that are not fully understood and without any focused preventive measures the importance of this organism declined, after a number of years, as quickly as it had previously risen. There are currently problems in many areas of the world due to strains of *S.aureus* which are resistant to a large number of antimicrobial drugs (MRSA). Detailed studies of these organisms have shown that even in quite small geographical areas, or indeed in a single hospital, there may be more than one type of MRSA. The consequence of this is that the approach to control of these infections is different from that which would be applied if they were thought to be due to a single common organism. This degree of precise characterisation also alters the perception of these organisms as emerging pathogens.

While it is possible that totally new pathogens may emerge as did the human immunodeficiency virus in the late 1970s, this will be a rare event. What is far more likely is that organisms currently known to exist will be identified in ever greater detail so that

changes in the prevalence of individual strains will be apparent. This is demonstrated by the changing pattern, for example, of Adenovirus 40 (see section 4). It is possible also that as the small round structured viruses (SRSV) are subjected to greater scrutiny, additional distinct groups will be identified.

Despite the enormous efforts devoted to the culture, identification and classification of micro-organisms there are still many that cannot be cultivated artificially and for which there is no useful animal model. Initially there were enormous problems in diagnosing legionnaires disease because the causative organism, *Legionella pneumophila,* could not be grown on artificial media. The greater understanding of *Cryptosporidium parvum* is currently compromised by the lack of satisfactory *in vitro* culture systems. Similarly, the microbial etiology of Haemolytic Uraemic Syndrome (HUS) went unrecognised until it was demonstrated that the strain of *E.coli* which was isolated from infected patients differed from other strains of the same species in that it did not ferment sorbitol. Culture media were then produced which took advantage of this characteristic, since when, the number of isolates reported has grown significantly. It is a matter of conjecture whether and to what extent, these changes relate to the ability of microbiologists to isolate the organism or to a genuine increase in its prevalence in the population. The fact that other verocytotoxin producing *E.coli*, which do not ferment sorbitol, have been identified in association with human disease and that serotypes other than *E.coli* 0157 have also been implicated serves to demonstrate the complexity of the situation, and the extent of surveillance resources required if clinically significant emerging pathogens are to be recognised in the early stages.

With water, as with other environments, there is a delicate balance between safety and a risk of potential infection for the user. The aim is to keep the risk of infection to a minimum. It could not have been envisaged that the creation of cooling towers and "modern" air conditioning systems might lead to the emergence of a previously undescribed disease due to a previously unknown organism. Similarly when the poultry industry, in an attempt to increase efficiency, started to feed chicken protein back to boiler and laying hens it was not appreciated that, with this practice, there would be an accompanying risk of perpetuating an infection cycle. Examples of this sort should serve to caution against any change in manufacture or processing practice without first considering the possible microbiological consequences. The same argument might be applied to future changes in water treatment processes.

In preparing this report the PHLS was asked to consider the possibility that one or more of a number of micro-organisms could emerge through the water distribution system as human pathogens. These were *Escherichia coli* 0157, the astroviruses, the caliciviruses, microsporidia, cyclospora and acanthamoeba. In addition we have considered, in varying degree, non 0157 vero cytotoxin producing *E.coli*, *Aeromonas* species, *Yersinia* species, hepatitis A and E and adenovirus 40 and 41.

This report was not asked to address cryptosporidia, giardia or the *Mycobacterium avium intracellularae* group of organisms all of which can be waterborne. Despite the absence of satisfactory *in vitro* culture systems our understanding of *Cryptosporidium parvum* has advanced considerably since the organism was first reported in 1983. Individual isolates of cryptosporidia can now be characterised with a considerable degree of discrimination. As a result certain strains may be shown to have a greater potential to cause human infections than others, analagous to the situation with other pathogens. Although many outbreaks of infection due to *Giardia lamblia* are reported from the United States and most of these are associated with water there are few similar reports from the UK. There is an increasing number of reports of infection due to *Mycobacterium avium intracellularae.* Most of them occur in the HIV positive population and there are recent reports of water being the source. Further work is required to determine the role of water in the transmission of these microorganisms to susceptible hosts.

Water has been implicated as the vehicle for infection in several outbreaks caused by *E.coli* 0157 (VTEC) either as a consequence of water in distribution becoming contaminated or because of inadequate chlorination. Present information indicates that the infective dose for this pathogen is low and therefore were VTEC to gain access to water in distribution where chlorination levels were low, it may be anticipated that outbreaks of infection would occur. Astroviruses do not, on current evidence, give cause for concern. During the past 10 years, the number of laboratory reports of astrovirus in England and Wales has remained constant at between 2 and 400 per year (Fig 2.5). It should be remembered that the technology for the identification of all virus groups detected by electron microscopy is developing and that improvements may lead to increased ascertainment. Although EM procedures for SRSV particularly have improved since the 1980's the recent rise in ascertainment rate for these

viruses is thought to be a genuine increase in incidence (Fig 2.6). However, epidemiological evidence of waterborne viral gastroenteritis does not depend on detailed virological knowledge. There is evidence for only a small number of outbreaks over the last twenty years and no obvious reason why this should increase in future. Continuing and effective surveillance by the PHLS of all waterborne outbreaks of infection will provide early information should any change occur. The likelihood of waterborne viral disease is less than that for protozoan infection, as enteric viruses are sensitive to the standard chlorination process and to the level of chlorine in the distribution system. The risk of waterborne viral gastroenteritis can be reduced to a minimum by good management of the sewage and drinking water distribution infrastructure.

With regards to microsporidia there are no reliable reports to date of outbreaks of human infection. However it should be borne in mind that the expertise to recognise these intracellular spore forming protozoa is, in general, lacking. They have only recently emerged as human pathogens. largely in patients suffering from AIDS. The level of scientific and medical understanding of these organisms and the infections for which they are responsible is currently low. Until more is known it is not possible to anticipate whether or not these organisms could be significant in the distribution system. A similar situation exists with regard to cyclospora. although a few outbreaks have been reported and one of these. in Chicago in 1990, implicated the water supply. The majority of cases reported so far have been sporadic. As with microspora little is known about these organisms at present. It is assumed that were they to get into the distribution system they would survive, in common with other protozoa because of their resistance to chlorine. At the present time it is unknown whether the presence of cyclospora in distributed water would pose a risk to the general population or not.

Free-living amoebae (FLA) occur in nearly all soil and aquatic environments. Certain species, *Acanthamoeba spp. Naegleria fowleri and Balamuthia mandrillaris,* cause infrequent but nevertheless severe, and often fatal disease in man. *Acanthamoeba* is one of the most common genera of free-living amoeba in soil and water habitats and is the cause of a potentially blinding infection of the cornea termed Acanthamoeba keratitis. At least 250 cases have occurred in the United Kingdom and the incidence is increasing, 3 cases were reported in 1989 compared with 60 in 1995. Contact lens wearers are most at risk from

infection, accounting for approximately 90% of reported cases. Poor lens hygiene habits and the use of tap water for the rinsing or storing of lenses are recognised risk factors in the acquisition of the disease. Epidemiological studies using DNA typing have implicated bathroom tap water, derived from attic storage tanks, as the source of infection in some acanthamoeba keratitis cases. *N. fowleri* and *B. mandrillaris* are not emerging pathogens.

With regards to the other organisms considered in this report adenovirus 40 and 41 with less than 200 isolates from England and Wales per year are not presently considered as significant emerging pathogens. Hepatitis A will not survive the standard levels of chlorine present in distributed water and there is in any case no evidence that the number of reports per year is changing. Hepatitis E is a new pathogen by recognition rather than by increased prevalence. It is transmitted primarily by the faecal oral route and contaminated drinking water has figured prominently in epidemic reports. However the geographical distribution of the virus is largely confined to developing countries and less than 10 cases per year, all imported, are reported in England and Wales.

Aeromonas species have been isolated from drinking water and they have been shown to colonise slow sand filters. Treated waters with or without disinfection can contain small numbers of aeromonas and regrowth is possible.. Currently the number of reported cases per year in England and Wales is 600 -700 Although the number of reports have increased during the last decade it is unlikely, on present evidence that this organism will emerge as a significant pathogen in the healthy population. The absence of outbreaks of infection due to this organism makes it difficult to determine its source A comparable situation exists with regards to yersinia which is rarely reported from the UK, although associated with human infection in parts of mainland Europe. It is not known whether these geographical differences are real or due to ascertainment. In England and Wales the number of *Yersinia enterocolitia* infections has declined over the last six years from a peak of 550 cases in 1989.

The techniques for the recovery of micro-organisms, of all sorts, from water are progressively improving. It would not be surprising therefore if an increased number and a wider range of organisms were isolated from water in distribution. On the basis of the information currently available it is most unlikely that such organisms would be a

significant cause of human illness. Current epidemiological techniques do not require the isolation of a potential pathogen from the water supply system in order to demonstrate an association with infection, if one exists.

The Public Health Laboratory Service keeps details of all outbreaks of infection which might have an association with water. The numbers are small and in the majority a pathogen is identified. The surveillance system was reviewed in 1995 and a revised protocol for investigating outbreaks in which water might have been the cause was introduced in January 1996 together with revised definitions on the strength of association for waterborne outbreaks. These were published in the Communicable Disease Report (23rd February, 1996). As a result of this there is every reason to believe that were a pathogen to emerge in the water distribution system it would be identified well before any problems became significant.

PHLS HQ (Environmental Services Unit)
WP/JLouvois/ep001.
Final 14th May 1996

APPENDIX I
Organisms which have recently been described as "emerging pathogens" directly or indirectly associated with water

Bacteria, Rickettsiae, and Chlamydiae

Agent	Related Diseases/Symptoms	Mode of Transmission
Aeromonas species	Aeromonad gastroenteritis, cellulitis, wound infection, septicemia	Ingestion of contaminated water or food; entry of organism through a break in the skin.
Campylobacter jejuni	Campylobacter enteritis: abdominal pain, diarrhoea, fever	Ingestion of contaminated food, water, or milk; fecal-oral spread from infected person or animal
Escherichia coli 0157:H7	Haemorrhagic colitis; thrombocytopenia; haemolytic uremic syndrome	Ingestion of contaminated food, esp. undercooked beef and raw milk
Helicobacter pylori	Gastritis, peptic ulcer, possibly stomach cancer	Ingestion of contaminated food or water, esp. Unpasteurised milk; contact with infected pets
Legionella pneumophila	Legionnaires' disease: malaise, myalgia, fever, headache, respiratory illness	Air-cooling systems, water supplies
Vibrio cholerae	Cholera: severe diarrhoea, rapid dehydration	Ingestion of water contaminated with the faeces of infected persons; ingestion of food exposed to contaminated water

Emergent Viruses

Agent	Related Diseases/Symptoms	Mode of Transmission
Hepatitis E	Fever, abdominal pain, jaundice	Mainly faecal-oral Contaminated water
Norwalk and Norwalk-like agents	Gastroenteritis; epidemic diarrhoea in children	Most likely faecal-oral; occasional drinking and swimming water, and uncooked foods
Rotavirus	Enteritis; diarrhoea, vomiting, dehydration, and low-grade fever	Primarily faecal-oral; faecal-respiratory transmission may also occur

APPENDIX II

Organisms which have recently been described as "emerging pathogens" with no apparent association with water

Bacteria, Rickettsiae, and Chlamydiae

Agent	Related Diseases/Symptoms	Mode of Transmission
Borrelia burgdorferi	Lyme disease: rash, fever, neurologic and cardiac abnormalities, arthritis	Bite of infective *Ixodes* tick
Chlamydia pneumoniae (TWAR strain)	TWAR infection: fever, myalgias, cough, sore throat, pneumonia	Inhalation of infective organisms; possibly by direct contact with secretions of an infected person
Chlamydia trachomatis	Trachoma, genital infections, conjunctivitis; infection during pregnancy can result in infant pneumonia	Sexual intercourse
Clostridium difficile	Colitis: abdominal pain, watery diarrhoea, bloody diarrhoea	Faecal-oral transmission; contact with the organism in the environment
Ehrlichia chaffeensis	Ehrlichiosis; febrile illness (fever, head-ache, nausea, vomiting, myalgia)	unknown; tick is suspected vector
Haemophilus influenzae bio-group aegyptius	Brazilian purpuric fever: purulent conjunctivitis, high fever, vomiting, and purpura	Contact with discharges of infected persons; eye flies are suspected vectors

Agent	Related Diseases/Symptoms	Mode of Transmission
Listeria monocytogenes	Listeriosis; meningoencephalitis and/or septicemia	Ingestion of contaminated foods; contact with soil contaminated with infected animal faeces; inhalation of organism
Mycobacterium tuberculosis	Tuberculosis; cough, weight loss, lung lesions; infection can spread to other organ systems	Exposure to sputum droplets (exhaled through a cough or sneeze) of a person with active disease
Staphylococcus aureus	Abscesses, pneumonia, endocarditis toxic shock	Contact with the organism in a purulent lesion or on the hands
Streptococcus pyogenes (Group A)	Scarlet fever, rheumatic fever, toxic shock	Direct contact with infected persons or carriers; sometimes ingestion of contaminated foods
Vibrio vulnificus	Cellulitis; fatal bacteremia; diarrheal illness (occasionally)	Contact of superficial wounds with seawater or with contaminated (raw or undercooked) seafood; ingestion (occasionally)

Emergent Viruses

Agent	Related Diseases/Symptoms	Mode of Transmission
Bovine Spongiform Encephalopathy (BSE)	Bovine spongiform encephalopathy in cows	Ingestion of feed containing infected sheep tissue

agent		
Chikungunya	Fever, arthritis, hemorrhagic fever	Bite of infected mosquito
Crimean-Congo hemorrhagic fever	Hemorrhagic fever	Bite of an infected adult tick
Delta virus	Fulminant hepatitis and accelerated progression of pre-existing HBV hepatitis	Contact with saliva, semen, blood, or vaginal fluids of an infected person; mode of transmission to children not known
Dengue	Hemorrhagic fever	Bite of an infected mosquito (primarily *Aedes aegypti)*
Filoviruses (Marburg, Ebola)	Fulminant, high mortality hemorrhagic fever	Direct contact with infected blood, organs, secretions, and semen
Hantaviruses	Abdominal pain, vomiting, hemorrhagic fever	Inhalation of aerosolized rodent urine and faeces
Hepatitis B	Nausea, vomiting, jaundice; chronic infection leads to hepatocellular carcinoma and cirrhosis	Contact with saliva, semen, blood, or vaginal fluids of an infected person; mode of transmission to children not known
Hepatitis C	Nausea, vomiting, jaundice; chronic infection leads to hepatocellular carcinoma and cirrhosis	Exposure (percutaneous) to contaminated blood or plasma; sexual transmission

Human herpes virus 6 (HHV-6)	Roseola in children, syndrome resembling mononucleosis	Unknown; possibly respiratory spread

Agent	**Related Diseases/Symptoms**	**Mode of Transmission**
Human immunodeficiency viruses HIV-1	HIV disease, including AIDS: severe immune system dysfunction, opportunistic infections	Sexual contact with or exposure to blood or tissues of an infected person; vertical transmission
HIV-2	Similar to above	Same as above
Human papillomavirus	Skin and mucous membrane lesions (often, warts); strongly linked to cancer of the cervix and penis	Direct contact (sexual contact/contact with contaminated surfaces)
Human parvovirus B19	Erythema infectiosum: erythema on face, rash on trunk; aplastic anaemia	Contact with respiratory secretions of an infected person; vertical transmission
Human T-cell lymphotropic viruses (HTLV-I and HTLV-II)	Leukaemia's and lymphomas	Vertical transmission through blood/breast milk; exposure to contaminated blood products; sexual transmission
Influenza	Fever, headache, cough pneumonia	Airborne (esp. in crowded, enclosed spaces)
Drift	Same as above	

		Same as above
Japanese encephalitis	Encephalitis	Bite of an infective mosquito
La Crosse and California Group viruses	Encephalitis	Bite of an infective mosquito

Agent	**Related Diseases/Symptoms**	**Mode of Transmission**
Lassa	Fever, headache, sore throat, nausea	Contact with urine or faeces of infected rodents
Measles	Fever, conjunctivitis, cough, red blotch rash	Airborne; direct contact with respiratory secretions of infected persons
Rabies	Acute viral encephalomyelitis	Bite of a rabid animal
Rift Valley	Febrile illness	Bite of an infective mosquito
Ross River	Arthritis, rash	Bite of an infective mosquito
Venezuelan equine encephalitis	Encephalitis	Bite of an infective mosquito
Yellow fever	Fever, headache, muscle pain, nausea, vomiting	Bite of an infective (*Aedes aegypti*) mosquito