Advisory Committee on the Microbiological Safety of Food

Advises the Government
on the Microbiological Safety of Food

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Microbiological Safety of Food

Report on FOODBORNE Viral Infections

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on the Microbiological Safety of Food

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CONTENTS

Summary

CHAPTER 1: INTRODUCTION

Background to the review	**1.1-1.2**
Working Group on Foodborne Viral Infections	**1.3**
Infections	
General background to foodborne viruses	**1.4-1.8**
Viruses	
Programme of work	**1.9**
Acknowledgements	**1.10**

CHAPTER 2: INFECTIOUS AGENTS, CLINICAL SPECTRUM AND PATHOGENESIS

Introduction	**2.1-2.2**
Viral gastroenteritis	**2.3-2.13**
Viral gastroenteritis and viral foodborne infections	2.8
Rotaviruses, adenoviruses, astroviruses and caliciviruses	2.9-2.10
The agents	2.11
Physicochemical properties	2.12
Biological properties	2.13
Clinical disease	**2.14-2.26**
Gastroenteritis	2.14
Pathogenesis	2.15
Resistance to infection	2.16
Management and control of viral gastroenteritis	2.17
General principles of management of cases of gastroenteritis and foodborne infection	2.18
Management of SRSV infection and outbreaks	2.19
Kaplan Criteria for suspecting an outbreak is due to SRSV	2.20
Clinical specimens in a suspected case/outbreak of SRSV gastroenteritis	2.21-2.23
Management of acute gastroenteritis	2.24
Control and containment of the acute episode/ outbreak and prevention of spread to others in hospitals/institutions	2.25
In the community and the home	2.26
Non-diarrhoeal viruses : viral hepatitis	**2.27-2.36**
Hepatitis A	2.27
Classification	2.28
Physicochemical properties	2.29

Biological properties	2.30
Pathogenesis	2.31
Resistance to infection	2.32
Clinical infection	2.33
Laboratory confirmation	2.34
Management	2.35
Prevention of person-to-person spread	2.36
Conclusions	**2.37**
Recommendations	**2.38-2.39**

CHAPTER 3: OCCURRENCE OF FOODBORNE VIRAL INFECTION IN THE UNITED KINGDOM

Reporting foodborne viral infections	**3.1**
Viral gastroenteritis	**3.2-3.19**
Laboratory reports	3.2-3.3
Reports of outbreaks	3.4-3.19
Hepatitis A foodborne infection	**3.20**
Discussion	**3.21-3.23**
Conclusions	**3.24**
Recommendations	**3.25-3.27**

CHAPTER 4: DETECTION METHODS FOR VIRUSES IN CLINICAL SAMPLES AND FOODS

Introduction	**4.1-4.2**
The methods for clinical samples (antigen/nucleic acid detection)	**4.3-4.9**
Electron Microscopy - negative staining of virus particles	4.3-4.4
Solid Phase Immune Electron Microscopy (SPIEM)	4.5
Immunoassays	4.6-4.7
Culture techniques	4.8
Animal models	4.9
Molecular methods for the detection of viruses in clinical samples and, potentially, food	**4.10-4.18**
Reverse transcriptase polymerase chain reaction (RT-PCR)	4.10-4.13
RT-PCR for other enteric viruses	4.14-4.15
Adapting molecular methods for detection of virus in food and the environment	4.16
Detection of viruses in shellfish	4.17-4.18
Antibody detection in human serum samples (sero-diagnosis)	**4.19-4.25**
Hepatitis A	4.20
SRSV	4.21-4.23
Astroviruses and caliciviruses	4.24

Rotavirus	4.25
Sampling	**4.26**
Food and environmental samples	4.26
Quality assurance and accreditation	**4.27-4.30**
Commercial Immunoassays	4.28
Non-reference in-house assays	4.29
Reference tests	4.30
Health and safety in the laboratory	**4.31-4.34**
Conclusions	**4.35**
Recommendations	**4.36-4.37**

CHAPTER 5: VIRAL CONTAMINATION OF FOOD : ROUTES OF SPREAD AND VEHICLES : PREVENTION AND CONTROL MEASURES

Introduction	**5.1-5.3**
Non-food vehicles or direct person-to-person spread	**5.4**
Water	**5.5-5.6**
Bivalve molluscan shellfish	**5.7**
Contamination of food crops	**5.8-5.10**
Infected food handlers	**5.11-5.26**
Prevention of contamination by infected food handlers	5.13-5.17
Food handlers' fitness to work	5.18-5.19
Pre-employment assessment	5.20
Symptomatic food handlers : exclusion and re-employment criteria	5.21
Good hygiene practices	5.22
Food handlers : legislative controls	5.23-5.26
Conclusions	**5.27-5.28**
Recommendations	**5.29-5.34**

CHAPTER 6: VIRAL CONTAMINATION OF SHELLFISH : PREVENTION AND CONTROL MEASURES

Introduction	**6.1-6.2**
Bivalve molluscan shellfish	**6.3**
Human health hazards	**6.4-6.8**
Commercial treatment processes	**6.9-6.10**
Statutory control measures	**6.11-6.15**
Water legislation affecting shellfisheries	**6.16-6.19**
Research	**6.20-6.27**
Conclusions	**6.28-6.29**
Recommendations	**6.30-6.33**

CHAPTER 7: SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

ANNEX A: ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD : MEMBERSHIP

ANNEX B: ORGANISATIONS AND INDIVIDUALS WHO SUPPLIED INFORMATION

ANNEX C: GUIDANCE ON CLEANING UP VOMIT

ANNEX D: CONTROL OF THE SPREAD OF HEPATITIS A VIRUS

ANNEX E: TREATMENT AND DISPOSAL OF SEWAGE SLUDGE

ANNEX F: BIVALVE MOLLUSCAN SHELLFISH : CHARACTERISTICS, INDUSTRY FACTS, LEGISLATION

GLOSSARY

REFERENCES

WORKING GROUP ON FOODBORNE VIRAL INFECTIONS

TERMS OF REFERENCE

The Working Group's terms of reference were to:-

- assess the extent of viral foodborne infection in the UK (with particular reference to small round structured viruses (SRSVs) and hepatitis A);
- describe the epidemiology, sources and mode of transfer of foodborne infection;
- identify the practical options that exist, or might be developed, for the prevention and control of foodborne transmission;
- assess the implications of the new technologies for public health;
- identify research priorities where it would be more valuable to have more information; and
- report on these matters by early 1997.

WORKING GROUP ON FOODBORNE VIRAL INFECTIONS

MEMBERSHIP

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SUMMARY

1 In this Report we have assessed the significance of viruses as agents of foodborne infection in humans. We have considered the scientific literature and both written and oral evidence from a variety of individuals and organisations. We have also considered information on the viruses of primary concern in respect of foodborne illness, their occurrence in the UK, sources and routes of transmission, and prevention and control measures for foodborne viruses. The Report is limited to the consideration of foodborne viral infection which presents in man as gastroenteritis or viral hepatitis.

2 The major cause of outbreaks of viral gastroenteritis is the small round structured viruses (SRSVs), identified by the electron microscope (EM) on the basis of their morphology. We recognise that, as a result of advances in the molecular characterisation of SRSVs, they are now classified within the Caliciviridae family. There nevertheless remain morphological and epidemiological differences between SRSVs and human caliciviruses, as well as important differences in their immunobiology, suggesting that these are distinct sub-groups within the family of human caliciviruses. In this Report, SRSVs are therefore considered separately from human caliciviruses.

3 SRSVs are spread by the faecal/oral route, by hand-to-mouth transfer of infected vomit from the contaminated environment, and, possibly, by ingestion of aerosolised vomit from an infected human. Foodborne viral disease occurs when food is inadvertently contaminated by material from an infected human source. From outbreak investigations it appears that the infectious dose is low, and illness may occur after ingestion of as little as 10-100 virus particles. Following an incubation period of 15-50 hrs (dose dependent), the onset of clinical symptoms - typically, abdominal cramps, projectile vomiting, diarrhoea, malaise and fever - is rapid.

4 The most common cause of foodborne viral hepatitis is hepatitis A virus. Following an incubation period of 2-6 weeks, the first symptoms of disease are malaise of 1-2 weeks duration, followed by acute hepatitis. Damage of the liver cells (hepatocytes) is responsible for the symptoms of hepatitis, including jaundice. Mild gastrointestinal disturbance may accompany the hepatitis. Foodborne incidents due to hepatitis A virus are rare in the UK. From 1992 to 1996, 19,147 reports of hepatitis A virus infections were received in England and Wales, and 0.5% (or 87) of these were associated with food. Infection mainly results from food handlers contaminating ready-to-eat food or, to a lesser extent, from consumption of raw or undercooked molluscan shellfish. In the UK, herd immunity to hepatitis A is declining, giving rise to the potential for future outbreaks. Effective vaccines are available and could be used for vaccinating food handlers. However, in the view of the Joint Committee on Vaccination and Immunisation (JCVI), neither food packagers nor food handlers in the UK have been associated with hepatitis A virus transmission sufficiently often to justify their immunisation as a routine measure.[1] We have recommended that the JCVI should keep this question under review.

5 Awareness of viral gastroenteritis is increasing. In England and Wales, laboratory reports of SRSVs increased sharply from about 400 cases in 1990 to 2,387 in 1996. In the same period, reports of SRSV infection in Scotland remained constant at between 78 and 94 cases per year whereas in N. Ireland an increase from 7 to 35 cases per year was recorded. These data probably significantly underestimate the true incidence of the disease.

6 SRSVs accounted for 7% (54) of the "mainly foodborne" outbreaks of gastroenteritis reported between 1992 and 1995 in England and Wales (785) but it is likely that the role of foodborne transmission is underestimated. There may be delays in the recognition of an outbreak due to the longer incubation period and the complexity of laboratory testing. There are difficulties with investigations of outbreaks of SRSV infection, which may be due to the agents being given lower priority than other pathogens, such as *Salmonella*, as the disease is usually less severe. The short duration of the illness, the high person-to-person transmission rates, and the difficulty and delay in laboratory confirmation of SRSVs are a considerable hindrance to effective control of outbreaks. We have therefore recommended that control measures for SRSV infection should be applied as soon as the diagnosis is suspected and should not be delayed until it is confirmed by laboratory tests.

7 Electron microscopy remains the only catch-all method of identifying viruses causing gastroenteritis. However, neither EM nor immunoassays are sufficiently sensitive for the detection of viruses in food. The molecular characteristics of SRSVs have allowed the development of a polymerase chain reaction (PCR) for diagnosis and this can be applied to the detection of viruses in food. We recommend that all laboratories using EM or molecular techniques for the investigation of viral diarrhoea should be accredited and should participate in quality assurance arrangements. Quality assurance (QA) schemes must be developed for molecular diagnostics and must be reintroduced for EM.

8 Despite the limitations of the surveillance data, there are currently two main patterns of SRSV foodborne outbreaks in the UK - those in which an infected foodhandler is suspected as the source and those due to contaminated bivalve molluscan shellfish. Foods contaminated by water in food processing have also been identified as the source of outbreaks, although this is rare. There may also be the risk of infection from food crops through the application of sewage sludge to land but this has not yet been established. Although most SRSV infections are caused by direct person-to-person spread, it should be remembered that food may have been the primary unidentified vehicle.

9 Food handlers who are symptomatic, i.e. with diarrhoea or vomiting at the time of handling food, present the greatest risk of transmitting foodborne SRSV infections. Outbreaks have also been attributed to contamination of food by food handlers up to 2-3 days after cessation of symptoms. Personal hygiene, together with the education and training of food handlers, are essential in reducing the risk of SRSV transmission. There are comprehensive legal controls available governing the personal hygiene of food handlers in food businesses but it may not

always be clear exactly what measures are needed to comply with the hygiene training requirement. We have therefore recommended that more guides to good hygiene practice in the food industry should be developed and steps should be taken to bring these and established guides to the attention of food businesses.

10 Bivalve molluscs are filter feeders. They concentrate microorganisms, including human viruses, in their tissues. When grown in sewage-polluted waters they can consequently present a significant health risk and they have been implicated in a number of outbreaks of foodborne viral infection. The principal concern is with oysters which are eaten raw or lightly cooked. We therefore recommend that the Government develops a national policy for the reduction of pollution-related illness associated with shellfish consumption. Such a policy should include a review of the effectiveness of the epidemiological surveillance of shellfish-associated incidents; the designation of classified shellfisheries as sensitive areas under the Urban Waste Water Treatment Directive;[2] their designation under the Shellfish Waters Directive;[3] and the reduction to a minimum, and the monitoring, of discharges from Combined Sewage Overflows (CSOs) into shellfish areas.

11 There are no proven recorded outbreaks of foodborne viral infection attributed to the use of sewage sludge spread on agricultural land as a fertilizer and soil conditioner. Although the application of sewage sludge to land is subject to the provisions of Regulations[4] and a Code of Practice,[5] we are uncertain whether the Code, and the way it is currently applied, affords full consumer protection from the contamination of produce by viruses. The amount of sewage sludge spread on agricultural land is increasing and even greater use of this method of disposal is likely when the sea disposal option is withdrawn after full implementation of the Urban Waste Water Treatment Directive[2] from 1998 onwards. In addition, the risk from imported produce is currently unknown. We welcome the recommendations of the Royal Commission on Environmental Pollution[6] relating to a review of the scientific basis of the controls and effective enforcement of the provisions of the Code of Practice for Agricultural Use of Sewage Sludge.[5] We also recommend that the importers of fresh fruit and salad crops take account of these hazards and ensure suitable precautions are taken to ensure food safety.

12 We have recommended that there should be research and surveillance in a number of specific areas. We recommend that the Government takes steps to improve the harmonisation of detection, reporting and surveillance of foodborne SRSV infections throughout the UK, with a view to establishing a comprehensive picture of the disease. We have also recommended the further development of the enhanced EM network surveillance better to define the problem. In the areas of research, we have recommended a better understanding of the physiology and behaviour of viruses, in particular their survival in sewage sludge, and their behaviour during sewage treatment processes, with a view to maximising virus removal. We have recommended research into the effectiveness of the washing methods used to remove viruses from fruit and vegetables. We

have also recommended the continued development of indicators of viral shellfish pollution and a pilot scheme of the F+ bacteriophage model.

13 The Report's conclusions and recommendations appear at the end of the respective chapters and are drawn together in Chapter 7.

CHAPTER 1

INTRODUCTION

Background to the review

1.1 In June 1993 the Advisory Committee on the Microbiological Safety of Food (ACMSF) discussed a literature review of foodborne viruses and subsequently asked the Steering Group on the Microbiological Safety of Food (SGMSF) to examine the adequacy of epidemiological investigations of foodborne viral disease. The Steering Group concluded that there was a need for research into extraction, detection, identification and isolation techniques for foodborne viruses.

1.2 Following a joint ACMSF/SGMSF workshop in October 1994,[7] at which the opportunities and priorities for research were considered, the ACMSF decided to set up a Working Group on Foodborne Viral Infections to determine the extent of the problem from the available data. The Working Group was asked to focus primarily on the small round structured viruses (SRSVs) and hepatitis A virus, the viruses thought to be of primary concern in respect of foodborne illness, and direct its attention to two areas considered to be important in their transmission:-

- problems associated with consumption of raw or lightly cooked bivalve molluscan shellfish; and
- problems resulting from the contamination of food by food handlers.

Working Group on Foodborne Viral Infections

1.3 The terms of reference of the Working Group are shown on page 5. Membership of the Working Group is shown on page 6. Membership of the ACMSF is shown in Annex A.

General background to foodborne viruses

1.4 Viruses are simple sub-microscopic organisms which, with a few exceptions, can only be seen with the aid of the electron microscope. They only replicate in the living cells of man, animals, plants or bacteria. The principal component of a virus is its nucleic acid (its genetic material), which is surrounded by a protein coat, or capsid. When a virus invades a host cell it directs that cell to produce more virus, which infects new cells.

1.5 Each virus is highly specific with respect to the cells it can invade, and in which it can replicate, and most viruses are not capable of infecting both man and other animal species. Consequently, foodborne viruses which cause human disease are not usually zoonotic and humans are the only source of infection.

1.6 The consumption of contaminated food is an important route of transmission of some viruses. Food may be contaminated indirectly by viruses in the faeces or vomit from an infected individual and disease results if the food is eaten uncooked or inadequately cooked. Viruses, unlike bacteria, do not replicate in food, which merely acts as a passive vehicle for their transmission. If an infected food handler contaminates foodstuffs large outbreaks of infection may result.

1.7 The number of viruses present in food is usually low but bivalve molluscan shellfish, which feed by filtering particulate matter from the surrounding water, can concentrate viruses when they are present. This occurs when estuarine water in which the shellfish are grown is contaminated by sewage. The problem is exacerbated because some shellfish are eaten raw or lightly cooked and bivalve molluscs, particularly oysters, have been implicated in many outbreaks of foodborne viral infection.

1.8 The viruses most commonly causing foodborne illness are the SRSVs, and, to a lesser extent, hepatitis A virus. Both are considered in this Report.

Programme of work

1.9 The Working Group first met in September 1995 and met subsequently on eleven occasions. Details of the visits carried out in the course of its investigation are at Annex B.

Acknowledgements

1.10 The Working Group wishes to thank all the organisations and individuals, detailed at Annex B, who provided it with information or gave oral evidence.

CHAPTER 2

INFECTIOUS AGENTS, CLINICAL SPECTRUM AND PATHOGENESIS

Introduction

2.1 Numerous, taxonomically distinct, enteric viruses infect man and replicate in the human gastrointestinal tract. It is now known that human enteric viruses are a significant cause of foodborne illness, although it is not possible at present to quantify the extent of the problem on a global scale. This is largely due to the lack of comprehensive surveillance systems allied to expert laboratory diagnosis in most parts of the world. Nevertheless, it is clear that enteric viruses are commonly recognised as a cause of human disease associated with foodborne transmission and are certainly under-ascertained on a worldwide basis.

2.2 The diseases caused by foodborne human enteric viruses fall into two major, but distinct, clinical entities which present in man as gastroenteritis or viral hepatitis. Foodborne viral gastroenteritis is increasingly recognised, whilst foodborne viral hepatitis is less common in the developed world, particularly in the United Kingdom.

Viral gastroenteritis

2.3 Diarrhoeal disease in man, microbiologically distinct from bacterial gastroenteritis, was initially described more than 50 years ago in the southern USA. This clinical syndrome was originally described in families where a high secondary attack rate occurred, suggesting a high infectivity. The illness was described as 'hyperemesis hiemis', or winter vomiting disease, reflecting its clinical presentation and seasonality.[8] Some forty years later, laboratory studies in the USA identified a small round virus in faecal samples by electron microscopy from infected patients, and the term Norwalk virus was coined (based on the geographical location of the outbreak) for this first ever description of a virus aetiology for the syndrome of winter vomiting disease.[9] Subsequently, this syndrome has become known as epidemic non-bacterial gastroenteritis.[10]

2.4 Following the success of establishing a virus aetiology for epidemic non-bacterial gastroenteritis, studies around the world established numerous other human faecal viruses which were subsequently shown to cause viral gastroenteritis in man. These included rotavirus, adenovirus, caliciviruses and astroviruses which were all thought to represent different families of viruses.[11] All these viruses were shown to be pathogens, mainly in populations of young children, in contradistinction to the Norwalk group of viruses which affected all age-groups and which were a common cause of outbreaks of gastroenteritis.[10]

2.5 As electron microscopy was the only means by which these viruses could be identified in the early 1970s, the method became established as the standard procedure for examining human faecal samples. The identification of each virus was dependent on the recognition of morphological features characteristic of each virus group.

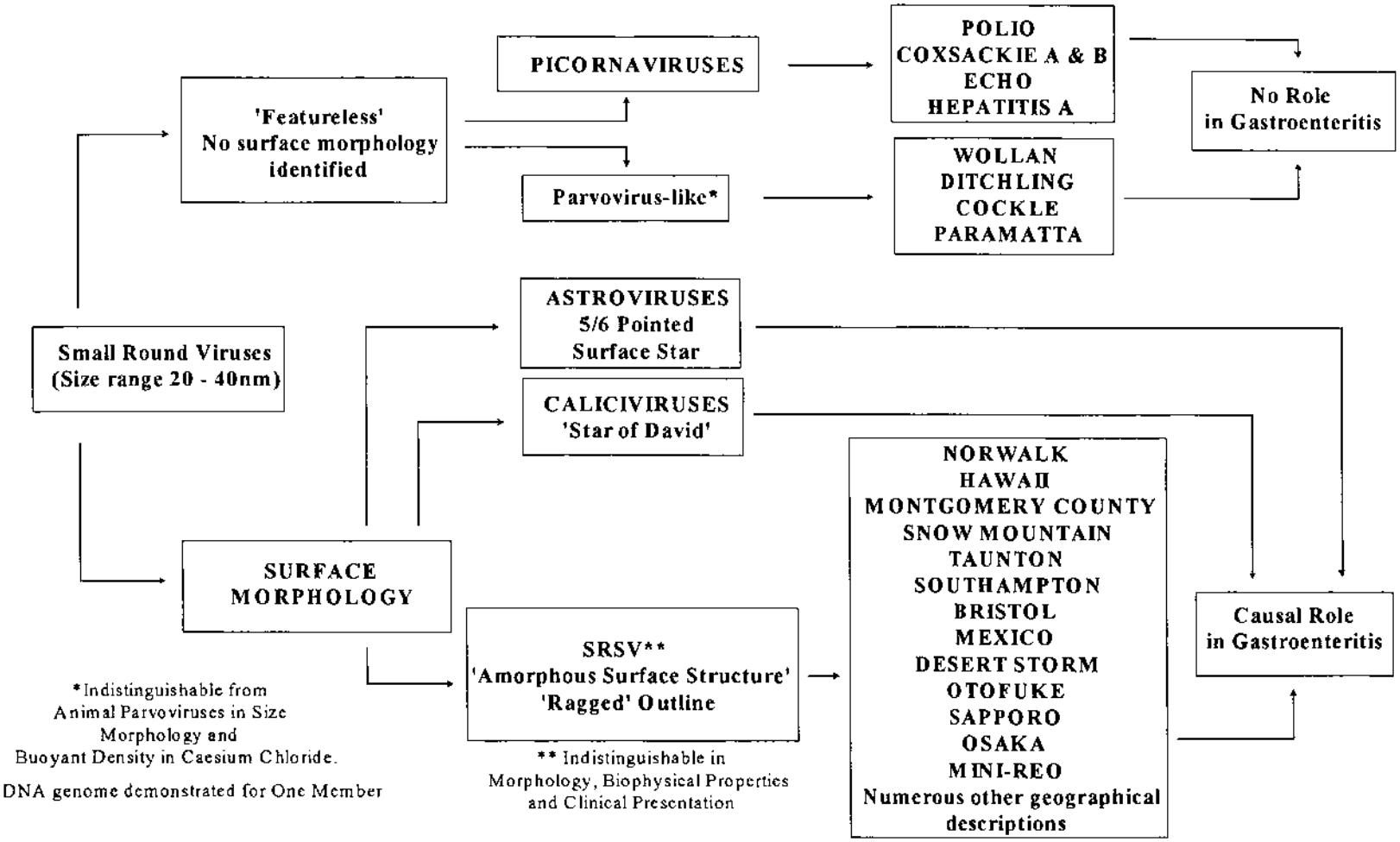
2.6 In order to improve the surveillance of these viruses as a cause of disease in man, it became necessary to establish criteria for their definitive identification in the electron microscope. This was particularly important for those viral pathogens which were described as 'small round viruses' and which included Norwalk virus, astroviruses and the classical caliciviruses. As a result, a comparative study of human and animal enteric small round viruses was carried out in the UK and morphological criteria were established to differentiate small round viruses from each other and which could then be used for the surveillance of these viruses in cases of gastroenteritis in the UK.[12] This classification scheme is shown in Figure 2.1 and has been the basis for reporting these viruses in the UK for many years.

2.7 Comprehensive, world-wide studies have now shown that the Norwalk group of viruses is represented by multiple serotypes and that all viruses within this group are morphologically indistinguishable in the electron microscope and are clinically indistinguishable in their presentation in man. In recognition of these multiple serotypes within the same family, it became apparent that the term Norwalk virus was inappropriate for these antigenically distinct, but morphologically identical, viruses. Thus, the term small round structured viruses (SRSV)[12] was used as an interim classification scheme for this group of viruses to aid surveillance purposes in the UK. It has been adopted world-wide. The terms SRSV, Norwalk and Norwalk-like viruses are therefore interchangeable. Recent molecular studies have established that the Norwalk group of viruses (SRSVs) can be sub-divided into two genogroups.

Viral gastroenteritis and viral foodborne infections.

2.8 All the small round viruses causing human gastroenteritis and which have been incriminated in foodborne gastrointestinal disease can be recognised and sub-divided by their characteristic structure when examined in the electron microscope (Figure 2.1). They all cause either sporadic or epidemic gastroenteritis in man but only SRSVs have been consistently implicated in foodborne gastroenteritis.[10,13,14,15,16,17,18,19,20,21,22,23,24]

Figure 2.1 : Classification of small-round viruses by electron microscopy (pre-molecular characterisation)



*Rotaviruses, *adenoviruses, *astroviruses and caliciviruses*

2.9 The current epidemiological evidence suggests that these are mainly paediatric infections giving rise to long term immunity into adult life.[10] For this reason, foodborne infections with these viruses are extremely rare (Chapter 3).

2.10 Rotavirus is the commonest cause of childhood diarrhoea but it is not usually foodborne. SRSVs are a major cause of epidemic non-bacterial gastroenteritis worldwide.[10,25] The other agents associated with viral gastroenteritis occur far less commonly (Chapter 3). As SRSVs are the only group of viruses commonly incriminated in foodborne viral gastroenteritis, the remainder of this Chapter concentrates on this group.

The agents

2.11 The viruses which cause most of the gastroenteritis in man, and their taxonomic status, are shown in Table 2.1, together with hepatitis A. World wide, only SRSV and hepatitis A virus are significant causes of foodborne illness.

Table 2.1: 'Small-round' human enteric viruses - taxonomic status.

Virus	Disease	Asymptomatic infection	Family	Nucleic acid	Genogroups	Serotypes
SRSV	Epidemic gastroenteritis (all age groups)	Not proven	Caliciviridae	Single stranded RNA	2	Multiple
Classical Calicivirus	Mainly paediatric gastroenteritis (Epidemic)	Yes	Caliciviridae	Single stranded RNA	2 (distinct from SRSV)	4
Astrovirus	Mainly paediatric gastroenteritis (Endemic)	Yes	Astroviridae	Single stranded RNA	1	7
Hepatitis A	Hepatitis	Yes	Hepatovirus	Single stranded RNA	1	1

Physicochemical properties

2.12 The major physicochemical properties of the agents, and their susceptibility to inactivating procedures and chemicals, are shown in Table 2.2.

Biological properties

2.13 In general, the routine diagnosis of all these viruses by isolation of the agents in laboratory cultures is not successful. Current knowledge of their biological properties is shown in Table 2.3. SRSVs do not naturally infect other animal species and naturally occurring infection of animals with antigenically-related viruses has not been described. As SRSVs have never been propagated in the laboratory, production of viral proteins for laboratory use has only been

* NB: these are not small-round viruses

Table 2.2: Bio-physical properties of small-round human faecal viruses

Virus	Nucleic Acid	Size	Inactivation by:				
			pH≤3	Ether/ Chloroform	Alcohol 70%	60°C'	Free Chlorine ≥10mg/L
SRSV" Norwalk/ Norwalk-like	RNA	32-38 nm	No	No	Partially	No (30 mins)	Yes
Calicivirus	RNA	32-38 nm	No	No	Partially	No (30 mins)	Yes
Astrovirus	RNA	28±1 nm	No	No	Yes	Yes (10 mins)	Yes
Hepatitis A	RNA	27-32 nm	No	No	Yes	No (60 mins)	Yes

' - Dependant on suspending media. " - Most data derived from studies on Norwalk virus

Table 2.3: Biogical properties of human gastroenteritis viruses

Agent	Multiplication in Cell Culture (Cell Lines)	Related Animal Viruses Cause Similar Illness in Animals
Adenovirus (Types 40 and 41)	Yes (HEK, Graham 293)	No
Astrovirus	Yes (HEK, LLCMK2, Ca Co-2)	Yes (Sheep, Cats, Calves)
Calcivirus	No	No
Rotavirus	Yes (Primary MK, MA - 104)	Yes (Rotavirus groups A - E)
SRSV	No	Yes (Newbury Agent - Calves)

possible since the advent of methods of genetic engineering. This has enabled the production of viral proteins which can be used to develop the necessary reagents for user-friendly laboratory tests in the future.

Clinical Disease

Gastroenteritis

2.14 SRSV-related gastroenteritis occurs throughout the year and re-infections are common. The clinical symptoms may be mild, with the result that infected individuals continue to work. As a result, the opportunities for infected food handlers to unwittingly spread infectious virus at work are increased. This also serves to increase the risk of spread to secondary cases, particularly in closed communities. Following an incubation period of 15-50hrs (dose dependent),[10] the onset of clinical symptoms is rapid (fever, malaise, abdominal cramps, projectile vomiting, diarrhoea) and the virus spreads easily from person to person by the faecal/oral route. Environmental contamination and spread by

vomit are common.[26,27] Spread by contamination of food by food handlers may also occur. Excretion of SRSVs occurs throughout the symptomatic period (usually 24-48hrs) and for at least 2 days after recovery. The clinical and epidemiological features of all the gastroenteritis viruses are shown in Table 2.4.

Table 2.4: Clinical and Epidemiological Features of Gastroenteritis Viruses

Agent	Mode of Spread	Incubation Period	Infectious Dose (Virus particles)	Transmissibility to close contacts	Symptoms	Duration of Viral Shedding (by EM)	Duration of Infectivity	Attack Rates in Close Contacts
Adenovirus (Types 40 and 41)	Faecal/oral	5-7 days	N.K.	Moderate	Watery diarrhoea and fever	5-7 days	5-7 days	Moderate
Astrovirus	Faecal/oral	3-4 days	<100	Moderate	Mainly[1] diarrhoea	2-3 days	2-4 days	Moderate
Calicivirus	Faecal/oral	24-48 hrs	10-100	High	Diarrhoea[1] and vomiting	1-2 days	2-4 days	High
Rotavirus	Faecal/oral	24-48 hrs	<100	High	Diarrhoea and vomiting	4-7 days	4-7 days	High
SRSV	Faecal/vomit /oral	15-50 hrs	10-100	High	Diarrhoea[11] and vomiting	1-2 days	2-4 days	High

[1] Subclinical infection common in children.

[11] Projectile vomiting occurs in >50% of cases.

Pathogenesis

2.15 Spread of these viral agents occurs by the faecal/oral route and, additionally, in SRSV infection, also by hand to mouth transfer of infected vomit from the contaminated environment and, possibly, by ingestion of aerosolised vomit from affected cases.[26] The human infectious dose in SRSV infection is thought to be as little as 10–100 virus particles,[8] which explains the ease of spread and the explosive outbreaks commonly recognised. Following ingestion, the viruses resist the acid pH of the stomach to reach the small intestine where they replicate in the mucosal epithelium. Viral replication in mucosal cells results in widespread damage of the epithelium with blunting of the intestinal folds (*villi*) and a decrease in certain digestive enzymes.[28] A delay in gastric emptying is also evident, particularly with SRSVs, and it is this abnormality of gastric function which is thought to be responsible for the nausea and projectile vomiting characteristic of SRSV infection.

Resistance to infection

2.16 Multiple serotypes of SRSVs are recognised.[25,29,30] This explains the epidemiology of the disease and the possibility of re-infections over time.

Volunteer studies suggest that resistance to infection with the same virus is complex and poorly understood[25] but may be achieved by two mechanisms:-

- some individuals appear to be resistant to clinical infection despite receiving doses of the virus which would normally cause disease in a susceptible person. In these cases, protection is not related to the level of serum antibody to the infecting virus. Defective viral attachment to the gut wall receptor, possibly genetically mediated, has been postulated as underlying this finding but, as yet, no evidence exists to support or refute this;
- in those patients who do become infected, serotype-specific serum antibodies i.e. capable of protecting against challenge with the homologous infecting virus, persist for between 6–14 weeks. However, there is no protection at any time against challenge with an antigenically different (heterotypic) virus. After 6–14 weeks, all protective immunity is lost and rechallenge with the original virus can produce just as severe disease.

Management and control of viral gastroenteritis.

2.17 Four aspects are necessary for the adequate control and management of viral gastroenteritis[31] in semi-closed communities or in foodborne outbreaks:-

- early clinical suspicion of viral gastroenteritis;
- containment and management of the acute episode/outbreak;
- prevention of person-to-person spread;
- rapid laboratory confirmation of the cause, where possible;

General principles of management of cases of gastroenteritis and foodborne infection

2.18 Although rotaviruses, adenoviruses, astroviruses and caliciviruses are well recognised as causes of paediatric gastroenteritis, they are uncommon in foodborne viral gastroenteritis infection and are not considered further. The essential clinical features are shown in Table 2.4.

Management of SRSV infection and outbreaks

2.19 Currently, the awareness, availability and access to good laboratory confirmation of SRSV (and other viral diarrhoea agents) may be variable. It may therefore be necessary to institute specific control and containment measures based on clinical suspicion alone. Control measures should not be delayed pending laboratory confirmation. The short duration of the illness, and the high person-to-person transmission rates, are a considerable hindrance to effective control of outbreaks. However, outbreaks associated with SRSVs have a pattern, if virological results and testing are not available, that can lead to strong suspicions of their involvement. Kaplan and colleagues defined the criteria which give a strong positive prediction.[13]

Kaplan Criteria for suspecting an outbreak is due to SRSV

2.20 The Kaplan Criteria for suspecting that an outbreak is due to SRSV are:-

- stool cultures negative for bacterial pathogens. It must be emphasised that, when available, electron microscopy can produce a definitive answer on the aetiology of an outbreak in less than 24hrs, whereas bacteriological examinations can take 24-48hrs;
- mean duration of illness 12-60 hours;
- vomiting in >50% of cases;
- incubation period (if known) of 15-50 hours.

Clinical specimens in a suspected case/outbreak of SRSV gastroenteritis

2.21 Increasingly, molecular techniques are being applied which are more sensitive than the traditional use of electron microscopy. The most crucial aspect of collecting specimens for laboratory confirmation of a suspected SRSV (or other gastroenteritis viruses) outbreak is to ensure that faeces are collected from those recently affected. Experience has shown that stools obtained more than 2 days after onset are rarely positive by electron microscopy (but may be suitable for molecular diagnosis). Specimens collected within 2 days of onset of the illness can be placed into any sterile container and examined as soon as possible, or stored at +4 $^{-}$C until they can be processed.

2.22 Although virus has been detected in vomit,[26] the titre is less than in faeces. In addition, the examination of vomit is more difficult than examination of faeces, and many laboratories do not routinely examine it. Solid phase immune electron microscopy (SPIEM) is a particularly suitable method for the examination of vomit.

2.23 Serum collected at the time of the illness and 4 weeks later may also be used to confirm the agent as an SRSV, if faecal samples have not been examined, although such methods are not routinely available. The complexity of interpreting antibody levels in individuals following infection is also a limiting factor. The mildness of the illness does not normally justify the venepuncture of patients and serological methods are therefore mainly used for retrospective epidemiological investigations.

Management of acute gastroenteritis

2.24 Most cases are self limiting and resolve within 48-72 hours without complications. Treatment of the abdominal cramps and gastrointestinal symptoms may be achieved symptomatically with bismuth subsalicylate. Oral fluid and electrolyte replacement with isotonic fluids usually suffices, but parenteral fluids may be necessary if severe dehydration occurs. The major problem, especially with SRSVs, is the rapid spread to other individuals, particularly in semi-closed communities. In an outbreak, anti-emetics may be used specifically to reduce vomiting and therefore spread of the virus through environmental contamination.

Control and containment of the acute episode/outbreak and prevention of spread to others in hospitals/institutions

2.25 The following steps should be taken to control and contain the acute episode/outbreak and to prevent its spread to others in hospitals/institutions:-

A. Containment of infectious individuals

- attempt to isolate/segregate symptomatic patients;
- affected staff should be off duty until fully recovered;
- try to avoid transfer of patients and staff to other wards;
- close the ward to prevent the introduction of new susceptibles.

B. Hand hygiene

- emphasize the importance of hand washing;
- provide alcoholic chlorhexidine hand rub as an alternative;
- gloves should be worn for dealing with excreta and vomit.

C. Environmental decontamination

- wearing disposable gloves and a plastic apron, cover vomit and faecal spillages with absorbent paper towels. On hard surfaces, the paper towel can be soaked in 500ppm hypochlorite. Envelope with solid material in the towels and discard into a clinical waste bag;
- hard surfaces should be then cleaned with general purpose detergent prior to disinfection with 500ppm hypochlorite solution;
- carpets will not withstand hypochlorite at this concentration and the recommendation is that, after removal of gross contamination, the carpet should be cleaned with hot water and detergent and, if possible, disinfected with an efficient steam cleaner.

In the community and the home

2.26 Broadly, the same principles to those detailed above apply to control and containment in the community. In the domestic environment, the guidelines set out in Annex C should be followed.

Non-diarrhoeal viruses : viral hepatitis

Hepatitis A

2.27 Viral hepatitis in man is caused by at least six taxonomically distinct viruses classified as types A-F. Type A, infectious hepatitis,[32] is the most common cause of hepatitis in man and is transmitted by the faecal/oral route to susceptible individuals

Classification

2.28 Hepatitis A is a member of the enterovirus family which has recently been ascribed to a new family, the hepatoviruses.[33] The viruses measure 27-32 nm and have a single stranded positive sense RNA viral genome. The viral genome encodes three viral capsid (coat) proteins to which most of the protective immune response is directed.

Physicochemical properties

2.29 In general, the virus resists inactivation by lipid solvents, extremes of pH and moderate temperatures. Table 2.2 documents the properties of hepatitis A virus.

Biological properties

2.30 The virus is spread by the faecal/oral route, either directly from person-to-person or by contaminated water and food, particularly filter feeding shellfish. Hepatitis A virus is difficult to propagate in *'in vitro'* culture and this method is not routinely used, although one strain has now been adapted to grow in cell culture.

Pathogenesis

2.31 Following ingestion, the acid-stable virus passes through the stomach to the small intestine where limited replication occurs before systemic spread and infection of the liver. Damage of the liver cells (hepatocytes) is responsible for the symptoms of hepatitis (including jaundice). Mild gastrointestinal disturbance may accompany the hepatitis.

Resistance to infection

2.32 Only one serotype of hepatitis A virus has been described. Human infection results in the production of neutralising antibody which persists for life and is protective against subsequent viral challenge. Epidemiological data suggest that 50% of adults over the age of 50 years are immune to hepatitis A in the UK. This relatively high herd immunity explains the infrequent foodborne outbreaks in the past. However, recent studies have indicated that the percentage of immune individuals in the UK is declining, as a consequence of improved standards of living, and this may give rise to explosive foodborne outbreaks in the future.

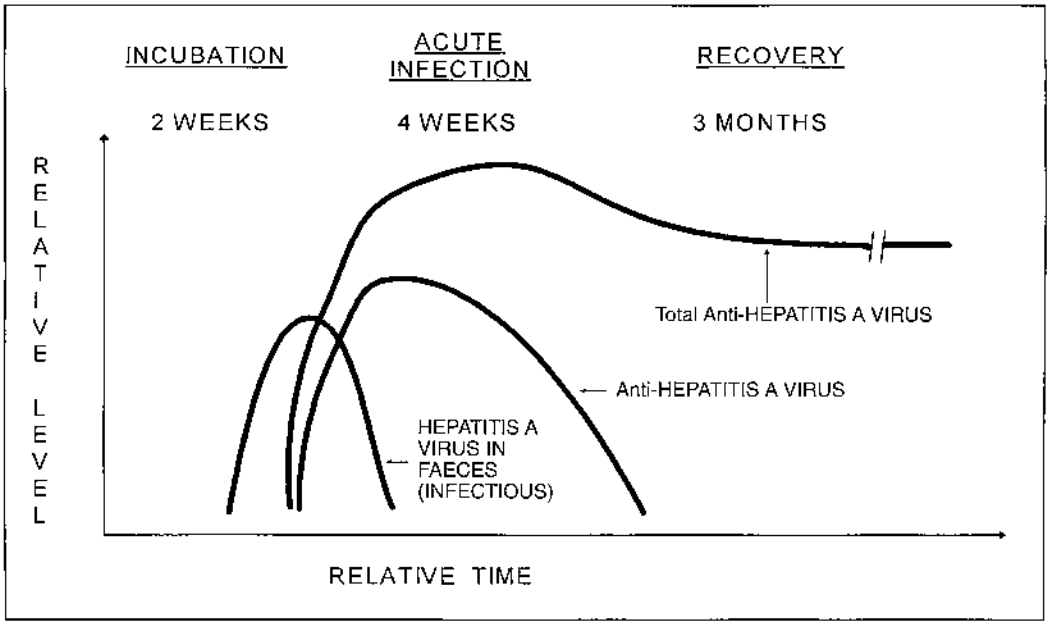
Clinical infection

2.33 The majority of infections acquired in childhood are asymptomatic. Adults are more likely to have symptomatic disease, which classically presents as prodromal malaise of 1-2 weeks duration, followed by acute hepatitis (Figure 2.2). The incubation period of the disease is 2-6 weeks and viral shedding lasts 3 weeks, peaking just prior to the onset of jaundice (Figure 2.2). Complications are few with less than 1% of cases developing overwhelming liver failure.

Laboratory confirmation

2.34 Good laboratory confirmation, by detection of specific hepatitis A IgM antibodies using commercial enzyme immunoassay kits, has been available for over 10 years. Originally, the antigen in the kits was produced from virus propagated in chimpanzees and marmosets. Nowadays, recombinant antigens are produced from genetically-engineered virus. Serum samples collected shortly after, and up to 8 weeks following, the onset of jaundice usually contain sufficient IgM to make a reliable diagnosis. The direct detection of the virus by electron microscopy, or by the use of immunoassays in faecal samples, is not appropriate.

Figure 2.2 : Serological profile of typical hepatitis A virus infection



Management

2.35 Acute cases of hepatitis A can be managed symptomatically at home. Hospital admission is not required unless deterioration of liver function occurs. Cases are less infectious once the jaundice has appeared. Management of foodborne outbreaks is dealt with in Chapter 5.

Prevention of person-to-person spread

2.36 Close contacts of a case in the 2 weeks prior to the appearance of jaundice are at risk of infection. Post exposure prophylactic measures, for those at risk, are available and include normal immunoglobulin and/or hepatitis A vaccine. Appropriate advice can be obtained locally from Consultant Microbiologists/ Virologists, Consultants in Communicable Disease Control - in Scotland, Consultants in Public Health Medicine (CD and EH), Consultants in Infectious Diseases or Occupational Health Departments. Effective vaccines are available for hepatitis A protection in food handlers. However, the Joint Committee on Vaccination and Immunisation have concluded that food packagers and food handlers in the UK have not been associated with hepatitis A virus transmission sufficiently often to justify their immunisation as a routine measure.[1]

Conclusions

2.37 We have concluded that:-

- awareness of foodborne virus gastroenteritis is increasing;
- a shortage of expert laboratory diagnostic facilities continues to hamper the accurate recognition of viruses causing foodborne illness;
- SRSVs are the major cause of such outbreaks;
- the most crucial aspect of collecting specimens for confirmation of an outbreak of foodborne viral infection is to ensure that faeces are collected from those recently affected. Stools obtained more than two days after onset are rarely positive by electron microscopy;
- faecal contamination of any food may occur, irrespective of whether it is raw or cooked;
- personal hygiene, and education and training of food handlers are essential to reducing the risk of SRSV transmission;
- effective management of SRSV cases and outbreaks, and implementation of control of infection measures, can be based on early clinical recognition;
- in the UK, herd immunity to hepatitis A is declining, giving rise to the potential for future outbreaks of foodborne hepatitis A;
- although effective vaccines are available for hepatitis A protection in food handlers, the JCVI have concluded that routine immunisation is not currently justified;

- outbreaks of foodborne infection associated with SRSVs follow a clear pattern ("Kaplan criteria") which, in the absence of virological results and testing, can lead to strong suspicions of their involvement.

Recommendations

2.38 We strongly recommend that, for cases of infection fulfilling "Kaplan criteria", control measures are instituted immediately without waiting for laboratory confirmation - although confirmation of diagnosis in due course is desirable (eg. for epidemiological and research purposes).

2.39 We recommend that the JCVI keep under review the question of the routine immunisation of food handlers against hepatitis A virus.

CHAPTER 3

OCCURRENCE OF FOODBORNE VIRAL INFECTION IN THE UK

Reporting foodborne viral infections

3.1 The two main sources of data in the United Kingdom on foodborne viral infections are:-

- the voluntary reports of laboratory diagnoses made by clinical laboratories.[34] In England and Wales these are made to the Public Health Laboratory Service Communicable Disease Surveillance Centre (PHLS CDSC), in Scotland to the Scottish Centre for Infection and Environmental Health (SCIEH) and in Northern Ireland to the Department of Health and Social Services (DHSS(NI));
- reports of outbreaks of gastrointestinal disease by Consultants in Communicable Disease Control (in Scotland, Consultants in Public Health Medicine), Environmental Health Officers and Microbiologists to PHLS, SCIEH and DHSS (NI).[33]

Viral Gastroenteritis

Laboratory reports

3.2 In England and Wales, laboratory reports of small round structured virus (SRSV) identified in faeces samples increased sharply from 418 cases in 1990 to 2,387 in 1996. Reports followed a seasonal pattern, with peaks in winter. A proportion of the increase in reports is believed by the PHLS to reflect increased awareness of SRSV, leading to increased referral of samples for electron microscopic investigation. In Scotland from 1990 to 1996, SRSV reports remained constant at 78 to 94 cases per year. In Northern Ireland, for the same period, between 7 and 35 cases per year were recorded, with peaks in 1992 and 1995.

3.3 These data probably significantly underestimate the true incidence of the disease. Most cases are mild, self-limiting, short duration illnesses and most patients will not seek medical attention. Even when they do, faeces sampling of sporadic cases for viral examination is unusual. Furthermore, in sporadic cases of viral infection, it is very unlikely that a source will be found.

Reports of outbreaks

3.4 Data on outbreaks of viral gastroenteritis are likely to be more complete than for sporadic cases but there are a number of reasons why these data understate the true position.

- the low sensitivity of current viral diagnostic methods means that a high proportion of suspected SRSV outbreaks are not confirmed microbiologically;
- recognition of viral foodborne outbreaks is subject to several potential biases. Outbreaks at social functions, affecting discrete groups, are more likely to be recognised than outbreaks associated with contaminated food from retail or wholesale outlets in which cases are dispersed in the community;
- investigations of outbreaks of SRSV infection may not be as successful as for outbreaks due to other pathogens such as *Salmonella*. The disease is usually less severe so they may be given lower priority; the longer incubation period and the longer time for laboratory testing may delay recognition of the outbreak, reducing the chance of eliciting accurate information; and the lower sensitivity of laboratory diagnosis makes definition of cases for epidemiological investigation less precise.

3.5 A further difficulty with data on outbreaks concerns classification of the mode of spread. The current surveillance system does not have explicit criteria for the mode of spread of an outbreak. The local investigator classifies the outbreak as mainly foodborne or person-to-person spread according to local judgement although several modes of transmission may operate in a single outbreak. For example, SRSV outbreaks have been reported following an episode of vomiting which releases large numbers of virus particles into the environment. Foodborne infection may be the cause of the first case, with subsequent cases resulting from exposure to vomit or faeces. Cases due to faeces/vomit contamination may lead to contamination of food and, thus, secondary foodborne cases. It is possible that a significant number of outbreaks in hospital, homes etc, often attributed to person-to-person spread, have an element of foodborne infection. One case of foodborne infection may lead, by person-to-person spread, to a large and prolonged outbreak.

3.6 Even when foodborne infection is suspected, confirmation of viral contamination of food is not possible routinely. Therefore, evidence to implicate a particular food will be solely epidemiological. Epidemiological investigation will seek to discover whether those affected were more likely than those not affected to have consumed a particular food or drink. Food or drink statistically associated with illness can be investigated by investigating food preparation and handling. In SRSV outbreaks it is not uncommon to find that several food items are independently associated with illness and that these have been handled by a particular foodhandler suffering from gastroenteritis.

3.7 The limitations of the available data on outbreaks outlined above need to be borne in mind when assessing the following statistics.

3.8 A review of SRSV outbreaks in England and Wales between 1981–90 identified 100 outbreaks affecting 4,746 cases. Half of the outbreaks (51%) occurred in hospitals or residential/nursing homes. These are likely to have a large

component of person-to-person spread by the faecal/oral route. Fourteen outbreaks were in hotels and 7 at receptions; these are more likely to be principally foodborne.

3.9 Enhanced surveillance of general outbreaks of infectious intestinal disease was introduced by the PHLS in 1992. From 1992-1995, 2,680 general outbreaks were reported in England and Wales and a minimum dataset was obtained on 2,149 (80%). 25% of the 2,149 outbreaks were due to *Salmonella* infection, 33% (710) to SRSV infection, 2% (53) to Rotaviruses, 0.5% (9) to astroviruses, 0.5% (10) to small round viruses (SRV) and there were two calicivirus outbreaks and one coronavirus outbreak. 20% (433) were of unknown aetiology. Of these 433 outbreaks of unknown aetiology, 56% (243) were suspected to be viral in origin. In 1994, the number of recorded SRSV outbreaks (155) exceeded the number of Salmonella outbreaks (107) for the first time.

3.10 In 1995 further enhancement of outbreak surveillance was conducted via the PHLS electron microscopy network. Eleven laboratories investigated over 900 outbreaks of infectious intestinal disease and SRSVs were identified as causal agents in over half. In a further 33% of outbreaks SRSVs were believed, on clinical and epidemiological grounds, to be the causative agent but specimens were collected too late to allow positive identification. 84% of these outbreaks occurred in hospitals or nursing/residential homes. The very large increase in outbreaks identified by this new system suggests very significant under-reporting of outbreaks in past years.

3.11 Of the 2,149 outbreaks of infectious intestinal disease identified between 1992-95 and referred to above, 39% (844) were described as "mainly foodborne", 50% (1,068) were "mainly person-to-person" and 6% (126) were mixed. SRSVs accounted for 54/844 (6%) of the "mainly foodborne" outbreaks.

3.12 Of the 785 outbreaks reported between 1992 and 1995 in England and Wales caused by a single virus, 8% (61) were reported to be "mainly foodborne" and affected over 2,120 people. (See Table 3.1).

Table 3.1: Aetiological agents associated with foodborne outbreaks of viral gastroenteritis, 1992-95

Virus	General outbreaks	Foodborne
SRSV	710	54
Rotavirus	53	1
SRV	10	4*
Astrovirus	9	2
Calcivirus	2	–
Coronavirus	1	–
Astro + SRSV	2	–
Rota + SRSV	2	–
Adenovirus serotype 40 + *Campylobacter spp*	1	–
Astro + SRSV + *Shigella sonnei*	1	–
SRSV + *Clostridium perfringens*	1	–

* SRV is not considered to be a cause of viral gastroenteritis and these cases were not confirmed.

3.13 In these 61 foodborne outbreaks the place of the outbreak was reported as:-

Restaurants/hotels	30
Other commercial catering sector	8
Institutions	15
Private Houses	6
Unknown	2

3.14 In only 35 (65%) of the 54 SRSV foodborne outbreaks was a food vehicle identified. The foods reported were oysters (11), salad (4), sandwiches (2), turkey (2), "Chinese food", carrots, custard slices, fish dinner, lobster tail, margarine, meat pies, melon and papaya cocktail, pasta salad, peach and raspberry gateau, prawn cocktail, raspberry syllabub, raw mushroom and stilton starter, vegetable soup and watercress. Virus was identified in the food in only two outbreaks (in both cases the virus was identified in oysters by reverse transcriptase polymerase chain reaction). Of the 35, the food was served raw in 18, including the 10 raw oyster outbreaks.

3.15 Infected food handlers were believed to have been implicated in 19 outbreaks, although laboratory confirmations of viral infection was obtained from food handlers in only 4 of these.

3.16 Ten outbreaks were reported to be due to SRV although SRVs are not generally regarded as causing viral gastroenteritis. Such outbreaks present features similar to those of SRSVs. Four (with 164 cases) were foodborne and occurred in a canteen, a hotel restaurant and a golf club. An infected foodhandler was believed to be the source in one of these, raw oysters were the vehicle of infection in another and sausages in a third outbreak. The vehicle was not known in the fourth outbreak.

3.17 Two foodborne astrovirus outbreaks were reported, one of which was associated with a visit to a sewage plant where visitors inadvertently drank non-potable water. In the other outbreak no further details were available.

3.18 One foodborne outbreak of rotavirus, with 23 cases, was reported and was associated statistically with eating chicken tikka masala.

3.19 In Scotland no viral foodborne outbreaks were reported from 1992 to 1995. In 1996, following introduction of an improved surveillance system, 25 outbreaks of viral gastroenteritis involving more than one family were reported and summary details were obtained for 20 of these. Two of the 20 were believed to be foodborne. In Northern Ireland from 1992-1995, 11 viral gastroenteritis general outbreaks were identified, 3 confirmed as SRSV, 1 as adenovirus (untyped) and one calicivirus. Three of the 11 were suspected to be foodborne infections.

Hepatitis A foodborne infection

3.20 Foodborne incidents of hepatitis A are rare in the UK. In 1978 a widespread outbreak covering the Midlands and the North of England was traced to inadequately cooked mussels. In 1980/81 another widespread outbreak, in the South of England, was attributed to undercooked cockles. Between 1987 and 1988 five further outbreaks of hepatitis A due to consumption of molluscan shellfish were recorded by CDSC. In 1985 several outbreaks of hepatitis A were traced to frozen raspberries, probably contaminated by workers during picking or weighing. During the 1980s there were five other outbreaks reported to CDSC, all attributed to contamination of food by infected food handlers. From 1992 to 1996, 19,147 reports of hepatitis A infections were received and in 87 cases (0.5%) a food (mainly shellfish) was reported as a possible source.

Discussion

3.21 Despite the limitations of the surveillance data, two patterns of SRSV outbreaks emerge - those due to raw oysters and those in which an infected food handler is suspected. Food handlers who are symptomatic, ie with diarrhoea or vomiting at the time of handling food, present the greatest risk but outbreaks have been attributed to contamination of food by food handlers up to 2-3 days after cessation of symptoms.[20,35,36] More rarely, presymptomatic transmission has been reported.[37] In the last case the food handlers have had family members with symptoms at the time of the incident so that mechanical contamination of hands or clothing from the cases at home to the food at work could not be excluded as the cause.

3.22 The available laboratory data on sporadic cases is likely to greatly underestimate the true incidence of the disease. The Department of Health funded Infectious Intestinal Disease (IID) Study will provide a more accurate picture. Preliminary results confirmed the views of the Foodborne Viral Infections Working Group on the contribution of SRSVs to foodborne disease and the Working Group decided not to delay completion of this Report pending receipt of the full analysis of the IID data. Outbreak data are also limited by a number of factors including lack of standardised reporting across the UK, lower priority for local investigation (which produces a knock-on effect nationally), less sensitive laboratory tests and incomplete reporting. Clinical and epidemiological criteria have been developed to classify such outbreaks as probable SRSVs in origin. Typical features include the occurrence of vomiting as well as diarrhoea in a high proportion of cases, a relatively long incubation period (15-50 hours) and a significant secondary household attack rate due to faecal/oral spread.

3.23 We have already recognised the need for more thorough systematic investigation of outbreaks in our earlier reports on *Salmonella* in Eggs[38] and on Poultry Meat.[39] Guidance on the management of outbreaks for foodborne illness has been published by the Department of Health,[40] by the Scottish

Office and by DHSS(NI). This guidance includes procedures for the epidemiological investigation of outbreaks.

Conclusions

3.24 The main conclusions for the epidemiological data are that:-

- nearly all reported foodborne viral infections are caused by SRSV;
- laboratory reports of isolations greatly underestimate the true incidence;
- identification of the source of infection and mode of transmission of reported outbreaks is difficult and it is likely that current data underestimate the number of cases involving transmission by food and infected food handlers;
- current foodborne SRSV infection in the UK falls into two patterns, namely outbreaks due to consumption of raw oysters and outbreaks due to contamination of foods by infected food handlers. In the latter type, any handled food or drink may be a vehicle of infection;
- foodborne infection with hepatitis A virus is rare. When it does occur, it is the result of infected food handlers contaminating ready-to-eat food or, even less frequently, from consumption of raw or under-cooked bivalve molluscan shellfish.

Recommendations

3.25 We recommend that the Government takes steps to improve harmonisation of detection, reporting and surveillance of SRSV infections throughout the UK.

3.26 We recommend that the Government encourages thorough investigation of viral gastroenteritis with a view to establishing a comprehensive and timely picture.

3.27 We recommend that Government maintains, develops and enhances surveillance systems throughout the UK, including the Electron Microscopy Network, in order to better define the problem.

CHAPTER 4

DETECTION METHODS FOR VIRUSES IN CLINICAL SAMPLES AND FOODS

Introduction

4.1 Historically, the difficulty of growing the viruses that cause human gastroenteritis has necessitated the use of electron microscopy (EM) for their detection and characterisation. This technique is only applicable to human clinical samples (faeces). It has no role in the examination of food as the method is insufficiently sensitive for the detection of the small numbers of virus particles which may be present. Whilst rotaviruses, adenoviruses, astroviruses, SRSVs and caliciviruses are all causes of human gastroenteritis, only SRSVs are important in foodborne gastroenteritis.

4.2 The complete genome of three strains of SRSV[29,41,42] and one strain of human enteric caliciviruses[43] have been sequenced. New molecular techniques now offer the possibility of detecting these viruses in clinical samples and shellfish. Although the sequence of the hepatitis A virus is known, these techniques are not necessary for the diagnosis of human infections as serological methods are adequate. They could, however, be applied to the detection of hepatitis A virus in shellfish.

The methods for clinical samples (antigen/nucleic acid detection)

Electron Microscopy - negative staining of virus particles

4.3 Examination of faeces by electron microscopy has been the major method used for the diagnosis and surveillance of these viruses in the UK[11] and remains the only method which is routinely available for the detection of SRSVs. EM has been used to characterise the appearance of all these viruses and provides a basis for their classification (see Chapter 2).

4.4 The method requires dedicated and highly skilled staff and is relatively insensitive requiring a minimum of 10^6 viral particles/g of faeces. For optimum results it is essential to have stool samples collected within 48 hours of onset of clinical symptoms. EM remains the only "catch all" tool for the detection of all the gastroenteritis viruses and the most rapid of all the available methods. EM methods vary considerably between laboratories, reflecting the particular preference of the individual operator. No single method has been shown to be significantly superior to other methods but all have in common a pre-examination procedure of concentrating viruses from the clinical sample. This may be achieved by ultra centrifugation, precipitation of the virus or by antibody capture methods.

Solid Phase Immune Electron Microscopy (SPIEM)

4.5 The method involves coating the EM grid with serum containing the antibodies to the virus. Again, methods vary but overall the use of SPIEM has greatly improved the detection of enteric viruses, particularly SRSVs. The method has been used to determine antigenic differences between closely related viruses and is the basis for the serotyping of astroviruses and caliciviruses, as well as SRSVs.[29,30,44,45] The method is limited to viruses for which antibody can be obtained. Viruses can be detected only if convalescent sera can be obtained from cases in an outbreak. Thus, the assays are limited by the requirement for reagents derived from non-replenishable clinical sources. These limitations should be overcome by the use of genetic engineering techniques to produce recombinant antigens and specific antisera i.e. polyclonal or monoclonal antibodies.

Immunoassays

4.6 Immunoassays to detect the gastroenteritis viruses fall into two categories:-

- *agglutination assays*, in which virus-specific antisera are absorbed onto a latex bead, provide a low cost and relatively specific detection system. Reaction with antigen (virus) causes clumping of the latex beads and this method has been successfully utilised in the detection of rotavirus and adenovirus from faecal samples.

- *enzyme immunoassays* (EIAs) rely upon labelling of the virus-specific antisera enzymatically. Labelled antiserum is reacted with the viral antigen in the stool or culture sample in a microtitre plate following which unbound excess antiserum is washed away. The amount of bound antiserum, which is proportional to the amount of antigen in the sample, is detected by the optical density of a colorimetric reaction produced when the substrate is added to the bound enzyme label. Commercially produced agglutination and EIA assays for the detection of rotavirus group A and adenovirus infections have been available for many years and use monoclonal or polyclonal antibodies specific for the virus being sought. Recently a monoclonal based astrovirus EIA has been developed[46] and is commercially available.

4.7 Immunoassays for the detection of the other small round RNA viruses (SRSV and caliciviruses) are not yet available for routine diagnosis. The following reference tests are available:-

- *Caliciviruses:* a solid phase Radioimmune Assay (RIA) using polyclonal sera, which has now been adapted to an EIA format, has been developed for the detection of caliciviruses[47] and has been shown to be more sensitive than EM.

- *Astroviruses:* monoclonal antibodies to the group antigen have been produced and been shown to detect all seven astrovirus serotypes in cell culture.[48] Modification of this assay has allowed the development of EIAs

which are as sensitive and specific as EM for the detection of astroviruses in stool samples.

- *SRSVs:* development of immunoassays for SRSVs is further behind, largely due to the variation of the gene encoding the main immunological target, the capsid protein. Recently, however, recombinant baculovirus capsid antigens have been produced from a number of strains of SRSVs.[42,49,50] Monoclonal and polyclonal antibodies to these laboratory-produced viral antigens are now being produced and will be available in the next few years for the development of an EIA for the detection of all SRSVs.

Culture techniques

4.8 Although some of the viruses (rotavirus, astrovirus and adenovirus) do replicate in cell culture, this technique has not proved useful for laboratory diagnosis.

Animal models

4.9 Although useful for research into the comparative pathogenesis of the related animal gastroenteritis viruses, and for the production of laboratory reagents, particularly specific antibodies, animal models are not useful for routine diagnosis.

Molecular methods for the detection of viruses in clinical samples and, potentially, food

Reverse transcriptase polymerase chain reaction (RT-PCR)

4.10 The recent characterisation of the complete genome (sequencing) of SRSVs by British[29,42] and American[41] workers has revolutionised the laboratory detection and diagnosis of this group of viruses. This has allowed the targeting of one region of the viral genome (RNA polymerase) and its amplification by the polymerase chain reaction and has been applied by researchers worldwide in the examination of human faecal samples. Sequencing methods have permitted the epidemiological linking of outbreaks and the source virus.

4.11 The method involves the reverse transcription of a cDNA copy of a region of the viral RNA genome (RNA polymerase). This is followed by amplification of a part of the cDNA by means of specific oligonucleotides (primers) which hybridise to the denatured target in the presence of a heat-stable polymerase. Cycling of the temperature of the reaction results in the synthesis of large numbers of copies of the target sequence (amplification), enabling detection either by polyacrilamide/agarose gel or by hybridisation and colorimetric detection using a microtitre format. It should be recognised that each PCR is an individual and distinct 'experiment' and no single method or set of primers are recognised as the 'gold standard' procedure.

4.12 RT-PCR is the most sensitive method for detecting SRSVs and is the method of choice for examining foodborne outbreaks. It does, however, suffer from the

limitation of naturally-occurring inhibitors in both human and food samples which interfere with the enzymatic reaction. RT-PCR, especially if it is an in-house assay, must be carried out in accordance with the accepted guidelines for good practice to avoid contamination and the production of false positives.

4.13 Primers were originally directed against the polymerase gene in anticipation that this was the most conserved region of the viral genome. However, subsequent nucleotide sequencing of a number of polymerase fragments suggests considerable diversity, both in this gene and in other parts of the genome, including the capsid gene.

RT-PCR for other enteric viruses

4.14 The complete genome characterisation (sequence) of both caliciviruses[43] and astroviruses[51] has recently been published.

4.15 Astroviruses and caliciviruses are clinically less of a problem than SRSVs in foodborne infection and are rarely incriminated in foodborne outbreaks. In addition, good immunological reagents already exist for the detection of astroviruses in human faecal samples which negates the need for RT-PCR diagnosis. Similar caveats apply to both adenoviruses and rotavirus, for which good immunological assays are commercially available for detection of virus in clinical samples. The need for detection of these viruses in food rarely arises, although a rapid and simple test might be applicable should one become available.

Adapting molecular methods for detection of virus in food and the environment

4.16 The vast majority of foodborne virus infections in the UK are due to SRSV and, to a small extent, hepatitis A. Similarly, many foodborne virus infections result from the consumption of bivalve molluscs from faecally-contaminated shellfish harvesting areas. The consumption of fresh or frozen food, incidentally contaminated by an infected food handler, may also cause an outbreak of foodborne virus infection and is increasingly recognised. Laboratory assistance in investigating the latter will depend largely on good diagnostic techniques for clinical samples. In the case of contaminated shellfish harvesting areas environmental surveillance of viruses, both in shellfish and in the water, are necessary for good control. The new molecular techniques are particularly useful in this regard.

Detection of viruses in shellfish

4.17 Electron microscopy has never been shown to be useful for the detection of SRSVs in either shellfish or water. This is because of the relatively low concentrations of virus and the presence of large amounts of contaminating protein. Surrogate models were employed to try to understand how viruses behaved in water and shellfish. Polio and other enteroviruses, including hepatitis A as well as F+ bacteriophage, have been used in experiments to investigate the effect of shellfish depuration on viruses. The enteroviruses are

easily assayed in tissue culture, whilst the F bacteriophages can be plaque-assayed in *Escherichia coli* or *B. fragilis* depending on the phage chosen. Recently, astroviruses have also been mooted as a good model because, although they rarely cause foodborne illness, they can be grown in culture.

4.18 Recent developments in RT-PCR for SRSVs have led to direct studies of these viruses in shellfish[52,53,54,55] and water. Methods for extracting the RNA from the shellfish have been optimised using the easily grown polio virus as a model. Recent studies have demonstrated the presence of SRSVs in shellfish implicated in outbreaks of gastroenteritis. Sequencing of the SRSV in human faecal samples, and 'matching' the sequence with that derived from the shellfish, has provided an elegant means of 'fingerprinting' the SRSV as the offending pathogen.[53] Thus, molecular epidemiology has provided a definitive method for monitoring the spread of SRSVs from shellfish to the consumer. Molecular techniques can, however, detect low numbers of viruses as well as viruses which are non-infectious (dead). It is, therefore, possible that these methods may over-estimate the problem in some circumstances. Further work is now needed to correlate detection of enteric viruses in these specimens with disease causing potential.

Antibody detection in human serum samples (sero-diagnosis)

4.19 This is not useful for the diagnosis of acute viral gastroenteritis infections as it is an invasive technique providing retrospective diagnosis and is rarely applicable, particularly in children. Other excellent methods for the detection of virus, antigen and nucleic acid are now available which provide a rapid diagnosis and assist clinicians in patient management.

Hepatitis A

4.20 In human infection with hepatitis A there is little antigenic variation of viral epitopes. One virus serotype appears to be responsible for all human infections. Good levels of IgM are produced in the acute phase of the infection and persist for up to 3 months. Thereafter, persistence of IgG antibodies is a good indication of past infection and denotes life-long immunity.

SRSV

4.21 Detection of antibody has been successfully used in seroprevalence studies of SRSVs. The first useful antibody based assays were developed and used for studying the epidemiology of Norwalk virus in America and other countries where a high prevalence in adult populations has been documented.[25]

4.22 Molecular techniques seem to indicate that there is little virus circulating in the community. This contrasts with the results of antibody assays, which might suggest that the latter are detecting a wide range of responses from antigenically distinct SRSVs.

4.23 More recently, recombinant baculovirus capsid antigens from a number of distinct antigenic strains of SRSVs representing genotypes 1 and 2 have been cloned and expressed in baculovirus and incorporated into an EIA format. Recent studies in the UK using a baculovirus-expressed Norwalk capsid protein have allowed more extensive and definitive sero-epidemiological studies.[56] The expression of other serotypes of SRSV in the future will extend knowledge on the sero-epidemiology of this group of viruses. Sero-epidemiological methods have no role in the management of foodborne outbreaks caused by SRSVs.

Astroviruses and caliciviruses

4.24 Sero-epidemiological assays have established high prevalences (greater than 80%) in children over the age of 5 years. This high prevalence in children correlates with the clinical data which shows that astroviruses are mainly an infection of paediatric populations.[10] With the development of monoclonal antibodies the serotype distribution of astroviruses has also been established.

Rotavirus

4.25 Specific serological tests for rotavirus have been widely reported and the data from these and future studies will be important in monitoring the efficacy of rotavirus vaccine when it is introduced into the UK.

Sampling

Food and environmental samples

4.26 Techniques involved in the extraction of viruses from shellfish are not standardised. Current recommendations are outlined in Chapter 6. Techniques for the extraction and detection of viruses from other foods have not yet been reported and may well be the most challenging future application of molecular technology. Although outbreaks of waterborne SRSV gastroenteritis have not been reported in the UK, considerable interest exists in applying molecular techniques to this substrate. Contamination of the environment with SRSVs, as a result of projectile vomiting in human infection, is currently being investigated as part of a research project.[57] Preliminary data from these studies reinforce the need for environmental decontamination. It is not envisaged that future routine sampling of the environment will be necessary for effective control of infection measures.

Quality Assurance and Accreditation

4.27 All laboratories undertaking diagnostic tests are required to be formally accredited by the CPA for good laboratory practice. Many of the molecular or electron microscopical investigations described here are carried out by reference or research laboratories and they are not currently required to participate in this accreditation scheme. This will certainly change in the near future if they intend to provide a diagnostic service. Many routine microbiology laboratories do, however, carry out viral antigen detection by EIA

for enteric viruses such as rotavirus and adenovirus antigen. In addition, detection of IgM or IgG to hepatitis A by commercial assays is commonly carried out by non-specialist laboratories.

Commercial immunoassays

4.28 All commercially produced assays are quality assured by the manufacturer. As part of this, positive and negative controls as well as internal standards will be included, as will instructions as to how to use the assay. Internal controls used by laboratories undertaking such tests are part of the National External Quality Assurance Scheme (NEQAS) and are necessary for accreditation.

Non-reference, in-house assays

4.29 Some of the more specialised, non-routine tests, e.g. EM, may be carried out in non-specialist laboratories. At present, there is neither external quality control of electron microscopy nor a requirement for accreditation of laboratories' performance of EM. The result is that EM diagnosis of viral gastroenteritis is variable, even in specialist reference centres, and often unavailable outside the specialist centres. London in particular is poorly resourced, although the Enteric and Respiratory Virus Laboratory at the PHLS Central Public Health Laboratory does offer routine examination of faecal samples from outbreaks which occur in the London catchment area.

Reference tests

4.30 All reference centres in the UK undertaking SPIEM, immunoassays and molecular detection of gastroenteritis viruses are attached to major centres of research excellence in the field. Whilst no formal quality assurance (QA) or accreditation of this work exist, other than basic in-house precautions, a certain scrutiny of standards comes from peer review of their published work. However, molecular techniques, in particular, would benefit from more formal QA. If expansion of the molecular techniques to non specialist laboratories does occur, proper external QA and standardisation of assays will be required.

Health and safety in the laboratory

4.31 The publication of revised Control of Substances Hazardous to Health Regulations (COSHH) in 1994,[58] and their associated Approved Code of Practice,[59] reinforced the need for laboratories to undertake risk assessments for all analytical procedures. The requirements are that:-

"An employer shall not carry out any work which is liable to expose any employees to any substance hazardous to health unless he has made a suitable and sufficient assessment of the risks created by that work to the health of those employees and of the steps that need to be taken to meet the requirements of these regulations."

4.32 The agents associated with viral gastroenteritis are classified as Hazard Group 2 by the Advisory Committee on Dangerous Pathogens: categorisation of

biological agents according to hazard and categories of containment.[60] Accordingly, they should normally be handled at Containment Level 2, with vaccination recommended for hepatitis A. There is currently no vaccine available conferring protection for the agents of viral gastroenteritis.

4.33 The risks to laboratory workers are those of laboratory acquired infection, particularly associated with a low infective dose. Preventative strategies should therefore be geared towards the prevention of ingestion. The wearing of disposable gloves and scrupulous hand washing are of paramount importance. This will be common to the handling of all diagnostic faeces samples independent of the choice of testing methodology. With the proven occurrence of aerosol-borne infection it would be appropriate to consider the use of a Class 1 Safety Cabinet, conforming to BS5726.

4.34 The extent of subsequent potential exposure will, however, be methodology dependent. The extent of manual manipulation is the single most important factor in this regard. Latex agglutination tests will by definition be more hazardous than ELISA methods. The use of guanidinium isothiocyanate in the extraction procedure for PCR effectively renders the virus non infectious for all subsequent procedures.

Conclusions

4.35 We have concluded that:-

- electron microscopical examination of human faecal samples remains the only catch-all method of identification of viruses causing gastroenteritis;
- numerous immunoassays have been developed for the identification of individual viral pathogens and are commercially available;
- neither EM nor immunoassays are sufficiently sensitive for the detection of viruses in food;
- the molecular characterisation of SRSVs has allowed the development of a sensitive PCR for diagnosis and can be applied to the detection of virus in food.

Recommendations

4.36 We recommend that all laboratories using EM and/or molecular techniques for the investigation of viral diarrhoea should be accredited and should participate in internal and external quality control arrangements.

4.37 We recommend that schemes for quality assurance must be developed for molecular diagnostics and must be reintroduced for EM.

CHAPTER 5

VIRAL CONTAMINATION OF FOOD : ROUTES OF SPREAD AND VEHICLES : PREVENTION AND CONTROL MEASURES

Introduction

5.1 Humans are the only reservoir of small round structured viruses (SRSVs) and hepatitis A virus. Unlike with many other foodborne pathogens, food animals do not act as a reservoir of the infectious agents. There is no evidence of a viral hazard from faecal pollution from any other animal. The viruses cannot multiply in food but they can remain viable, even in frozen foods. Hepatitis A is a very rare foodborne infection in the UK, but routes of spread and preventative measures are similar to those for SRSV. Where hepatitis A differs from SRSV infection we indicate this specifically below.

5.2. This Chapter addresses five potential routes of spread:-

- non-food vehicles;
- water;
- bivalve molluscan shellfish;
- contamination of growing food crops; and
- infected food handlers.

5.3 For the foodborne routes we identify the critical points at which the chain of infection can be broken in order to prevent disease. The wider issues concerning bivalve molluscan shellfish are taken up in more detail in Chapter 6. Annex C contains information on cleaning and sanitisation in outbreak control where preventative measures have failed. Whilst Chapter 3 describes the occurrence of foodborne viral infections in the UK, this Chapter draws examples from wider afield.

Non-food vehicles or direct person-to-person spread

5.4 A food vehicle is not essential to the spread of viral infection. Most SRSV infections are spread by way of aerosols or through non-food vehicles. Once an outbreak of SRSV has become established in a closed environment (eg. in a residential home or hotel, or on board a ship), these routes of infection may play a major role in its escalation. It will often be impossible to identify the relative significance of spread through either non-food or food vehicles. Indeed, there will be cross-contamination from one to the other and both must be tackled for effective control of the outbreak. Infections that are not foodborne are outside the scope of this report, although some outbreak control measures described in Annex D may be relevant.

Water

5.5 Public water supplies in Great Britain have rarely, if ever, been implicated in the transmission of viral pathogens. Internationally, the literature contains occasional reports of outbreaks linked to contaminated well water or other natural sources of drinking water and others where water was used in food processing or food production. There is usually evidence of sewage contamination. Examples include:-

- in Czechoslovakia (1963),[61] over 400 people suffered viral hepatitis after consuming dairy products. Fracture of sewage pipes led to contamination of the water supply to a local dairy. The water supply was used for equipment cleaning and coincidentally there was a malfunction in pasteurising equipment in the dairy;
- well water was used to produce ice commercially and caused illness among an estimated 5,000 persons in a multi-State outbreak of viral gastroenteritis in the USA;[62]
- water cress grown in polluted water caused an outbreak of viral hepatitis in the USA during the early 1970s;[63]
- celery used in chicken salad caused an outbreak of foodborne viral gastroenteritis involving 1,500 cadets and staff at the US Air Force Academy.[64] The celery had been washed and soaked for an hour in water from a hose that had previously been used to unclog floor drains after sewage had backed up in the kitchen.

5.6 The few waterborne cases in the literature only serve to reinforce the basic point that all water used in food production, including that used for cleaning, must be potable. Water is not discussed further in this Report.

Bivalve molluscan shellfish

5.7 Currently, significant quantities of sewage are discharged directly into UK coastal waters, often with only primary treatment - screening (see Annex E). There is thus a significant risk that filter feeding shellfish harvested from these waters will be contaminated. Outbreaks of viral gastroenteritis linked to the consumption of bivalve molluscs are commonly recorded in most parts of the world. We look in greater detail at shellfish in Chapter 6.

Contamination of food crops

5.8 There is a potential hazard if growing crops are exposed to faecal contamination, particularly those crops that are eaten raw, for example, salad vegetables and fruit. Although there are no proven recorded outbreaks of foodborne viral infection attributed to faecal contamination of such food, we have considered the possible role of sludge derived from human sewage as a source of such contamination. Treated sewage sludge is valuable, both as a fertilizer and as a soil conditioner, and water companies are active in developing

markets for it. With the phased implementation of the Urban Waste Water Treatment Directive,[2] production of sewage sludge is expected to rise to around 1.5 million tonnes annually. As a result of the ending of sea dumping in 1998 and reduced amounts going to landfill sites, the quantity recycled to agricultural land is predicted to double to 1 million tonnes (66% of sludge) by 2005. The area of agricultural land treated would rise from the current 0.5% to around 1%. However, there are neither standards nor routine monitoring for viral contamination in treated sludge. Published research suggests that the best available treatment techniques will only reduce the load by 2-4 logs.[65,66,67,68] In the UK, the main sewage stream is treated by the water companies before disposal (see Annex E). A limited quantity of human waste from private cesspools and septic tanks may receive no treatment before disposal.

5.9 The application of sewage sludge to agricultural land is controlled by the Sludge (Use in Agriculture) Regulations 1989[4] which are complemented by a Code of Practice.[5] The Regulations, amongst other controls, prohibit the application of sludge to land where fruit or vegetable crops are growing, and to ground intended for the cultivation of fruit and vegetable crops (which are normally in direct contact with the soil and normally eaten raw) for a period of ten months preceding harvest. However, in the course of our work, questions were raised about the effectiveness of the Regulations and their implementation. Of necessity they cover a range of issues apart from foodborne viral hazards, including other microbial pathogens, heavy metals and organic toxins. In addition, much of the Regulations are intended to protect those involved in the process of sludge application, those living nearby and livestock that may subsequently graze the land. Questions arise about the scientific basis for the periods specified in the Regulations between sludge application and cropping or grazing and there is little evidence that the provisions of the Regulations are actively enforced. Whilst we reiterate that the literature contains no proven outbreaks of foodborne viral infection attributable to this route of spread, we believe that the issue needs to be reviewed. As indicated, foodborne viral infections are only one of the potential hazards and a comprehensive review is outside the scope of this report. We welcome the recommendation of the Royal Commission on Environmental Pollution[6] that the scientific basis for the specified periods between use of sludge and planting or harvesting of crops, and/or livestock grazing, should be reviewed and the Government's decision to commission such a review. The ACMSF has been asked to assist with the microbiological aspects of the review which will include potential foodborne viral infections.

5.10 There are also questions about the risk from imported produce. There is now a global market for fresh fruit and vegetables, with significant quantities being imported into the UK from all around the world. We have little information about the use of human waste material in agriculture but there are reports that the use of "night soil" (ie. untreated human waste) is customary in some parts of the world. Although there have been no proven cases of infection there have been suspicions about this route of spread in a number of cases, including some

viral outbreaks.[69] Inability to test for viral pathogens in food means that there is no effective monitoring at the point of entry into the EU. This puts the onus on the importers of such produce to satisfy themselves of its safety. We recommend that they take account of these hazards and ensure suitable precautions for food safety.

Infected food handlers

5.11 When a symptomatic infected individual is engaged in food handling the potential for transmission of virus via food is great. Post-symptomatic individuals will remain a hazard for about 48 hours after SRSV infection. In the case of hepatitis A infection, food contamination may occur a few days pre-symptomatically. Epidemiological data tend to be subject to ascertainment bias towards shellfish outbreaks because such outbreaks have been much easier to identify. In fact, contamination of food by symptomatic food handlers may be the most common route for viral pathogens. Analysis of two reports of outbreaks of SRSV food poisoning implicating foods other than shellfish showed that food handlers had evidence of infection in all outbreaks[70] or in most outbreaks.[24]

5.12 Where infection is spread by infected food handlers the type of food is usually quite incidental. The risk factor is more likely to be how the food is prepared and served, rather than the nature of the food itself. So, hot foods are less likely to be implicated than cold foods and there appears to be a higher association with foods that are subject to more handling in preparation. Foods which include no processing or control step from farm to table, such as fruits or salad vegetables, may present a particular hazard. Anyone handling food at any stage in the food chain may be a source of infection. For example, agricultural workers engaged in raspberry picking were thought to be the source of hepatitis A virus infection.[71,72] Table 5.1 gives examples of outbreaks attributed to infected food handlers, to illustrate the very wide range of foods that may be implicated.

Prevention of contamination by infected food handlers

5.13 The same basic precautions needed to prevent the spread of viral infections will also be effective against many other foodborne diseases. The key preventative measure is to ensure good personal hygiene by anyone handling food from harvest through to the final consumer. Food handlers who are suffering gastroenteritis pose a special threat and must be excluded from contact with food. Particular care is needed for foods which are eaten cold, especially if their preparation involves significant handling. The peculiar feature of many SRSV infections is the sudden and explosive onset of projectile vomiting. Individuals may become ill with little warning and it is not unknown for this to occur in sensitive areas such as food preparation rooms. Projectile vomiting will carry an aeorosol of highly infectious material over a very wide area. All equipment and food in the vicinity must be dealt with accordingly. Advice is included in Annex C.

Table 5.1: Foodborne viral infections spread by food handlers

(To illustrate the wide range of foods that may be implicated and the scale of the outbreaks that may result.)

Food	Comment
Sandwiches[73]	Customers of a sandwich bar suffered SRSV gastroenteritis from food probably contaminated by a symptomatic food handler.
Melon/vermicelli pasta[74]	Diners at two banquets within three weeks in the same hotel suffered SRSV gastroenteritis
Various foods[75]	Guests at four separate functions in a hotel suffered SRSV infection. There was evidence of poor food handling practices, and one kitchen worker suffered diarrhoea 24 hours before the first incident.
Salads[76]	287 were ill with SRSV infection after eating on a university campus. A symptomatic food handler had prepared salad vegetables.
School lunches[77]	Over 3,000 pupils and staff at nine schools suffered SRSV illness attributed to school meals produced at a central facility. A food handler had continued to work whilst symptomatic.
Hot food (hamburgers/ french fries)[78]	130 school students had SRSV gastroenteritis. There was a clear link to hot foods handled at the servery after cooking by two symptomatic food handlers.
Salads[35]	220 cases of SRSV infection were associated with eight banquets over a six day period, all served from a central restaurant employing a symptomatic food handler. There was evidence that she had contaminated food in the later banquets 24-48 hours after her own symptoms had cleared.
'Frosted' cakes[79]	Bakery worker suffered onset of diarrhoea and vomiting on his way to work. Also, five episodes of diarrhoea and two of vomiting during a six hour shift. Despite this, he made 76 litres of buttercream to coat 10,000 individual cakes. Estimated that >3,000 people were infected with SRSV gastroenteritis.
Various foods (including sandwiches)[80]	68 persons became ill after eating foods from the same restaurant. Traced to one cook who had poor personal hygiene, used IV drugs, and diagnosed acute hepatitis A just after ending his employment.
Bread[81]	50 people contracted hepatitis A from bread. A local baker continued to handle food although symptomatic.
Ice slush drinks[82]	57 persons contracted hepatitis A from ice slush drinks served at a market stall. A flavoured syrup was mixed manually, prior to freezing, by an employee reported to be jaundiced 3-4 weeks before the outbreak.
Raspberries[71, 72]	Two outbreaks of hepatitis A, affecting four and twenty four persons respectively, were traced to frozen raspberries. There was evidence that the raspberries were contaminated by infected pickers.
Strawberries[83]	A multi-state outbreak of hepatitis A in the USA affected 189 people who ate strawberries distributed frozen. The contamination was attributed to an infected picker.
Potato salad[84]	47 guests at a wedding reception in hotel became ill with SRSV gastroenteritis. The source of the outbreak was traced to a kitchen assistant who suddenly became ill on the eve of the reception and vomited into a sink used to prepare vegetables. The sink was cleaned with a chlorine-based disinfectant and used the next morning to prepare a potato salad.

5.14 Individuals with hepatitis A infections are usually most infectious in the few days before symptoms appear. Excluding staff when they become ill may be too late to prevent the spread of infection. Good hygiene practice all of the time, even by apparently fit individuals, is vital. Food handlers who may have been exposed to hepatitis A virus can take additional precautions if they are aware of early symptoms. They should be advised to look out for dark urine, joint pain, abdominal pain, or, if they smoke, an aversion to tobacco. If they experience any of these symptoms in the two months after exposure they should not visit the food preparation area and instead should report to their manager who should exclude them in accordance with current legislation. Further advice on controlling the spread of hepatitis A virus is given in Annex D.

5.15 Contamination may occur at any stage of the food chain and there have been a number of outbreaks linked to primary production. Adequate toilet and sanitary facilities should be made available for food handlers engaged in the harvesting, grading or packing of susceptible crops such as salads and soft fruits. Also, "Fitness to Work" criteria should be applied (see below). Later in the 'food chain', food handlers working in the food industry, or in the catering or retail trades, must also follow the normal disciplines of good food hygiene. Indeed, the same is true for anyone preparing food in the home. Although legal constraints may not apply, the same principles of food hygiene should be followed. Key amongst these is to keep infected individuals away from food and food preparation areas.

5.16 In both domestic and commercial food preparation, a step that removes or reduces contamination (such as washing, peeling, sanitisation or cooking) should be included in the preparation of every food wherever possible. However, we recognise that the sanitisation of food surfaces is technically complex and there is no clear evidence about the effectiveness of washing or sanitisation treatments against bacteria let alone viral pathogens. The literature, including the ICMSF Report,[85] contains only a limited amount of information on viral inactivation that is of practical benefit. There is good evidence that some disinfectants will inactivate some viruses in some situations. None of these studies, apart from a very small number of volunteer trials, have assessed SRSV because of the difficulties in culturing the virus. Other studies show that many factors affect the effectiveness of treatments including:-

- the virus;
- the sanitiser;
- the form of the sanitiser (eg different forms of chlorine have different effects);
- the concentration;
- the contact time;
- the substrate;
- inactivation of the sanitiser;
- protection of the virus; and

- the presence of other substances, such as detergents, salts etc, which may either enhance or nullify the disinfectant.

5.17 Against this background we recommend that Government funds research into effective methods of food sanitisation, especially for fruit and vegetables, to remove or inactivate viruses.

Food handlers' fitness to work

5.18 The document "Food handlers' fitness to work"[86] gives advice on the personal hygiene of food handlers. It covers general issues and some specific points relevant to viral food poisoning. There is advice in three key areas:-

- pre-employment assessment;
- advice on good hygiene practices; and
- exclusion and re-employment criteria for food handlers who are ill.

5.19 The primary document is backed up by a booklet, "Food handlers fitness to work - guidelines for food business managers",[87] and a pamphlet, "Food handlers fitness to work - your responsibilities as a food handler."[88] These documents are recommended reading and the following points are drawn from them.

Pre-employment assessment

5.20 New staff should be questioned about their recent medical history. This may take the form of a simple written questionnaire. Individuals who report recent or current symptoms will be unsuitable for immediate deployment as food handlers. Contact with persons infected with hepatitis A is not a reason to exclude an individual from work as a food handler. They should, however, be advised to look out for the early symptoms described above. The Joint Committee on Vaccination and Immunisation does not believe that routinely vaccinating food handlers against hepatitis A virus is justified.[1] However, we have recommended in Chapter 2 that they should keep the matter under review.

Symptomatic food handlers : exclusion and re-employment criteria

5.21 Preventative action depends upon food handlers reporting their illness. They have a legal obligation[89] to do so and they should be reminded of this when they start work. In some businesses it is a condition in the contract of employment. They must be given clear guidance on what types of illness are relevant. Any food handler who reports ill must be excluded immediately from food handling duties. He or she should not be allowed to return to work until 48 hours after symptoms of diarrhoea or vomiting have cleared. Food handlers with confirmed hepatitis A infection must remain off work until at least seven days after the onset of symptoms, usually jaundice. If in any doubt, medical advice should be sought. The doctor should be made aware that the patient works as a food handler.

Good hygiene practices

5.22 Staff must observe all the normal rules of good hygiene in any food preparation environment. The premises must be properly equipped with sanitary facilities and hand wash basins to allow this. The basic principles are described in "Food handlers' fitness to work"[86] and many other guides. We will only expand on the issue of gloves because of the importance of direct handling of food in the transmission of foodborne viral infection. There is still no clear consensus on the use of disposable gloves by food handlers. Gloves may prevent contamination of the food from the hand of an infected food handler but a gloved hand is just as likely as an ungloved hand to transfer contamination from contaminated food or surfaces. The application of basic hygiene principles is important whether or not gloves are worn. (Gloves are recommended in outbreak control, to protect any staff who have the job of cleaning fouled areas – see Annex C).

Food handlers : legislative controls

5.23 The legislative control of food hygiene is contained primarily in the Food Safety Act 1990[90] which applies to almost all sectors of the food industry. This places legal obligations, aimed at safe food production and distribution, on both the proprietors of food businesses and on those employed by them. There is a general requirement to produce and sell safe food.

5.24 More detailed provisions are included in regulations made under the Act. Key amongst these are the Food Safety (General Food Hygiene) Regulations 1995[89] which include the following:-

- *hazard analysis:* food businesses must identify and control the specific hazards relevant to their operation. Implicitly, this would include control of viral hazards;
- *reporting illness, and exclusion:* individual food handlers suffering from any disease or infection which may be transmitted through food must report the fact to the proprietor of the business. Proprietors must not let anyone suffering from such an illness work in a food handling area;
- *personal hygiene:* businesses must ensure that staff working in a food handling area maintain a high degree of personal cleanliness and wear appropriate clothing. Toilet and washing facilities must be provided;
- *training:* underpinning the whole issue of personal hygiene is the need to train individual food handlers to understand the basic principles and requirements. With the implementation of Directive 93/43/EEC,[91] this is now a specific legal requirement. Food handlers must be supervised, instructed and/or trained in food hygiene. The proprietor of a business is responsible for assessing the level of training necessary and for ensuring that it is carried out. The content of the training should include the information needed to control viral hazards.

5.25 We do see a problem that the General Food Hygiene Regulations,[89] perhaps of necessity, set broad goals. It may not always be clear exactly what measures are needed to comply, for example, with the hygiene training requirement. But we are also aware that Guides to Good Hygiene Practice[92] (developed in accordance with Articles 5-7 of Council Directive 93/43/EEC[91] and Regulation 8(2)(c)(ii) of the General Food Hygiene Regulations)[89] provide an opportunity to clarify the provisions specific to particular industry sectors. An Industry Guide for Catering[93] has already been published and guides for bakers and retail shops are about to be published. Several others are in preparation.

5.26 These Guides, produced by industry in consultation with Government, enforcers and consumers, must be officially recognised by the Secretary of State. They have special status compared to previous industry Codes of Practice. Although the guidance is not legally enforceable in itself it will relate to specific legal requirements in the Regulations and we believe that these Guides have an important role in effective and consistent enforcement. We would also expect to see rigorous enforcement of the Regulations making an important contribution to safe food preparation

Conclusions

5.27 Much SRSV infection may be transmitted through non-food vehicles and there are occasional reports of viral illness from wells and other natural water sources.[61,62,63,64] Although there are no recorded outbreaks, there are potential hazards from contamination of growing crops. During the course of our work questions were raised about the effectiveness of the Regulations[4] and Code of Practice regulating the application of sewage sludge to agricultural land[5] and their implementation. We welcome the Government's review of the scientific evidence relating to the controls on the agricultural use of sewage sludge. There must also be a potential hazard from imported produce. Importers should take a responsible interest in the systems of production to ensure the safety of the food. We also believe that there is a need for research into effective measures of food sanitisation, especially for fruit and vegetables, to remove or inactivate both bacteria and viruses.

5.28 Contamination by food handlers appears to be an important route of spread of foodborne viral infection. We believe that control simply requires the application of good basic food hygiene. We have considered the legal provisions governing the personal hygiene of food handlers in food businesses. We believe that there are comprehensive legal controls available provided, of course, that food business managers as well as individual food handlers, recognise their responsibilities to ensure high standards of personal hygiene and act on them. We draw particular attention to the importance of food hygiene training. Rigorous enforcement of Food Hygiene Regulations[89] is necessary if they are to be effective.

Recommendations

5.29 We recommend that systems of sewage sludge treatment and the Code of Practice for the agricultural use of sewage sludge[5] be reviewed to ensure the scientific basis of the controls and the effective enforcement of the provisions of the Code. If necessary, there should be more research into the effectiveness of viral inactivation.

5.30 We recommend that the importers of fresh fruit and salad crops take account of these hazards and ensure suitable precautions for food safety.

5.31 We recommend that Government funds research into effective measures of food sanitisation (especially for fruit and vegetables) to remove or inactivate viruses.

5.32 We recommend that there should be effective enforcement of Food Hygiene Regulations.[89] This may be facilitated by 'Guides to Good Hygiene Practice' developed in accordance with Articles 5–7 of Council Directive 93/43/EEC.[91]

5.33 We recommend that such Guides should be developed for more sectors of the industry. They should provide clear interpretation of exactly what is needed by way of training, personal hygiene standards and effective exclusion of symptomatic and post-symptomatic food handlers. Guides which do not provide clear guidance in these areas should not be recognised.

5.34 In addition, we recommend that when guides have been recognised, steps are taken to bring them, or at least the key points from them, to the attention of food businesses. The status, enforceability and effectiveness of guides should be kept under review.

CHAPTER 6

VIRAL CONTAMINATION OF SHELLFISH : PREVENTION AND CONTROL MEASURES

Introduction

6.1 As discussed in Chapter 5, contamination of food by infected food handlers appears to be the major source of contamination of food by viral pathogens. In such cases the type of food is not of itself important. Bivalve molluscan shellfish are an exception to this rule. As filter-feeders they accumulate microorganisms, including human pathogenic bacteria and viruses, when grown in sewage-polluted waters and can present a significant health risk when consumed raw or lightly cooked.

6.2 In this Chapter we review the background to the public health issue with regard to bivalve molluscs, which in the developed world is predominantly viral in nature, and consider the means by which viral contamination of shellfish may best be prevented or controlled. We also take stock of the research work currently underway which is directed towards improving consumer safety.

Bivalve molluscan shellfish

6.3 Annex F (paragraphs F.1-F.8) contains background information on those species of bivalve molluscan shellfish - oysters, mussels, clams, cockles and scallops - most commonly exploited commercially in Europe, their characteristics and habitats and levels of production and consumption of, and trade in, them. In the UK mussels are the primary farmed species. Pacific oysters are next in importance followed by native oysters. Cockles make up the highest proportion of landings from wild fisheries - 39% by volume but only 8% by value. Landings from natural mussel fisheries are also important. Scallops make up 70% of the total value of landings. Oyster landings (almost wholly native oysters) account for around 4% by value. The consumption of bivalve molluscs is currently low in the UK, a large proportion going for export.

Human health hazards

6.4 Human health hazards associated with the consumption of bivalve molluscs predominantly reflect the fact that they are filter feeders. This means that they concentrate and retain human pathogens derived from sewage contamination of their shallow in-shore growing waters. They can also accumulate toxic algae and naturally occurring pathogenic bacteria (such as some vibrio species) in the same way. These hazards are compounded by the traditional consumption of

bivalve shellfish either raw or only lightly cooked and by the consumption of the whole animal, including the viscera which contain the majority of the contaminants.

6.5 The main health hazard is therefore associated with oysters, as they are often eaten raw and whole. Mussels present a lower risk as they tend to be cooked, albeit lightly. Scallops, which are harvested from less polluted off-shore waters, and where the viscera are generally not consumed, present little infectious disease hazard. Most cockles and some mussels are cooked commercially before they are sold (see Annex F, paragraph F.9). Provided such heat treatment is carried out effectively it is sufficient to render the product safe.

6.6 Single shelled molluscs such as winkles and whelks are not filter feeders and do not present the same degree of infectious disease hazard as the bivalves. In the UK other non-filter feeding shellfish such as the crustacea (crabs, lobsters, etc.) are not normally associated with a microbiological hazard derived from harvesting area contamination.

6.7 Bivalve molluscs require specific and targeted control measures to contain the health risk which they pose. Human health problems arising from the consumption of bivalve molluscs are well recognised internationally and have been recorded since medieval times. The association of shellfish-transmitted infectious disease with sewage pollution became well documented in the late 19th and early 20th centuries, with numerous outbreaks of typhoid fever in several European countries, the US and elsewhere. These hazards have been documented as a cause of concern by various international agencies. The United Nations, in their comprehensive report on the marine environment[94] in 1990, stated that "the present state of knowledge indicates that the most clearly identified health risk associated with coastal pollution by urban waste water is the transmission of disease by the consumption of shellfish harvested in contaminated areas".

6.8 Disease outbreaks can occur on an epidemic scale as graphically illustrated by an outbreak of hepatitis A in Shanghai, China in 1988.[95] Almost 300,000 cases were traced to the consumption of contaminated clams. Disease outbreaks have been reported in many countries including in Europe and the US and have been extensively reviewed.[96] In the developed world, outbreaks of known aetiology are predominantly caused by small round structured viruses (SRSVs) of the Norwalk or Norwalk-like family giving rise to gastroenteritis. A smaller proportion of cases is caused by hepatitis A virus and only a few cases are caused by bacterial agents of food poisoning such as *Salmonella*. For those outbreaks where the cause is not conclusively known, the clinical symptoms are mostly consistent with viral gastroenteritis. The available evidence therefore suggests that in the developed world viral not bacterial infections are the predominant cause of infectious disease associated with shellfish consumption.

Commercial treatment processes

6.9 Two different forms of commercial process are conventionally used to reduce

the disease hazard from contaminated shellfish - heat treatment and self purification ie. depuration and relaying (see Annex F, paragraphs F.9-F.14). As noted above, heat treatment has been shown to be fully effective provided it is properly carried out.

6.10 While self purification procedures appear to be effective in removing bacteria from shellfish, human viruses are not rapidly removed by current depuration practices. This is problematic given the fact that, under statutory control measures aimed at limiting the microbiological burden of bivalve molluscs entering the food chain (see Annex F, paragraphs F.15-F.22), quantification of risk relies on traditional bacterial indicators of faecal contamination.

Statutory control measures

6.11 The UK's system of statutory controls for molluscan shellfish is determined by European legislation - specifically Council Directive 91/492/EEC.[97] The Directive's provisions are enacted in domestic legislation under the Food Safety (Live Bivalve Molluscs and Other Shellfish) Regulations 1992.[98] The Directive lays down end product standards which all shellfish sold to the consumer must meet, either directly from the harvesting area or following commercial processing.

6.12 In addition to the end product standard, the Directive[97] requires all harvesting areas to be classified according to the degree of faecal pollution, assessed by microbiological analysis of shellfish flesh. The arrangements for classifying shellfish harvesting areas in England and Wales, Scotland, and Northern Ireland are described in detail in Annex F, paragraphs F.23-F.26.

6.13 MAFF issues annual classification listings on the basis of bacteriological monitoring undertaken by Local and Port Health Authorities. Monitoring programmes, sampling procedures and testing methodologies[99] are performed according to agreed protocols to ensure national consistency. Sampling for established harvesting areas is currently conducted monthly. Although the performance of any microbiological surveillance programme can be improved through more frequent monitoring, the resource cost has to be balanced against the likely gains. Experience has shown that regular monthly monitoring is generally sufficient both to establish baselines and to detect significant deterioration. However, it may not always be sufficient to detect intermittent contamination. Increased monitoring in 'satisfactory' harvesting areas implicated in outbreaks, where there are concerns about contamination or where unusual results are obtained could be a useful development of the standard approach. Monitoring programmes in England and Wales are funded by local government and despite financial constraints faced by local authorities routine monitoring of harvesting areas has always been carried out. Monitoring programmes in Northern Ireland are funded in part by DHSS(NI) and in part by district councils.

6.14 The limitations of current shellfish depuration practices for virus removal is one of the key reasons underlying the classification of shellfish harvesting areas

under Directive 91/492.[97] This limits the degree of pollution of live shellfish entering the processing chain, in effect providing a safety cap. It is therefore imperative for consumer safety that monitoring regimes accurately reflect pollution status and hence the potential virus load. Although properly conducted bacterial monitoring programs generally provide an adequate assessment of shellfish pollution, they do suffer from limitations in some respects. Sampling protocols and testing methodologies must be performed according to standard agreed procedures to avoid the introduction of bias[99] and they must be performed over a sufficient period of time to build a reliable picture of the shellfish pollution status. It is also important to ensure, through good quality assurance programmes, that laboratory results are reliable. However, even with these precautions wide fluctuations in individual results can be anticipated and must be allowed for in result interpretation. These fluctuations reflect the short period of survival of *Escherichia coli* and faecal coliforms in the marine environment and their rapid uptake and removal by shellfish. However, the weight of evidence suggests that human enteric viruses contaminating shellfish are probably not subject to the same rapid fluxes and also survive much longer in the marine environment. Hence, bacterial monitoring may under some circumstances be misleading. Examples of such situations may be where sites are subject to intermittent pollution (such as storm or emergency discharges), where the discharge is remote from the harvesting area such that differential inactivation of bacterial indicators and enteric viruses may occur, or where limited data are available on which to make an assessment. Evaluation of alternative 'viral indicators' to help address these and other issues is described below.

6.15 Harvesting area classification makes a positive contribution to shellfish product safety. Moreover, as a result of this statutory requirement, an account of the routine pollution status of all commercial bivalve beds is now available in the UK. This new information would provide a solid basis for enabling discharge improvements to be targeted at vulnerable shellfisheries. We note, however, that there are currently no plans to target improvement of shellfisheries in this way. We understand that the absence of readily available relay sites for shellfish harvested from Category C areas and harvesting bans in prohibited areas have caused significant tensions and enforcement problems in some affected areas. Maps of current UK shellfish harvesting areas are at Annex F.

Water legislation affecting shellfisheries

6.16 In addition to the restrictive legislation governing shellfish hygiene, there are a number of other major pieces of legislation which impact upon water quality. These govern bathing water standards, urban waste water treatment and shellfish waters (see Annex F, paragraphs F.27–F.34). There are two major changes that could be made in the way these Directives[100,2,3] are implemented which would have a beneficial effect on the pollution status of shellfish harvesting areas. First, the Urban Waste Water Treatment Directive[2] provides for the designation of sensitive areas. Any sewage discharged into these areas

must receive tertiary treatment. Shellfish harvesting areas are not designated as sensitive areas for the purposes of this Directive[2] but would clearly benefit from being so designated. Second, the Shellfish Waters Directive[3] allows for the designation of waters considered in need of protection or improvement in order to support shellfish life and growth. Only 29 sites have been designated (18 in England and Wales, 10 in Scotland and 1 in Northern Ireland). The view held by the Department of the Environment, Transport and the Regions (DETR) in the past has been that this Directive is primarily designed to protect shellfish and their larvae and that, since the advent of the Shellfish Hygiene Directive,[97] it has no function in respect of the protection of public health. This is not the view of the Shellfish Association of Great Britain which lodged a formal complaint with the European Commission in 1996 about the failure of the United Kingdom Government to fully implement the Directive. The Secretary of State for the Environment subsequently enacted the Surface Waters (Shellfish) (Classification) Regulations[101] which place a duty on the Environment Agency to uphold the water quality standards laid down by the European Community for designated shellfish waters in England and Wales. DETR has indicated that there will be further consultation on aspects of implementation, including the designation of waters, and applications have now been made by the Shellfish Association of Great Britain for the designation of a further 31 sites in England and Wales. Consultations are taking place in respect of similar legislation in Scotland. We are not in a position to make a judgement on the merits of the legal arguments but we believe that delays in designation are detrimental to public health. We recommend that all commercial shellfish harvesting areas should be designated throughout the United Kingdom without further delay.

6.17 Continuous discharges arise from sewage pumping stations (in the case of crude discharges) or treatment works (where some level of treatment is applied). In general, the continuous nature of the discharge will result in the extent of contamination of an impacted shellfish bed being reflected by the classification monitoring programme and an appropriate level of processing of the shellfish concerned will therefore be defined in shellfish hygiene legislation. Given the known limitations on removal of viruses from shellfish, however, reduction of the extent of initial contamination, either by diversion of the discharge away from the shellfishery or by an increase in the level of sewage treatment, could be expected to reduce the potential health risk arising from shellfish consumption.

6.18 Intermittent discharges may be due to the operation of combined sewer overflows (from joint foul and rainwater systems) or storm overflows (rainwater only) during periods of rain, the operation of emergency overflows due to failure of plant at sewage treatment works, or decreases in the level of sewage treatment due to planned maintenance. Intermittent discharges are of particular concern as they discharge untreated sewage and their impact may not be reflected by the classification of the shellfish harvesting area which is based on monthly sampling. In Australia, outbreaks of gastroenteritis have

been linked to the operation of storm-associated discharges. In 1978, a nationwide outbreak involving 2,000 people occurred following heavy rainfall which caused increased sewage pollution of waterways in the Sydney area and an increase in the level of contamination of oysters.[102] Norwalk virus was diagnosed in a number of the those infected and oysters were clearly shown to have been implicated epidemiologically. In 1990, in another major incident involving oysters from the same area, fifty seven separate outbeaks of oyster-associated viral gastroenteritis occurred over an eighteen day period.[103] The incident followed a period of heavy rainfall, with the resultant flooding of the sewage system and discharge of large amounts of crude sewage.

6.19 Implementation of the Urban Waste Water Treatment Directive[2] does include a requirement to limit pollution from storm water overflows and this has resulted in investment likely to contribute to a reduction in Combined Sewage Overflow (CSO) problems affecting shellfish harvesting areas. More strict controls exist for CSOs affecting designated shellfish waters. No new CSOs should discharge into designated waters and spills from existing unsatisfactory overflows should be minimised.

Research

6.20 Having described the various controls and their implementation it is appropriate to consider how much protection they afford consumers and whether this could be improved. In this context, it is important to bear in mind that risk assessment is currently based on the bacterial indicators *E. coli* and the faecal coliforms, whereas most disease associated with bivalve shellfish is caused by viral pathogens. Bacteria behave differently to viruses in the marine environment, particularly with regard to their survival times, with viruses generally being much more persistent.[104] Under some circumstances this may limit the ability of bacterial monitoring to predict viral contamination in bivalve shellfish. In addition, disease outbreak data show that meeting the *E. coli* (or faecal coliforms) standard in depurated shellfish does not provide a guarantee of consumer safety. Indeed, in the UK an *E. coli* failure in purified shellfish causing disease is rare, reflecting the success of existing shellfish depuration practices in the removal of bacteria from shellfish. Given this, it is important that controls against bacterial contamination are maintained. However, these observations beg the question why legislation relies solely on bacterial indicators of faecal pollution for sanitary purposes in bivalves. The simple answer is that there are really no other practical alternatives. This fact is acknowledged in the opening statements to EC Directive 91/492.[97]

6.21 In the light of this background, research funding by MAFF over the past few years has been focused in two priority areas:-

- first, the development of faecal pollution indicator organisms more representative of the behaviour of viral pathogens than the bacterial indicators currently in use; and
- second, the development of methods for the direct detection in shellfish of the viral pathogens causing human illness.

These were identified as key research recommendations by the Richmond Committee.[105]

6.22 With regard to the first priority, several projects have been funded exploring the potential of various types of bacteriophage for better indication of viral hazard. MAFF work has focused on the use of male specific (F+) bacteriophage. This potential 'viral indicator' was chosen because it shares many characteristics with the human viral pathogens of concern. Its genome is single-stranded RNA, like the viral pathogens, it is of a very similar size and it has other similar characteristics such as its hardiness to environmental stresses. In addition, it is common in sewage and is cheap and easy to assay, making it a practical proposition for routine use. The behaviour of this indicator has been studied in shellfish both in the marine environment and during the depuration process. Laboratory studies have consistently shown that F+ bacteriophage is removed much more slowly than *E. coli* during depuration of shellfish previously contaminated by exposure to sewage.[106] This effect has been observed in both oysters and mussels and under a variety of depuration conditions.

6.23 This differential elimination of bacterial and viral indicators is consistent with observations from disease outbreaks which suggest that human viruses, unlike *E. coli* and the faecal coliforms, are not efficiently removed during depuration. Consequently, the absence of *E. coli* from purified shellfish is no guarantee of either product safety or that the processing has been appropriately conducted in order to maximise any potential viral removal. Studies are now in progress to determine whether F+ bacteriophage monitoring following depuration would offer any greater degree of consumer protection. Initial results have been encouraging and a working hypothesis is being developed along the lines that absence of F+ bacteriophage from bivalves may equate to absence of disease risk. The F+ bacteriophage system has also been utilised to examine the mechanism of virus contamination in shellfish and, in particular, to explore means of promoting virus removal from bivalves during depuration.

6.24 Other work has explored various forms of somatic bacteriophage as 'viral indicators' for shellfish. It has been reported that depuration for three days had no significant impact on the levels of F+ bacteriophage or a particularly resistant somatic phage (which attacks *E. coli*), although the numbers of *E. coli* were markedly reduced.[107] Relaying into biologically cleaner estuarine waters for up to two weeks reduced the levels of both bacteriophages and of *E. coli*. However, the somatic phage was removed much more slowly than either the F+ bacteriophage or *E. coli*. The number of F+ bacteriophages was reduced by 90 per cent within 10-14 days of relaying, whereas six to eight weeks were required to effect the same reduction with the somatic bacteriophage. It remains to be seen whether F+ or somatic bacteriophages will more closely represent the behaviour of human enteric viruses causing gastroenteritis. These studies offer a potential way forward for addressing some of the inherent inadequacies of *E. coli* as an indicator for shellfish. However, further studies are required to demonstrate the utility of this approach in a commercial setting.

6.25 The F+ bacteriophage is currently being used as a model to investigate the fate of sewage-derived viruses in bivalves. It has been suggested that bacterial titre reductions in bivalves may be a combination of utilisation as a food source, partial digestion, egestion and other factors. In contrast, viral titre reductions may be removed solely by egestion in faeces.[108] If the F+ bacteriophage model confirms this hypothesis the EC standards[97] (as currently based on an assay of viable bacteria) may lead to a substantial over-estimation of the true rates of removal of microbial pollutants during purification.

6.26 MAFF studies have also explored the behaviour of F+ bacteriophage in the context of the classification of harvesting areas. Initial studies have indicated that F+ bacteriophage levels in shellfish may not be subject to the same marked variability as *E. coli*. This may prove valuable for more accurate determination of faecal pollution in sites subject to intermittent or remote pollution, or where limited data are available. The use of such alternative indicators as an adjunct to *E. coli* may help provide additional confidence in the accuracy of pollution monitoring for molluscan shellfish but requires substantial further evaluation before it could be proposed in a statutory context. These limitations of bacterial monitoring may also be overcome by developing methods for directly detecting in shellfish the viral pathogens responsible for illness. For many years such developments were hindered by an inability to culture SRSVs in the laboratory. In recent years, however, molecular biology techniques such as polymerase chain reaction (PCR) have offered new prospects. MAFF work has addressed the severe practical difficulties that needed to be overcome before the power of these techniques could be harnessed for detection of viral pathogens in shellfish. Initial work used enteric viruses such as poliovirus as models for developing the application of these techniques to shellfish. A major obstacle was the presence of potent PCR amplification inhibitors in shellfish extracts. This required the development of extensive shellfish processing procedures prior to PCR.[109] The method developed was capable of tolerating 2-10 g of shellfish flesh, with a sensitivity of down to 10 infectious virus particles, which was sufficient for most practical purposes. Application to polluted field samples showed, on a limited number of samples, that the method was at least as sensitive as conventional enterovirus isolation.[110]

6.27 Subsequent studies concentrated on the application of the method to detection of the SRSVs causing human gastroenteritis. These studies were undertaken by the Centre for Environmental, Fisheries and Aquaculture Science (CEFAS) in collaboration with scientists at the Enteric and Respiratory Virus Laboratory at the PHLS Central Public Health Laboratory. Initial seeding experiments showed that the method for removal of amplification inhibitors was equally applicable to the detection of SRSVs in shellfish. Further studies on polluted field samples and on shellfish associated with human outbreaks of gastroenteritis showed that SRSVs could be detected in polluted shellfish.[52] This research continues, with many technical difficulties such as optimum PCR primer design, quantitation and method sensitivity still to be resolved. However, the studies do show for the first time that it is possible to detect

human enteric viruses causing gastroenteritis in molluscan shellfish. These advances are proving invaluable for investigation of disease outbreaks and for studying harvesting area contamination with viruses. These techniques are also now available for studying the behaviour of SRSVs during depuration and relaying and for evaluating the efficacy of alternative 'viral' indicators such as the F+ bacteriophage.

Conclusions

6.28 Shellfish such as crustacea and single-shelled molluscs such as winkles and whelks are not filter feeders. This fact, in association with other factors such as their habitat, mean that they are not normally associated with foodborne microbial infections. In the UK oysters are usually eaten whole and raw and, consequently, of all the bivalve molluscs, they probably present the most significant health risk to consumers who should be reminded of this fact. It seems obvious that the most direct and effective approach to reducing viral contamination of shellfisheries would be to limit the extent of contamination of shellfish beds in the first place, rather than attempting to remove contamination from bivalve molluscs once it has occurred. Given that the presence of pathogenic viruses results from human faecal contamination, such an approach would need to address the impact of sewage discharges on shellfisheries. At present, there is no requirement to take shellfisheries into account when urban waste water treatment works are undertaken and they may therefore become polluted. Implementation of new schemes for purposes of improving bathing beaches or for urban waste water treatment compliance yields opportunities for remedial action and additional consideration of the position of shellfisheries during the planning of such schemes would maximise the benefits which could be obtained. The designation of commercial shellfish harvesting areas under the Shellfish Waters Directive[3] would also be a positive and desirable step.

6.29 Despite the fact that viruses are generally more persistent in the marine environment than bacteria,[104] bacterial indicators are used for the quantification of the health risk from shellfish. The removal of *E. coli* and the faecal coliforms during depuration is no guarantee of the removal of viral pathogens. The use of alternative indicators and the development of methods for directly detecting viruses in shellfish, together with the other elements described above, may ultimately lead to better shellfish processing techniques for virus removal, routine tests for better determination of disease hazard in shellfish sold for consumption and further improvement of harvesting area monitoring, particularly for better determination of virus risk. Current research on the development of alternative indicators and the removal of viruses from shellfish shows promise and should continue to be supported so that methods are available to reduce the risk to the consumer.

Recommendations

6.30 We recommend that the Government should remind the public of the risks

from eating raw oysters, of the potential dangers from collecting molluscan shellfish from beaches, and of the need to cook molluscan shellfish thoroughly.

6.31 We recommend that investment plans for improving the quality of bathing waters and urban waste waters should be required to take account of the impact on commercially important shellfisheries.

6.32 We recommend that the Government develops a national policy for the reduction of pollution-related illness associated with shellfish consumption. Such a policy should contain the following elements:-

- procedures for the epidemiological surveillance of shellfish-associated incidents should be reviewed with a view to ensuring that they are effective and comprehensive;
- all classified shellfisheries should be designated as sensitive areas under the Urban Waste Water Treatment Directive[2] and we recommend the designation without further delay of all commercial shellfish harvesting areas throughout the United Kingdom under Council Directive 79/923/EEC[3];
- the Department of the Environment, Transport and the Regions, and the Environment Agency, in consultation with the Ministry of Agriculture, Fisheries and Food, and the Department of Health, should formulate a policy to reduce to a minimum the discharges from CSOs into shellfish areas. Frequency of discharges should be monitored and summary results should be published annually to enable a view to be taken of the trend in discharges into classified shellfish harvesting areas;
- CSOs should not be directed into Class A or B shellfish harvesting areas;
- water companies should provide the local Food Authorities with summaries of the operation of storm discharges in the vicinity of shellfish beds and of all emergency discharges immediately they occur. Following a discharge, Food Authorities should take sufficient samples to determine the extent of contamination so that, if necessary, they can prevent harvesting for a period, either by voluntary agreement from harvesters or by using statutory powers.

6.33 The Committee also recognises the importance of maintaining appropriate research in order to enhance current knowledge of foodborne viruses and calls upon the Government and industry to continue to fund research in this area. This in particular should be aimed at:-

- developing methods for the isolation and detection of viruses in shellfish, particularly SRSVs;
- continuing to fund the development of alternative viral indicators of shellfish pollution, in particular their practical application in the classification of harvesting areas, depuration and end product assessment, with a view to incorporating these as standards in EC hygiene control measures as soon as possible;

- investigating the behaviour of viruses during sewage treatment processes with a view to maximising virus removal; and
- investigating the behaviour of viruses during the depuration process in order to maximise virus removal and with a view to issuing guidance to operators on depuration requirements.

CHAPTER 7

SUMMARY OF CONCLUSIONS AND RECOMMENDATIONS

Conclusions

7.1 The principal conclusions reached by the Working Group on Foodborne Viral Infections during the course of our investigations are that:-

- awareness of foodborne virus gastroenteritis is increasing;
- a shortage of expert laboratory diagnostic facilities is hampering the recognition of viruses causing foodborne illness;
- laboratory reports of isolations greatly underestimate the true incidence;
- electron microscopical examination of human faecal samples is the only catch-all method of identifying viruses causing gastroenteritis;
- early collection of specimens is essential. Stools obtained more than two days after onset are rarely positive by electron microscopy (EM);
- nearly all reported foodborne viral infections are caused by small round structured viruses (SRSVs);
- effective management of SRSV and implementation of infection control measures can be based on early clinical recognition;
- in the absence of virological results and testing, Kaplan Criteria can lead to strong suspicions of the involvement of SRSVs in outbreaks of foodborne infection;
- although there are numerous commercially available immunoassays for the identification of individual viral pathogens, there are none for SRSVs;
- neither EM nor immunassays are sufficiently sensitive for the detection of viruses in food;
- the molecular characterisation of SRSVs has allowed the development of a sensitive PCR for diagnosis and can be applied to the detection of virus in food;
- current foodborne SRSV infection in the UK falls into two patterns - outbreaks due to consumption of raw oysters; and outbreaks due to contamination of food by infected food handlers. In the latter type, any handled food or drink may be a vehicle of infection;

- identification of the source of infection and mode of transmission of reported outbreaks is difficult. It is likely that current data underestimate the number of cases involving transmission by food and infected food handlers;
- faecal contamination of any food may occur, irrespective of whether it is raw or cooked;
- foodborne infection with hepatitis A virus is rare in the UK. When it does occur it is the result of infected food handlers contaminating ready-to-eat food or, even less frequently, from consumption of raw or under-cooked bivalve molluscan shellfish;
- effective vaccines are available for hepatitis A protection in food handlers but routine immunisation is not regarded as currently justified by the Joint Committee on Vaccination and Immunisation;[1]
- herd immunity to hepatitis A in the UK is declining, giving rise to a potential for future outbreaks of foodborne hepatitis A;
- there are potential hazards from contamination of growing crops. Questions have been raised about the effectiveness of the Code of Practice regulating the application of sewage sludge to agricultural land[5] and we welcome the Government's review of the scientific evidence;
- there must be a potential hazard from imported produce. Importers should take a responsible interest in production systems, to ensure the safety of food;
- the literature contains only a limited amount of information on viral activation that is of practical benefit and there is a need for research into effective measures of food sanitisation to remove or inactivate bacteria and viruses;
- prevention of contamination by food handlers requires the application of good basic food hygiene. Personal hygiene, education and training of food handlers are essential to reducing the risk of transmission of foodborne viral infection;
- there are comprehensive legal controls available governing the personal hygiene of food handlers. However, it is essential that food business managers as well as individual food handlers recognise their responsibilities to ensure high standards of personal hygiene and act on them;
- we draw particular attention to the importance of food hygiene training;
- rigorous enforcement of Food Hygiene Regulations[89] is necessary if they are to be effective;
- shellfish such as crustacea and single shelled molluscs are not normally associated with foodborne microbial infections;
- the nature and habitat of oysters, and the fact that they are usually eaten whole and raw, means that, of all the bivalve molluscs, they probably

present the most significant health risk to consumers who should be reminded of this fact;

- the most direct and effective means of reducing viral contamination of shellfisheries is to limit the extent of contamination of shellfish beds. This involves addressing the impact of sewage discharges on shellfisheries;
- additional consideration of the position of shellfisheries during the planning of new schemes to improve bathing beaches or for urban waste water compliance would maximise the benefits, as would designation of commercial shellfish harvesting areas under the Shellfish Waters Directive[3];
- bacterial indicators are used to quantify the risk from shellfish despite the fact that viruses are more persistent in the marine environment than bacteria;
- the removal of *E. coli* and the faecal coliforms during depuration is no guarantee of the removal of viral pathogens;
- the use of alternative indicators and the development of methods for the direct detection of viruses in shellfish may lead to better processing techniques for virus removal, routine tests for determining disease hazard in shellfish, and further improvement of harvesting area monitoring.

Recommendations

7.2 Against the background of the above conclusions, we make the following recommendations:-

Infectious agents, clinical spectrum and pathogenesis

Paragraph 2.38: we strongly recommend that, for cases of infection fulfilling Kaplan criteria, control measures are instituted immediately without waiting for laboratory confirmation - although confirmation of diagnosis in due course is desirable (eg. for epidemiological and research purposes).

Paragraph 2.39: we recommend that the Joint Committee on Vaccination and Immunisation keep under review the question of the routine immunisation of food handlers against hepatitis A virus.

Occurrence of foodborne viral infection in the UK

Paragraph 3.25: we recommend that the Government takes steps to improve harmonisation of detection, reporting and surveillance of SRSV infections throughout the UK.

Paragraph 3.26: we recommend that the Government encourages thorough investigation of viral gastroenteritis with a view to establishing a comprehensive and timely picture.

Paragraph 3.27: we recommend that Government maintains, develops and enhances surveillance systems throughout the UK, including the Electron Microscopy Network, in order to better define the problem.

Detection methods for viruses in clinical samples and foods

Paragraph 4.36: we recommend that all laboratories using EM and/or molecular techniques for the investigation of viral diarrhoea should be accredited and should participate in internal and external quality control arrangements.

Paragraph 4.37: we recommend that schemes for quality assurance must be developed for molecular diagnostics and must be reintroduced for EM.

Viral contamination of food : routes of spread and vehicles : prevention and control measures

Paragraph 5.29: we recommend that the systems of sewage sludge treatment and the Code of Practice for the agricultural use of sewage sludge[5] be reviewed to ensure the scientific basis of the controls and the effective enforcement of the provisions of the Code. If necessary, there should be more research into the effectiveness of viral inactivation.

Paragraph 5.30: we recommend that the importers of fresh fruit and salad crops take account of the hazards from contamination of growing crops by human waste material and ensure suitable precautions for food safety.

Paragraph 5.31: we recommend that Government funds research into effective measures of food sanitisation (especially for fruit and vegetables) to remove or inactivate viruses.

Paragraph 5.32: we recommend that there should be effective enforcement of Food Hygiene Regulations.[89] This may be facilitated by Guides to Good Hygiene Practice, developed in accordance with Articles 5-7 of Council Directive 93/43/EEC.[91]

Paragraph 5.33: we recommend that Guides to Good Hygiene Practice should be developed for more sectors of the industry. They should provide clear interpretation of exactly what is needed by way of training, personal hygiene standards and effective exclusion of symptomatic and post-symptomatic food handlers. Guides which do not provide clear guidance in these areas should not be recognised.

Paragraph 5.34: we recommend that when guides have been recognised, steps are taken to bring them, or at least the key points from them, to the attention of food businesses. The status, enforceability and effectiveness of guides should be kept under review.

Viral contamination of shellfish : prevention and control measures

Paragraph 6.30: we recommend that the Government should remind the public of the risks from eating raw oysters, of the potential dangers from collecting molluscan shellfish from beaches, and of the need to cook molluscan shellfish thoroughly.

Paragraph 6.31: we recommend that investment plans for improving the quality of bathing waters and urban waste waters should be required to take account of the impact on commercially important shellfisheries.

Paragraph 6.32: we recommend that the Government develops a national policy for the reduction of pollution-related illness associated with shellfish consumption, containing the following elements:-

- procedures for the epidemiological surveillance of shellfish-associated incidents should be reviewed to ensure they are effective and comprehensive;
- all classified shellfisheries should be designated as sensitive areas under the Urban Waste Water Treatment Directive[2] and we recommend the designation without further delay of all commercial shellfish harvesting areas throughout the United Kingdom under Council Directive 79/923/EEC[3];
- DETR and the Environment Agency, in consultation with MAFF and DH, should formulate a policy to reduce to a minimum the discharges from Combined Sewage Outflows (CSOs) into shellfish areas. Frequency of discharges should be monitored and summary results should be published annually to enable a view to be taken of the trend in discharges into classified shellfish harvesting areas;
- CSOs should not be directed into Class A or B shellfish harvesting areas;
- water companies should provide the local Food Authorities with summaries of the operation of storm discharges in the vicinity of shellfish beds and of all emergency discharges immediately they occur. Following a discharge, Food Authorities should take sufficient samples to determine the extent of contamination so that, if necessary, they can prevent harvesting for a period, either by voluntary agreement from harvesters or by using statutory powers.

Paragraph 6.33: we recognise the importance of maintaining appropriate research in order to enhance current knowledge of foodborne viruses and call upon the Government and industry to continue to fund research in this area. This, in particular, should be aimed at:-

- developing methods for the isolation and detection of viruses in shellfish, particularly SRSVs;
- continuing to fund the development of alternative viral indicators of shellfish pollution, in particular their practical application in the classification of harvesting areas, depuration and end product assessment, with a view to incorporating these as standards in EC hygiene control measures as soon as possible;
- investigating the behaviour of viruses during sewage treatment processes with a view to maximising virus removal; and
- investigating the behaviour of viruses during the depuration process in order to maximise virus removal and with a view to issuing guidance to operators on depuration requirements.

ANNEX A

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

MEMBERSHIP

CHAIRMAN

Professor D L Georgala	Independent scientific consultant. Retired Director of the Institute of Food Research

MEMBERS

Mrs F Anderson (b)	Nurse tutor and consumer representative
Mr D Boon (b)	Director of Environmental Health and Trading Standards, London Borough of Croydon
Mr D Clarke	Senior Accreditation Officer (Food), United Kingdom Accreditation Service
Dr T Clayton (c)	Technical Executive, Food Technology Team, Marks and Spencer plc
Professor R Gilbert	Head of Food Safety Policy Development, Public Health Laboratory Service. Visiting Professor at the Royal Veterinary College, University of London
Mrs P Jefford (a)	Environmental Health Services Manager for Gravesham Borough Council
Dr A M Johnston	Senior Lecturer, Royal Veterinary College, University of London
Mr D Kilsby (a)	Head of Food Microbiology Research Laboratory, Unilever plc, Colworth
Ms E Lewis	Computer consultant. Consumer representative
Dr M J Painter	Consultant in Communicable Disease Control, Infection Control and Surveillance Unit, Public Health Laboratory Service (North West)
Professor S Palmer	University of Wales College of Medicine
Dr T Roberts	Food Safety Consultant, Retired Head of Microbiology, Institute of Food Research, Reading Laboratory
Dr N Simmons	Emeritus Consultant in Microbiology to the Guy's and St Thomas' Hospital Trust; Honorary Senior Lecturer in Microbiology, St Bartholomew's and the Royal London School of Medicine and Dentistry

Professor W C S Smith	Reader in Public Health, University of Aberdeen and Honorary Consultant in Public Health Medicine, Grampian Health Board, Aberdeen
Ms B Saunders (d)	Consumer consultant
Mr R Southgate (b)	Technical Executive, Northern Foods plc
Dr G Spriegel (d)	Director of Scientific Services, J Sainsbury plc
Dr M Stringer (d)	Director of Food Science Division, Campden & Chorleywood Food Research Association
Dr J Stevens (a)	Group Technical Director, Unigate European Food
Mrs B W Thomas (c)	Senior Policy and Development Officer, National Consumer Council
Dr T D Wyatt (a)	Consultant Clinical Scientist at Mater Hospital Trust, Belfast

ASSESSORS

Mr P J R Gayford	Ministry of Agriculture, Fisheries and Food
Dr R J Harding	Ministry of Agriculture, Fisheries and Food
Dr C H McMurray	Department of Agriculture for Northern Ireland
Dr P Madden	Scottish Office Department of Health
Dr E Mitchell	Department of Health and Social Services, Northern Ireland
Dr R Skinner	Department of Health
Mr D Worthington	Welsh Office

SECRETARIAT

Medical Secretary

Dr J Hilton	Department of Health
Dr A Wright (c)	Department of Health

Administrative Secretary

Mr C R Mylchreest	Ministry of Agriculture, Fisheries and Food

Minutes Secretary

Mr P Hayes (f)	Department of Health
Mr G Robb (e)	Department of Health

(a) From 1 April 1998
(b) Until 31 March 1998
(c) From 1 January 1998
(d) Until 31 December 1997
(e) Until May 1996
(f) From June 1996

ANNEX B

ORGANISATIONS AND INDIVIDUALS WHO SUPPLIED INFORMATION

Organisations and individuals representing a wide range of interests and expertise were invited to supply the Working Group with information. Those who responded with information are detailed below.

Organisations and individuals who gave oral evidence to the Working Group

Centre for Environmental, Fisheries and Aquaculture Science (CEFAS)
Department of the Environment
Department of Health
Environment Agency
Dr R I Glass, Chief, Viral Gastroenteritis Unit, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
London School of Hygiene and Tropical Medicine
Public Health Laboratory Service

- Dr H Appleton
- Dr D Brown
- Dr J Heptonstall
- Dr R Eglin

Shellfish Association of Great Britain
Water Services Association

Organisations and individuals who supplied information to the Working Group

Association of Metropolitan Authorities
British Medical Association
Centers for Disease Control and Prevention, Atlanta, Georgia, USA
Dutch Health Inspection Service
Fishmongers' Company
Health Canada Bureau of Infectious Diseases
IFREMER
Institute of Food Science and Technology
Institute of Virology & Environmental Microbiology
Manchester Metropolitan University
North East Wales Institute
Norwegian College of Veterinary Medicine
Plymouth Marine Laboratory

Public Health Laboratory Service
- Exeter Public Health Laboratory
- PHLS Headquarters

Portuguese National Health Institute
Rank Organisation
Royal Institute of Public Health and Hygiene
J Sainsbury plc
Swedish Institute for Infectious Disease Control
United Kingdom Warehousing Association
Universities
- Queen's, Belfast
- Southampton
- University of Wales College of Cardiff
- York

Veterinary Laboratories Agency
World Health Organisation

Visits undertaken by the Working Group

Abbotsbury Oyster Farm, Ferrybridge, Weymouth
Billingsgate Fish Market
MAFF Fish Diseases Laboratory, Weymouth (CEFAS)
Water Services Association - Budds Farm sewage treatment plant

ANNEX C

GUIDANCE ON CLEANING UP VOMIT

General principles

Spread of Infectious material

C.1 Vomit from someone with SRSV is highly infectious. When someone with SRSV infection vomits the virus particles may be spread some distance from the site of impact. If the area is not adequately decontaminated it can create a long-term problem.[111] Precisely how far the infectious material may spread will vary, but, as a rule of thumb, the most significant levels of contamination are probably going to be within 2 metres of the site of impact.[112]

The clean up process

C.2 In order to avoid putting themselves at risk of infection, people who clear up vomit should be advised, or preferably shown, how to do it safely. The essential principles are:-

- those concerned should protect themselves by wearing disposable gloves, a plastic disposable apron and, if available, a face mask;
- they should attempt to keep the area of contamination as small as possible.

C.3 If the area that has been contaminated is made of an impermeable substance then sodium hypochlorite solution (bleach) may be poured over the spill in accordance with instructions on the bottle. Care must be exercised when doing this as sodium hypochlorite is corrosive to skin and metal surfaces. It will also bleach furnishings and soft fabrics.

C.4 Alternatively, chlorine-releasing granules containing sodium dichloroisocyanurate may be applied directly to the vomit instead of sodium hypochlorite. This should only be carried out in a well ventilated area as large amounts of chlorine gas are produced. The manufacturer's directions must be strictly followed in order to avoid over-dosing, with consequent excessive production of chlorine.

C.5 The vomit should be covered with paper towels or tissues to help soak up excess liquid and prevent contamination from spreading over a wider area.

C.6 As an alternative, sawdust may be spread over the contaminated area and left long enough for the liquid to be absorbed.

Disposal

C.7 Once as much liquid as possible has been absorbed, the material should be carefully removed for safe disposal. In the domestic setting, a plastic dustpan is a useful scoop and the material should be flushed down the toilet. In non-domestic

settings, the material should be placed in a plastic bag which should then be sealed and disposed of as contaminated waste.

C.8 After cleaning, the face mask (if used), the plastic apron, the disposable gloves and any cloths should be placed in a plastic refuse sack for disposal, ideally by incineration.

C.9 When as much material as possible has been removed, the area and any equipment used should be thoroughly washed with detergent and hot water.

C.10 ***The operator should always wash and dry his or her hands thoroughly after the operation.***

Treatment of specific materials

C.11 After clearing vomit from an area, soiled materials may be treated as follows:-

- bed linen should be carefully removed, to avoid generating further aerosols, before being washed on as hot a cycle as the fabric will withstand;
- carpets should be cleaned with a proprietary carpet shampoo or steam cleaner, if available. In the absence of this type of equipment, a thorough cleaning with hot water and detergent may have to suffice;
- curtains that have been visibly soiled should be removed for cleaning, ideally by a hot wash;
- soiled impermeable surfaces should be sanitised using sodium hypochlorite diluted 1 in 10 with water;
- horizontal surfaces in the vicinity of the soiled area should be wiped with a disposable damp cloth;
- furniture with wooden frames, and all soft furnishings, should also be wiped over with a disposable damp cloth;
- toilet rims, seats and handles, together with taps, should be cleaned with a suitable proprietary cleaner.

Food preparation areas

C.12 If the vomit is in a food preparation area special precautions are necessary and professional consideration must be given to the clean up process. Professional advice may be sought from the local Environmental Health Department of the relevant Local Authority. The following guidance applies:-

- using the general principles stated above, any vomit should be carefully removed from the area;
- all hard surfaces in the food preparation area should be sanitised using a hypochlorite based cleaner that releases 500 parts per million of available chlorine;

- in addition to all horizontal surfaces, any vertical surface which may have been contaminated should also be cleaned and sanitised;
- food that may have been contaminated by virus should be destroyed.

ANNEX D

CONTROL OF THE SPREAD OF HEPATITIS A VIRUS

D.1 Someone who has been exposed to, and infected with, hepatitis A virus is likely to start to become infectious in the two weeks before they become ill. On average this means two weeks after being infected. The ratio of symptomatic to asymptomatic cases means that, often, many people are infected before the diagnosis of hepatitis is suspected.

D.2 In closed and semi-closed settings, it is possible to carry out a serological survey to quantify how many people are immune, have evidence of recent infection or are susceptible. Serology is usually carried out on blood samples, but it is now possible to achieve the same end using salivary samples, which are easier to collect and more likely to achieve a high compliance. In practice, however, such an investigation is rarely feasible due to various inherent delays.

Passive immunisation[1]

D.3 Administration of gammaglobulin to those who are susceptible, and have recently been exposed to hepatitis A, can prevent or modify infection. The effect of gammaglobulin is immediate but protection declines with time and is usually non-existent within a few months of administration.

D.4 When given post-exposure, gammaglobulin should be administered within two weeks of exposure if it is to be effective. In practice this is often not feasible.

Active immunisation[1]

D.5 Vaccines are now available to give long-term protection against hepatitis A infection. It takes about 10–14 days after the first dose of vaccine before any immunity develops. A subsequent dose of vaccine given between six and twelve months later provides a level of immunity which may offer protection for at least ten years.

D.6 The use of hepatitis A vaccine may help control community outbreaks if given to a clearly defined population at an early enough stage.

D.7 Vaccination of food handlers against hepatitis A may be considered if local circumstances indicate that it is likely to be cost effective.

Action to be taken after a food handler is diagnosed as having hepatitis A[11,13]

D.8 It is possible that other, susceptible, food handlers in the food preparation area could have been infected by the index case. This gives rise to the possibility that they in turn may become cases and so infect other colleagues or contaminate food. To reduce the risks of this happening, all staff who have not been immunised against

hepatitis A, and who were in contact with the index case whilst he or she was symptomatic, should be offered immediate gammaglobulin (250 mg by intramuscular injection).

D.9 The use of hepatitis A vaccine in addition to gammaglobulin has not yet been evaluated in this situation but there is no contraindication to it being given at the same time as gammaglobulin, but in a different anatomical site.

D.10 Staff should be advised about the importance of maintaining good hygiene at all times, in particular, handwashing after visiting the toilet. They should also be informed of the early symptoms of hepatitis A, which include dark urine, joint pains, abdominal pain and, if smokers, an aversion to tobacco. It should be made clear that, if they experience any of these symptoms in the two months after exposure, they should not enter the food preparation area but should report to their manager who should exclude them in accordance with current legislation.

ANNEX E

TREATMENT AND DISPOSAL OF SEWAGE SLUDGE

Sewage treatment

E.1 Most sewage treatment plants have three or four process stages to treat sewage. These are preliminary, primary, secondary and tertiary treatment.

Preliminary treatment

E.2 Preliminary treatment removes large solids from the sewage flow to prevent damage to mechanical equipment and to protect subsequent treatment processes from blockage or overloading. The large solids are macerated or removed by screens. Inorganic grit is settled out.

Primary Treatment

E.3 In primary treatment most of the solid particles settle out by gravity in a settlement tank. Sludge is periodically drawn off from the bottom of the tank to undergo sludge treatment (see below).

Secondary Treatment

E.4 Secondary treatment is a biological treatment stage where microorganisms oxidise the settled sewage. Taking place in a biological filter tank, the settled sewage is sprinkled over a bed of stone or slag media which provide a habitat supporting the growth of microorganisms. This is followed by a further settlement stage to remove the waste products of oxidation. In activated sludge plants the microorganisms are kept in suspension in the sewage (or mixed liquor) which is aerated. Solids are again separated in a final settlement stage to undergo sludge treatment. Sludge containing the active bacteria is recycled to sustain the mixed liquor.

Tertiary Treatment

E.5 Tertiary treatment may be needed to enhance the effluent quality. This may include disinfection to reduce bacterial levels or removal of residual solids, nitrate, ammonia and phosphorus to reduce nutrient enrichment of watercourses.

Sewage sludge

E.6 Around one million tonnes dry weight of sewage sludge are produced in England and Wales each year (35 million tonnes if the associated water is included). About 30% is discharged to sea, although by 1998 this disposal route will be closed. Other disposal routes include application as a fertiliser to agricultural land (50%), incineration (10%), and landfill (10%). Small quantities are used for land

reclamation, forestry and as compost. While sewage sludge contains valuable plant nutrients and organic matter, it may also contain human parasites, pathogenic microorganisms, and chemicals.

E.7 Land application of sludge is covered by Regulations[4] and a Code of Practice[5] which are designed to provide barriers to the transmission of human, animal and plant pathogens whether by direct or indirect contact with it or with products produced from the treated land. Approximately 70% of the sludge applied to land is treated to reduce the level of sewage-derived microbes. Examples of effective sludge treatment processes are included in the Code of Practice.[5]

Sludge treatments

E.8 Sewage sludge produced from domestic sewage requires treatment to:-

- reduce organic matter and water content;
- remove unpleasant odours resulting from the incomplete oxidation of organic matter; and
- reduce pathogenic loads.

The principal methods of treatment are as follows.

Anaerobic digestion

E.9 The reduction of pathogenic microorganisms by anaerobic digestion is both time and temperature dependent (ie. longer retention times and higher temperatures produce greater pathogen reduction). Anaerobic digestion is usually carried out at mesophilic temperatures (i.e. temperatures in the range 22-38°C). Sludges are mixed and retained under anaerobic conditions for at least 12 days primary digestion in the temperature range 35°C ± 3°C or for at least 20 days in the temperature range 25°C ± 3°C. This is followed by a secondary stage with a retention period of at least 14 days. About 32% of sludge used on agricultural land is subject to anaerobic digestion.

Dewatering and storage

E.10 Untreated sludge may be conditioned with lime or other coagulants or subjected to primary mesophilic anaerobic digestion. It is then dewatered and the cake stored for a minimum period of 14 days (treated sludge) or 3 months (untreated sludge). About 16% of sewage sludge is treated in one of these ways.

Liquid storage

E.11 Untreated liquid sludge is stored for a minimum period of 3 months. About 8% of sewage sludge is treated in this way.

Other methods

E.12 Other methods of sludge treatment defined in the UK Code of Practice for Agricultural Use of Sewage Sludge[5] include thermophilic aerobic digestion,

composting, lime stabilisation and pasteurisation. About 10% of sewage sludge is treated by one of these methods or by methods, such as heat drying and gamma radiation, which are not included in the Code of Practice. A further 7% of sludge is treated by methods which are loosely covered by the Code of Practice but which do not meet the defined criteria in the Code. 26% of sludge used on agricultural land is untreated.

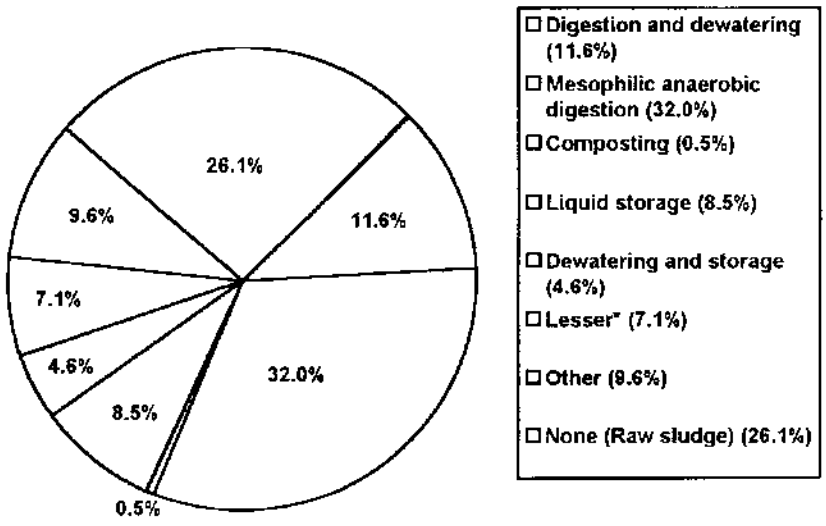
Table E.1: Summary of microbial reduction during sludge treatment[114]

		Log reduction		
Treatment	% sludge treated by method	Bacteria	Viruses	Parasites
Mesophilic anerobic digestion	32	0.5–4	0.5–2	0
Aerobic digestion	0.14	0.5–4	0.5–2	0
Composing	0.5	2–>4	2–>4	2–>4
Air drying	*	0.5–>4	0.5–>4	0.5–>4
Lime stabilisation	0.05	2–>4	>4	0

*Information is not available on the percentage of sludge treated by air drying or on the reduction of pathogens following treatments such as digestion and dewatering, or dewatering and storage.

E.13 It should be noted that, despite a 2-4 log decrease in bacterial and viral numbers, significant concentrations of pathogens are likely to remain after sludge treatment.[115]

Figure E.1 : Treatment of sludge used on UK agricultural land



*"Lesser" includes methods which are loosely covered by the Code of Practice[5] but which do not meet the defined criteria of the Code (ie. there is some element of, eg., anaerobic digestion or dewatering etc but the full conditions of the Code are not met[5].

ANNEX F

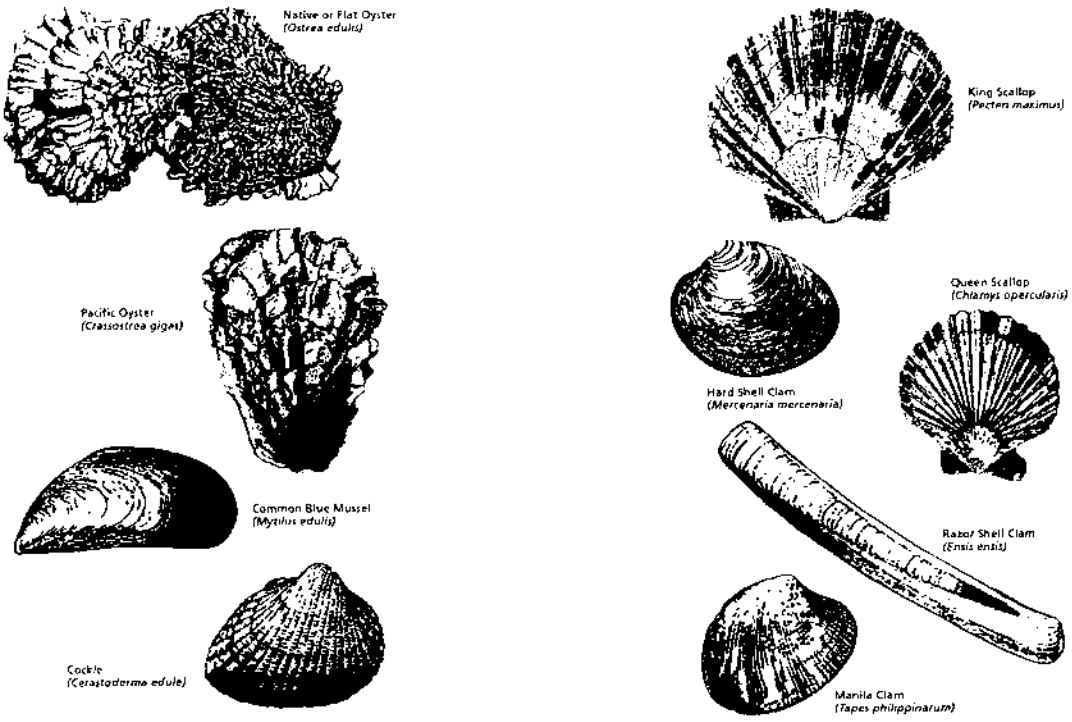
BIVALVE MOLLUSCAN SHELLFISH : CHARACTERISTICS, INDUSTRY FACTS, LEGISLATION

Bivalve molluscan shellfish

F.1 Bivalve molluscs are a type of shellfish that have two shell halves which hinge together. Species commonly commercially exploited in Europe include:-

- the native or flat oyster (*Ostrea edulis*);
- the pacific oyster (*Crassostrea gigas*);
- the common blue mussel (*Mytilus edulis*) and Mediterranean blue mussel (*Mytilus galloprovincialis*);
- cockles (*Cerastoderma edule*);
- king scallops (*Pecten maximus*) and queen scallops (*Chlamys opercularis*); and
- various clams, including the native clam or palourde (*Tapes descussatus*), the hard shell clam (*Mercenaria mercenaria*), the manila clam (*Tapes philippinarum*), and the razor shell clam (*Ensis* spp.).

Illustrations of the species commonly exploited in Europe appear below.



F.2 With the exception of scallops, these are normally static animals that attach themselves to, or bury themselves in, the sea bed or other submerged surfaces. They feed by filtering small particles such as algae from the surrounding water. Many of the commercial species are common in inshore estuaries or similar shallow or drying areas where nutrient levels are high and the waters are sheltered. Dense beds of the animals can occur in productive areas and have been an important source of food since prehistoric times. Indigenous species such as cockles, mussels and the native oyster continue to be harvested from natural populations. However, the characteristics of bivalve molluscs also make them suitable for cultivation. Nowadays the cultivation of indigenous species such as mussels and oysters is supplemented by breeding and farming introduced species such as pacific oysters and manila clams.

Characteristics and habitats

F.3 Bivalve mollusc species vary greatly in their characteristics and habitats. Those adapted to drying conditions close their shells tightly when out of the water to retain a marine environment around their fleshy internal parts. Such species (oysters, mussels and clams) can survive for extended periods out of the water and can be traded as live animals. Other species such as cockles are less hardy and are normally processed soon after harvest. However, they may also be traded as live animals if carefully handled. Scallops and other species not adapted to drying conditions soon die out of water and are normally handled as chilled or processed fishery product.

F.4 Species adapted to drying conditions can be cultivated in the intertidal range which facilitates handling and harvest. Oysters are frequently grown in bags or similar containers raised off the foreshore on trestles. However, they may also be cultivated broadcast directly on the sea bed if conditions are suitable. Manila clams can be cultivated in containers in a similar way. Oysters, clams and scallops can also be cultivated suspended in the water column in lantern nets. Mussels can be cultivated loose on the sea bed in the intertidal region or in deeper waters but are also commonly grown attached to ropes for ease of mechanical harvesting. Such ropes can be suspended from floating structures in deeper waters or hung from poles in the intertidal range. Other indigenous species (cockles and various clams) bury themselves in the substratum and feed through siphon tubes. Such species grow in both the intertidal range and continually submerged as do indigenous populations of native oyster and mussel. Stocks may be harvested whilst submerged, using various forms of dredge towed from a boat, or may be raked or gathered when exposed. Harvesting methods vary from sophisticated expensive high capacity mechanical devices to small scale local hand racking and gathering. Scallops are the only mobile bivalve species commonly harvested. Although there is a small scallop farming industry most are harvested from wild stocks by trawl or diver in deeper off-shore waters. In addition to their varying habitats, bivalve molluscs vary greatly in such physiological attributes as their filtration, growth and activity rates and in their response to environmental stress. This variability has important ramifications for various aspects of health monitoring and control.

Production, consumption and trade

F.5 Over 50,000 tonnes of bivalve molluscs were landed in the UK in 1994 and over 6,000 tonnes were produced by farming (see Tables F.1 and F.2).

F.6 The value of the industry in Great Britain is estimated to total approximately £35 million at first hand sale values, made up of almost £31 million from landings from wild fisheries in 1994 and almost £4 million from farmed shellfish. There are 282 shellfish farms in England and Wales, 315 in Scotland and 17 in Northern Ireland. Mussels are the primary farmed species, contributing over 80% of the volume and over 37% of the value. Pacific oysters are next in significance, making up over 10% of volume and over 30% of the value. Native oysters are third in importance in farmed production, making up rather less than 5% of the volume but over 25% of the value.

Bivalve Mollusc Production in the UK in 1994

Table F.1: Total landings in the UK in 1994

Type	Tonnes	Value (£000s)
Cockles	22,300	3,100
Mussels	10,300	2,000
Oysters	538	1,181
Winkles	2,300	1,600
Queens	3,000	1,600
Scallops	14,000	21,200
Total	**52,438**	**30,881**

Table F.2: Production (tonnes) of farmed bivalve molluscs in the UK

Type	Scotland	England and Wales	Northern Ireland	UK Total
Pacific oyster	168.0	332.0	189.4	689.4
Native (flat) oyster	11.4	139.0	109.3	259.7
Scallops	23.9	–	–	23.9
Queens	38.2	–	–	38.2
Mussels	715.6	4,431.0	62.7	5,209.3
Clams	–	34.1	–	34.1
Total	**957.1**	**4,936.1**	**361.4**	**6,254.6**

NB: These figures do not include stock produced for on-growing.

F.7 Landings are more widely spread across species in terms of volume. Cockles are the most significant at 42% of the total but only constitute 11% by value. The value of landings is dominated by scallops which make up 70% of the total. Oyster landings are almost all of native oysters and make up almost 4% of the total value.

F.8 The consumption of bivalve molluscs in the UK is currently low compared to many other countries. A large proportion is exported, the majority going to France and Spain. In 1994 the UK exported over 30,000 tonnes of shellfish and shellfish preparations whilst imports amounted to 8,000 tonnes. While there appears to be potential for further development of mollusc cultivation in the UK, the

requirements of the Shellfish Hygiene Directive[97] impose constraints in terms of the availablity of sites of suitable water quality.

Commercial treatment processes

F.9 Conventionally two different forms of commercial process are available for reducing the disease hazard from shellfish subject to pollution. For shellfish sold as a processed product heat treatment (cooking) may be used. Various heat treatment processes are available, varying from pasteurisation through to sterilisation by canning. Research in the UK has shown that HAV can be inactivated by raising the temperature of shellfish meats to 90°C and holding that temperature for 90 seconds.[116] Commercial heat treatments providing adequate health safeguards are laid down by Commission Decisions 93/25/EEC[117] and 96/77/EC.[118]

F.10 Heat processing is not, however, applicable for shellfish sold live, which constitute the bulk of the infectious disease hazard. Here self-purification either in tanks (depuration) or in the natural environment (relaying) can be used.

F.11 For depuration harvested animals are transferred to tanks of clean seawater where they continue to filter feed for a period during which time sewage contaminants are purged out by the normal physiological processes. Depuration periods commonly vary from 2 to 7 days. In the UK a period of 42 hours is stipulated as a minimum requirement for all depurated shellfish. Depuration systems also vary and include processes where water is static or changed in batches, through to systems where seawater is flushed through continually or recycled through a steriliser. Water sterilisation processes include ozone, chlorination, ultra violet (UV) irradiation and iodophores. In the UK virtually all commercial systems employ recycling seawater sterilised by in-line UV irradiation.

F.12 The approval of shellfish depuration plants in England and Wales is carried out by Local Authorities in cooperation with MAFF. MAFF conducts a technical assessment of all new plants and specifies conditions for their approval. Local Authorities issue approvals for plants with regard to this specification. Similar duties are carried out by SOAEFD officials in Scotland and by DHSS officials in Northern Ireland. Conditions attached to approvals cover such aspects as shellfish loading capacities, sea-water volumes, UV tube specification and maintenance, sea-water re-use limitations, minimum purification times, water temperatures, etc. The definition of conditions follows Hazard Analysis Critical Control Point (HACCP) principles and does not rely simply on compliance with the *E. coli* (or faecal coliform) end-product standards. This is aimed at ensuring that depuration efficiency is maximised for all microbial contaminants including viruses. However, this approach has been disputed by some operators who argue that regulation should not extend beyond that required for simple *E. coli* compliance. In addition, some Local Authorities have argued that local considerations should take precedence over general nationally set criteria. We understand that these issues have caused difficulties in some local areas which are currently still unresolved.

F.13 Depuration is widely used in many other countries too, including Australia, France, Italy and Spain. It is less widely used in the US. Depuration is also often used to 'add value' to shellfish recognised as fit for direct consumption by harvesting area criteria (see below).

F.14 Relaying involves the transfer of harvested animals to cleaner estuaries or inlets for self-purification in the natural environment. This process can be used as an alternative to depuration. Shellfish can only be held for relatively short periods in depuration tanks but can be maintained for much longer periods in the natural environment. This makes relaying more suitable for treating heavily polluted shellfish where longer periods may help the removal of contaminants such as enteric viruses causing gastroenteritis. In the UK the main disadvantages of relaying are the limited availability of suitable unutilised clean coastal areas and of obtaining ownership rights to those areas, the difficulty of controlling water quality and other water parameters and the susceptibility of stock to poaching. Relaying is less commonly utilised than depuration in the UK and in other countries with limited availability of suitable coastline but is more common in the US.

Statutory Control Measures

F.15 Control measures are aimed at limiting the microbiological burden and hence the infectious hazard risk of bivalve molluscs entering the human food chain. Quantification of risk relies on traditional bacterial indicators of faecal contamination such as the faecal coliforms or *Escherichia coli.* These can be measured in the shellfish themselves (the European Community approach) or in the shellfish growing waters (the US FDA approach). EC standards for these indicators are based on a 5-tube 3-dilution most probable number (MPN) test.

F.16 In the UK statutory controls for molluscan shellfish are now determined by European legislation (Council Directive 91/492/EC)[97] enacted in domestic legislation under the Food Safety (Live Bivalve Molluscs and Other Shellfish) Regulations 1992.[98] It is generally accepted that the most effective and reliable approach to control is to harvest shellfish from areas with good water quality. Removing contamination by mollusc processing - for areas where coastal populations cause water quality deterioration - is a less effective option although one still widely used in many countries.

F.17 Internationally most agencies recognise that shellfish which meet the microbiological standard - of less than 230 *E. coli* or 300 faecal coliforms in 100g of shellfish flesh - are fit for direct human consumption. This together with standards for specific pathogens (such as Salmonella), chemicals and algal biotoxins is the 'end-product' standard set out in EC Directive 91/492.[97] All shellfish sold to the consumer must meet this defined standard either directly from the harvesting area (EC Category A) or following commercial processing. In addition to this 'end product' standard the controls require all harvesting areas to be classified or graded according to the degree of faecal pollution as judged by microbiological analysis of shellfish flesh. This classification underpins the sanitary controls for bivalves and aims to ensure that:-

- water quality is adequate in areas from which bivalves are harvested for direct human consumption;
- where processing procedures are required to render molluscs fit for consumption contamination levels do not exceed safe limits for the particular process; and
- mollusc harvesting is prohibited where pollution levels are excessive.

F.18 The cleanest areas (EC Category A) must directly comply with the 'end product' standard of less than 230 *E. coli* or 300 faecal coliforms and may be harvested for direct human consumption. Shellfish cannot be harvested for direct consumption from shellfish growing areas exceeding this level of contamination. They may, however, be taken for heat processing, depuration or relaying. Depuration is not, however, completely effective, particularly for the removal of the human pathogenic viruses responsible for the bulk of shellfish associated infections. A number of instances of viral gastroenteritis have been documented following consumption of purified shellfish which meet specified safe bacteriological limits.

F.19 The regulations place an upper threshold on the degree of contamination beyond which it is not sensible to employ short-term purification. This upper threshold is defined as EC Category B. Shellfish from such areas must contain less than 4,600 *E. coli* or 6,000 faecal coliforms per 100g of shellfish flesh in 90% of samples. Protracted relaying should more effectively remove viruses from highly contaminated shellfish and is incorporated as an option in EC legislation.

F.20 Shellfish contaminated up to the level of Category C must be relaid for a minimum period of 2 months to reduce contaminants to acceptable levels before they can be placed on the market. Shellfish from Category C areas must contain less than 60,000 faecal coliforms per 100g of shellfish flesh. Relaying may be combined with depuration if relaying alone is not sufficient to meet the microbiological end-product standard.

F.21 For shellfish sold cooked the previously described heat cook parameters (paragraph F.9) must be adhered to. These parameters have proved very effective at inactivating pathogens. Since their introduction as a statutory requirement, we are not aware of any infectious disease incidents associated with commercial shellfish cooked according to such approved procedures. Because of their effectiveness when properly applied, cooking by an approved process is permitted for shellfish harvested from both lightly polluted (Category B) and more heavily polluted (Category C) harvesting areas. Shellfish growing areas exceeding these proscribed levels of contamination, or areas for which harvesting area classification has not been conducted, are prohibited for harvesting for human consumption. EC (and hence UK) legislation also contains clauses to suspend harvesting from classified areas following a pollution emergency. The various classifications categories are summarised in Table F.3.

Table F.3

Classification of shellfish harvesting areas

Shellfish treatment required	EC Classification	Microbiological standard per 100g shellfish
None required	Category A	All samples <230 *E. Coli* or all samples <300 FCs
Purification, relaying or cooking by an approved method	Category B	90% of samples <4,600 *E. coli* or 90% of samples <6,000 FCs
Protracted relaying (>2 months) or cooking by an approved method	Category C	All samples <60,000 FCs
Harvesting not allowed	Prohibited	>60,000 FCs or at discretion of member states

NB: FCs=faecal coliforms

F.22 In addition to the end-product standard and the criteria for harvesting area classification, the regulations also cover depuration, heat-treatment and relaying practices, general hygiene standards for buildings, a movement document system to ensure bivalve traceablility and specifications for equivalent standards for third country imports into the EC.

Implementation of EC legislation

Classification of shellfish harvesting areas

F.23 In England and Wales, implementation of EC Directive 91/492[97] (the so called 'Competent Authority' arrangements) is a shared responsibility between Local and Port Health Authorities (the "Food Authorities"), the Department of Health and MAFF. MAFF issues annual classification listings on the basis of bacterial monitoring undertaken by Local and Port Health Authorities.

F.24 The classification listing now covers more than 70 main production areas around the coast of England and Wales. The most recent classification listing, effective from 1 September 1996, shows that 9% of designated beds within these production areas fall into category A, 62% fall into Category B, 24% fall into Category C, and 5% are designated prohibited for harvesting. A 'provisional' classification is given where monitoring data for various reasons is incomplete but sufficient to indicate a probable trend. 'Seasonal' classifications can be given where monitoring results show a deterioration only during a non-commercial harvesting period and where a sufficient period has elapsed between the poor quality period and the resumption of commercial harvesting. MAFF has comprehensive maps of all harvesting areas which include information on the location of shellfish beds, agreed monitoring points and known sewage discharges. On the basis of this mapping information, and on data generated by the shellfish monitoring programmes, precise

geographical descriptions of designated harvesting areas (Classification Zones) have been defined. These mapped zones complement the annual classification listing of beds and are intended to aid and inform both the shellfish industry and local food authorities.

F.25 In Scotland similar arrangements exist to those described for England and Wales, with the Local Food Authorities and the Scottish Office Agriculture, Environment and Fisheries Department (SOAEFD) being the responsible bodies. The listing issued to the trade and local authorities in January 1996 contained details of 172 classified production areas in waters around the coast of Scotland of which 51% fall into category A, 24% into category B, 5% into category C and 20% split A-B. 'Seasonal' classification can be given which allows the farmer to harvest during a non-A period if he or she wishes and place produce on the market. The shellfish must be relaid and/or purified as required in order to meet the end product standards laid down in the Directive[97] and national implementing legislation.

F.26 In Northern Ireland Directive 91/492/EEC[97] is implemented under legislation produced by the Department of Health and Social Services and operated in liaison with the Department of Agriculture for Northern Ireland (DANI) and district councils. Sampling and testing is carried out on all production areas around the coast of Northern Ireland to keep classification under regular review. New production beds are subject to a monitoring and testing regime to determine the category of classification before the produce is allowed to be sold commercially. As at July 1996, there were 11 beds classified, 25% of which fall into category A and 75% into category B.

Water legislation affecting shellfisheries

F.27 Three pieces of legislation, governing bathing water standards, urban waste water treatment and shellfish waters, all impact upon water quality.

Bathing Water standards

F.28 The EC Bathing Water Directive 76/160[100] places obligations on EU Member States to achieve certain standards at identified bathing waters of which there were 472 in the UK at the time of the 1996 bathing season. Mandatory standards are set for total coliforms (10,000 or less per 100ml) and faecal coliforms (2,000 or less per 100ml). 95% of samples taken during the bathing season must achieve these standards. The Directive is implemented in England and Wales through the Bathing Waters (Classification) Regulations 1991,[119] in Scotland through the Bathing Waters (Classification) (Scotland) Regulations 1991[120] and in Northern Ireland through The Quality of Bathing Water Regulations (Northern Ireland) 1993.[121]

F.29 To achieve compliance with the total and faecal coliform standards at bathing waters not already meeting them sewerage undertakers were required to carry out improvement schemes. These might include the provision of primary and/or secondary sewage treatment, extension or relocation of outfalls away from a bathing water, or additional treatment including disinfection. Improvements under the bathing water compliance programme are likely to have a beneficial effect on

shellfish water quality although the extent of any improvement on shellfish quality would be difficult to assess until post completion monitoring had taken place.

Urban waste water treatment

F.30 The aim of the Urban Waste Water Treatment Directive (91/271/EEC),[2] which is implemented in the UK by Regulation,[122,123,124] is to protect the environment from the adverse effects of sewage disposal. Implementation of the Directive is designed to produce significant general improvements to the quality of rivers, and estuarine and coastal waters. The Directive sets priorities for the treatment of sewage according to the nature and sensitivity of the area receiving the sewage discharge and the size of the discharge. The Directive specifies secondary treatment as the norm but provides for higher standards of treatment for discharges to sensitive areas and at least primary treatment for discharges to areas with high natural dispersion characteristics. For many areas even primary treatment will represent a significant improvement on the quality of existing discharge. Among the Directive's requirements is the need to reduce and finally cease by the end of 1998 the disposal of sewage sludge to surface waters through pipes or from ships at sea. Introduction of the sewage treatment provisions in the Directive will be phased from 1998 to 2005, dependent upon the size of the discharge and the status of the receiving water.

Shellfish Waters

F.31 The Shellfish Waters Directive[3] requires Member States to designate waters considered to be in need of protection or improvement in order to support shellfish life and growth. The UK implemented this Directive in 1980 by administrative guidance and has to date designated 29 shellfish waters. This non-legislative transposition has now been replaced by the Surface Waters (Shellfish) (Classification) Regulations 1997,[101] together with Directions to the Environment Agency.

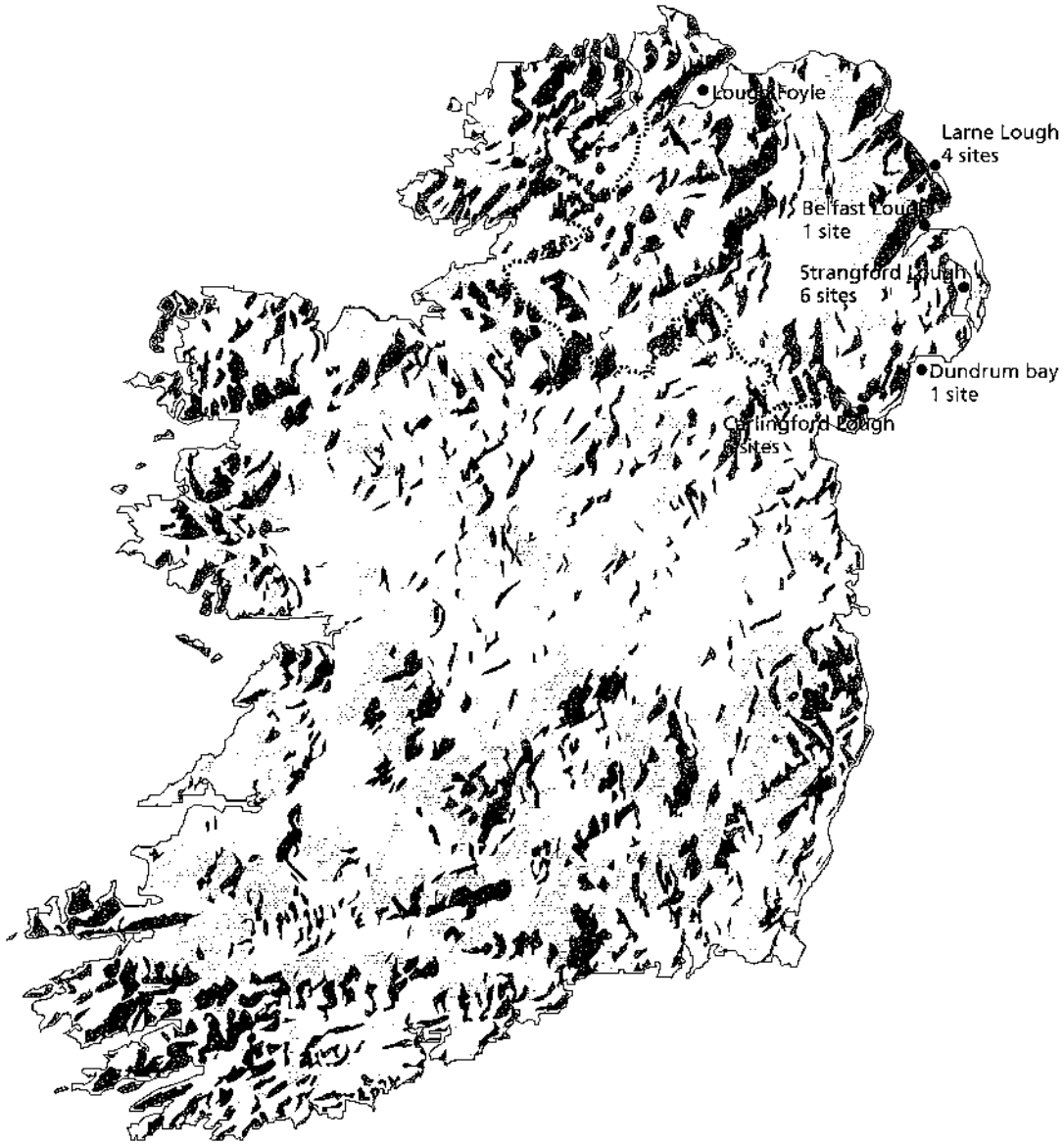
F.32 The mandatory parameters of pH, colour, suspended solids, salinity, dissolved oxygen and petroleum hydrocarbons must be measured in the water. Organohalogenated substances and heavy metals may be measured in shellfish water or in the shellfish flesh, though, as the mandatory requirement is that the level must not exceed that which has harmful effects on shellfish and larvae, measurement in the water would be more suitable.

F.33 The faecal coliform parameter of 300/100ml shellfish flesh and intervalvular fluid whilst only having guideline status in the Annex to the Directive[3] was, pending the adoption of the Shellfish Hygiene Directive,[97] to be considered mandatory in waters in which there were live shellfish directly edible by man.

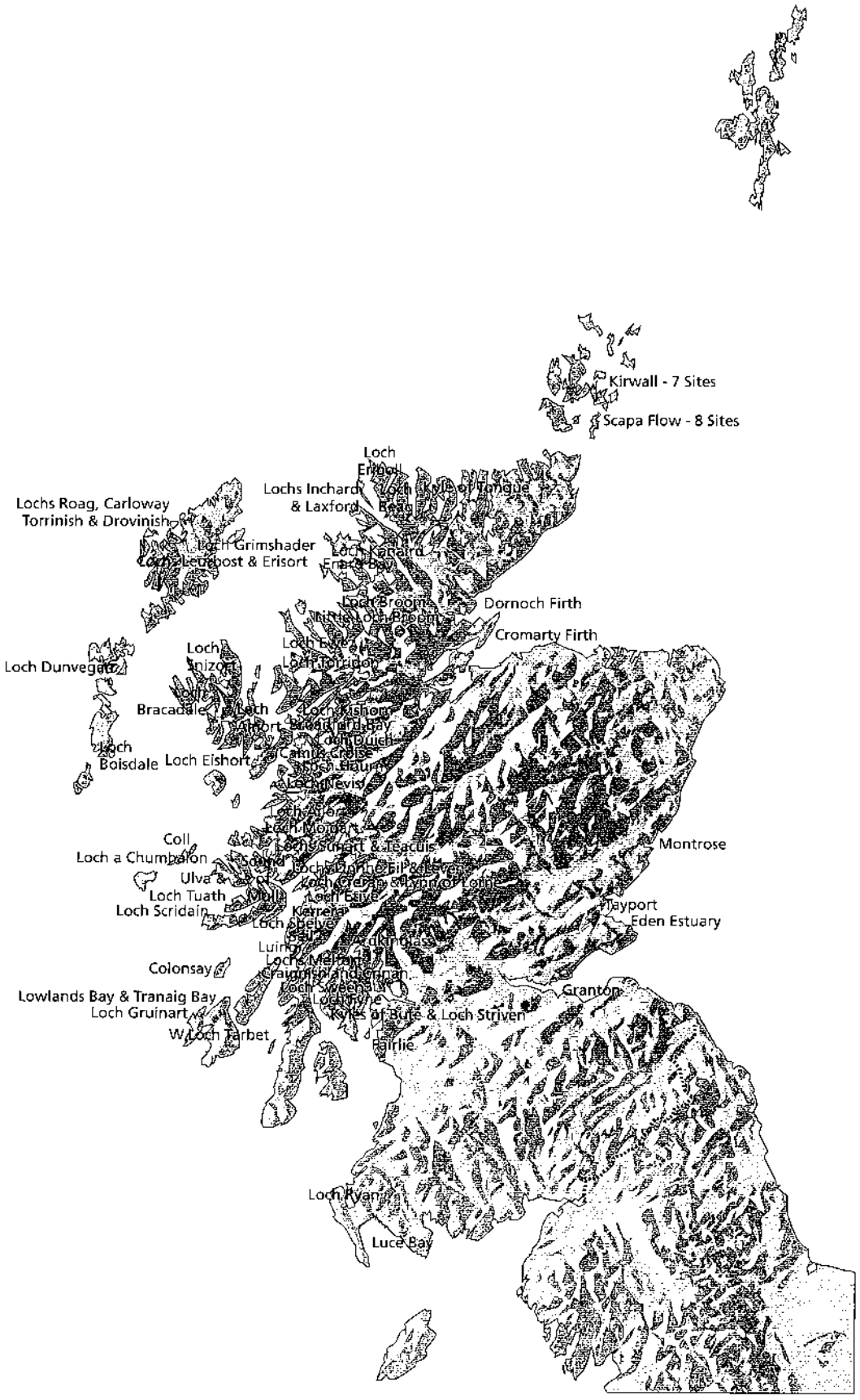
F.34 The European Commission, in its development of an overall water policy, has indicated the likely repeal of both the Shellfish Waters[3] and Freshwater for Fish[125] Directives.

Shellfish Harvesting Areas in England and Wales

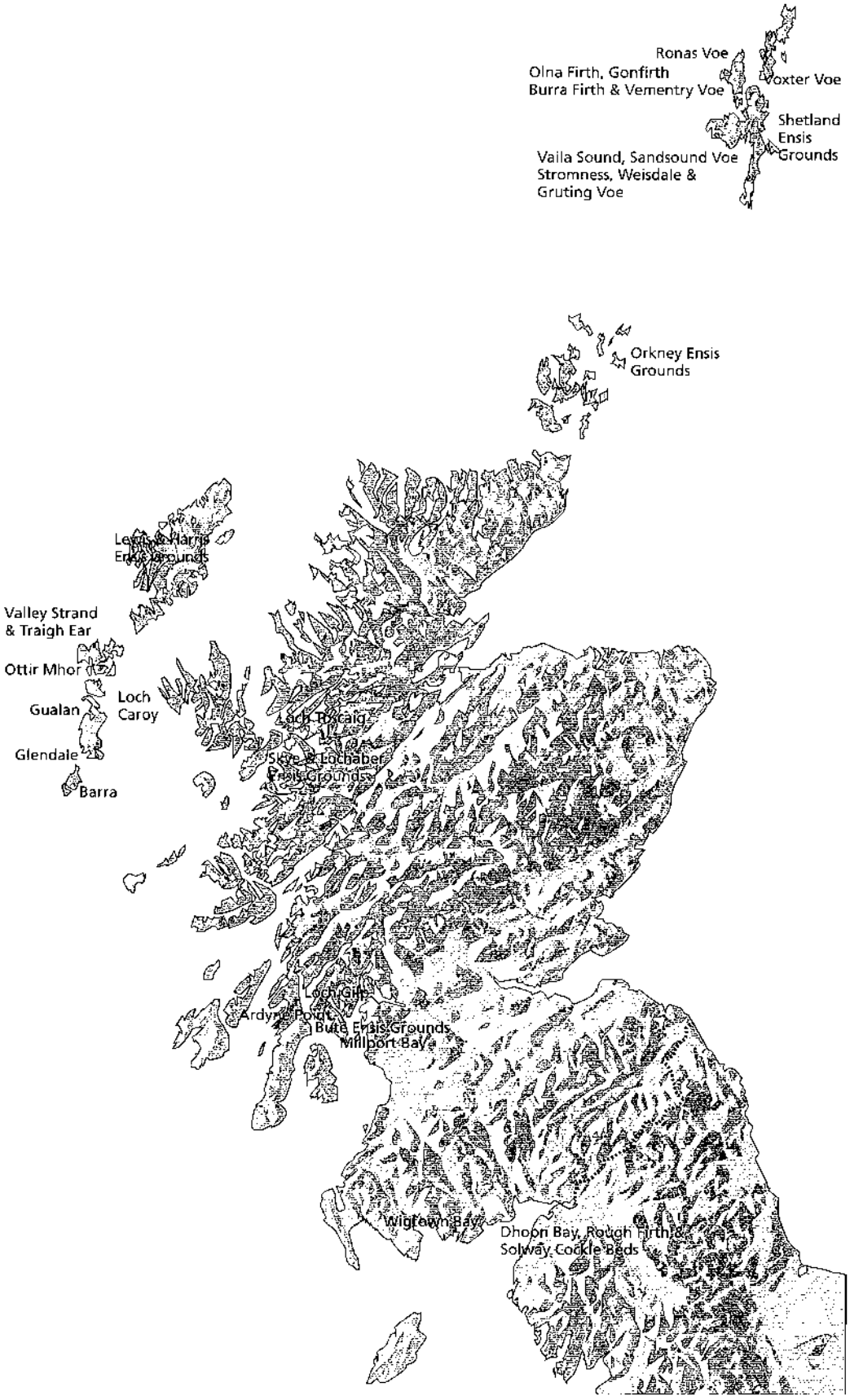
Shellfish Harvesting Areas: Northern Ireland



Shellfish Harvesting Areas: Scotland (map 1)



Shellfish Harvesting Areas: Scotland (map 2)



GLOSSARY

including abbreviations

This glossary is intended as an aid to the reading of the main text and should not be regarded as definitive.

AEROSOL: the suspension of particles in airborne water droplets.

AETIOLOGY: the study of the causation of disease.

AGGLUTINATION: the clumping together of antigens by antibodies so that a visible precipitate is formed.

AMORPHOUS: without definite shape or structure (eg. amorphous virus).

AMPLIFICATION INHIBITOR: see PCR amplification inhibitor

ANTI-EMETICS: drugs that counteract nausea and sickness.

ANTIBODY: a protein formed in direct response to the introduction into an individual of an antigen. Antibodies can combine with their specific antigens eg. to neutralise toxins or destroy bacteria.

ANTIGEN: a substance which elicits an immune response when introduced into an individual.

ANTISERUM: a solution which contains antibodies.

ASSAY: the determination of the content or the concentration of a substrate.

ASYMPTOMATIC INFECTION: an infection with a microorganism where the person infected does not suffer any resulting symptoms or disease.

BACTERIOPHAGE (PHAGE): a virus that infects bacteria.

BACTERIUM: a microscopic organism with a rigid cell wall - often unicellular and multiplying by splitting in two - which has the ability to live freely.

BACULOVIRUS: a recombinant virus expression system.

CAPSID: the protein coat of a virus particle.

CASE: a person in the population identified as having a particular disease.

CASE CONTROL STUDY: an epidemiological study in which the characteristics of persons with disease (eg. their food histories) are compared with a matched control group of persons without the disease or infection.

CCDC: Consultant in Communicable Disease Control.

cDNA: complementary DNA; copy DNA. A DNA molecule obtained by reverse transcription of an RNA molecule.

CDSC: Communicable Disease Surveillance Centre.

CEFAS: Centre for Environmental, Fisheries, and Aquaculture Science.

COLIFORMS: any of a group of bacteria associated with the colon, typified by *Escherichia coli*.

COLONISATION: the phenomenon of a population of microorganisms becoming established in a certain environment (especially in the intestinal tract of humans or animals) without necessarily giving rise to disease.

CPA: Clinical Pathology Accreditation scheme.

CSOs: combined sewage outflows.

DANI: Department of Agriculture for Northern Ireland.

DEOXYINOSINE: the deoxygenated form of the purine ribonucleoside inosine, containing the base hypoxanthine.

DEPURATION: a commercial treatment process used for shellfish. Harvested animals are transferred to tanks of clean seawater where they continue to filter feed for a period during which time sewage contaminants are purged out by normal physiological processes.

DETR: Department of the Environment, Transport and the Regions.

DH: Department of Health

DHSS(NI): Department of Health and Social Services (Northern Ireland).

DNA: Deoxyribonucleic acid, the genetic material of humans, bacteria, some viruses, etc. It is a polymer of nucleotides connected by sugars.

DNA HYBRIDISATION: a powerful and widely used technique which exploits the ability of complementary DNA sequences to pair.

DNA PROBE: A DNA fragment which has been labelled with a marker to indicate when DNA hybridisation has occurred.

D-VALUE: the time required at a given temperature to reduce the number of viable cells or spores of a given microorganism to 10% of the initial number, usually quoted in minutes.

EIA: see enzyme immunoassay

ELECTRON MICROSCOPY: microscopy that uses a beam of electrons as the radiation source for viewing a specimen.

ELISA: see enzyme-linked immunosorbent assay.

ENTERIC VIRUS: any virus which enters the body through the gastrointestinal tract, multiplies there, and is usually transmitted by the faecal/oral route.

ENTEROVIRUS: any virus which enters the body through the gastrointestinal tract, multiplies there, and has a tendency to invade the central nervous system.

ENZYME: a protein which acts as a highly efficient and specific biological catalyst.

ENZYME IMMUNOASSAY (EIA): any immunoassay in which an enzyme is used (as a marker) to indicate the presence of specific antigens, antibodies, etc.

ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA): an assay in which an enzyme is used (as a marker) to indicate the presence of specific antigens or antibodies.

EPIDEMIC: a disease occurring in a larger number of individuals than would normally be expected in a community at the same time.

EPIDEMIOLOGY: the study of factors affecting health and disease in populations and the application of this study to the control and prevention of disease.

EPITHELIAL CELLS: cells which form the layer (the epithelium) lining the inner surface of the intestines.

EPITOPE: an antigenic determinant. Any region of a macromolecule with the ability or potential to elicit, and combine with, specific antibody.

EU: European Union

FDA: (United States) Food and Drug Administration.

GAMMAGLOBULIN: a concentrated solution of the antibody fraction of the blood which has proved of great value in providing immunity against certain infectious diseases.

GASTROENTERITIS: inflammation of the stomach and intestine, usually due to infection by bacteria, viruses, or food poisoning toxins, causing vomiting and diarrhoea.

GENETIC ENGINEERING: the techniques involved in altering the characteristics of an organism by inserting genes from another organism into its DNA.

GENOME: the genetic material of an organism (eg. the DNA or RNA of a virus).

GENOTYPE: the genetic constitution of an organism (ie. the organism's content of genetic information).

HACCP: Hazard Analysis Critical Control Point.

HERD IMMUNITY: the collective immunity or resistance to a given disease exhibited by a community or population (human or animal) in the setting of its own environment.

HETEROLOGOUS: derived from, or associated with, a different species than that being referred to.

HETEROTYPIC: like to unlike - applied to the binding of adhesion molecules.

HOMOLOGOUS: derived from, or associated with, the same species as that being referred to.

HYBRIDISATION: the pairing of complementary RNA and DNA strands.

IgA, IgG, IgM: different types of immunoglobulin found in body fluids.

IMMUNITY: the body's ability to resist infectious disease, afforded by the presence of circulating antibodies and white blood cells.

IMMUNOASSAY: any procedure in which the specificity of the antigen-antibody reaction is used for detecting or quantifying antigens, antibodies or substances.

IMMUNOCOMPROMISED: an individual who is unable to mount a normal immune response.

IMMUNOGLOBULINS: a group of structurally-related proteins which are antibodies found in body fluids.

IMMUNOLOGICAL TESTS: tests based on antigen-antibody reactions.

INCIDENT: one or more cases.

INCUBATION PERIOD: the time interval between the initial entry of a pathogen into a host, and the appearance of the first symptoms of disease.

INDEX CASE: the first case in an outbreak of infectious disease.

INFECTIOUS DOSE: the amount of infectious material, eg. number of viruses, necessary to produce an infection.

IN VITRO: literally, "in glass", ie. in a test tube, plate etc. Used to describe biological processes made to happen in laboratory apparatus, outside a living organism.

IODOPHOR: an iodine-based antimicrobial/detergent complex.

JAUNDICE: the yellowing of the skin, or the whites of the eyes, indicating excess bilirubin (a bile pigment) in the blood.

LIPID: any of a large group of organic compounds that are esters of fatty acids, usually insoluble in water, but soluble in other organic solvents.

MAFF: Ministry of Agriculture, Fisheries and Food.

MICROFLORA: the microbial population of an area such as the gastro-intestinal tract.

MONOCLONAL (OR POLYCLONAL) ANTIBODIES: immunoglobulins derived from a single clone (or multiple clones) of plasma cells.

NIGHT SOIL: human excrement used as a fertilizer.

OLIGONUCLEOTIDES: short length polynucleoside chains, usually less than 30 residues long.

OUTBREAK: two or more cases of disease linked to a common source.

PAEDIATRICS: the branch of medical science concerned with children and their diseases.

PARENTERAL: administered by any route other than through the mouth.

PASTEURISATION: a form of heat treatment which kills vegetative pathogens and spoilage bacteria in milk and other foods.

PATHOGEN: any biological agent which can cause disease.

PATHOGENESIS: the manner in which a disease develops.

PATHOGENICITY: the ability to behave as a pathogen.

PCR: (see polymerase chain reaction)

PCR AMPLIFICATION INHIBITOR: a naturally-occurring substance which suppresses the generation of multiple copies of one or more genes during a PCR reaction.

pH: an index used as a measure of acidity or alkalinity.

PHAGE: see bacteriophage.

PHLS: Public Health Laboratory Service

PHYLOGENETIC: relating to the evolutionary history of a species or taxonomic group.

PLASMA: the fluid part of the blood in which the cells are suspended.

POLIOMYELITIS - "POLIO" (Infantile paralysis): an infectious food/water-borne viral infection affecting the central nervous system, resulting in mild to extreme paralysis.

POLYCLONAL ANTIBODIES: see monoclonal antibodies.

POLYMERASE CHAIN REACTION (PCR): an *in vitro* technique which enables multiple copies of a DNA fragment to be generated by amplification of a target DNA sequence.

PREVALENCE: the proportion of a population having a specific disease at a given point in time.

PRODROMAL: relating to the period of time, following the incubation period, when the first symptoms of illness appear.

PROPHYLACTIC: treatment, usually immunologic, designed to protect an individual from the future development of a condition or disease.

QA: quality assurance.

RADIOIMMUNE ASSAY (RIA): a highly sensitive immunoassay by which antigens and antibodies are quantified using radioactive labelling.

RECOMBINANT: DNA which contains sequences from different sources, brought together as a single unit to form a DNA sequence that is different from the original sources. Commonly used specifically for DNA molecules which have been constructed *in vitro* using various genetic engineering techniques.

RELAYING: a commercial treatment process used for shellfish. Harvested animals are transferred to cleaner estuaries or inlets, for self-purification in the natural environment.

REVERSE TRANSCRIPTASE (RT): an RNA-dependent DNA polymerase which synthesises DNA on an RNA template.

RIBONUCLEIC ACID (RNA): the genetic material of some viruses in the absence of DNA. Involved in protein synthesis in bacteria, humans, etc.

RNA POLYMERASE: an enzyme that catalyses the synthesis of RNA.

SCIEH: Scottish Centre for Infection and Environmental Health.

SEQUENCING: techniques used to determine the specific order of nucleotide residues in a nucleic acid.

SERODIAGNOSIS: identification of a microorganism by means of serological tests.

SEROLOGY: the study of antigen-antibody reactions *in vitro*.

SEROPREVALENCE: the persistence of serotype-specific serum antibodies, following infection with a given pathogen (eg. virus), which are capable of protecting against challenge with the same virus type (but there will be no protection against an antigenically different virus).

SEROTYPE: the antigenic characteristics of a pathogen.

SERUM: essentially similar to plasma (the fluid part of the blood), but lacking fibrinogen and other substances active in the coagulation process.

SERUM ANTIBODIES: antibodies found in the fluid fraction of coagulated blood.

SEWAGE SLUDGE: residual sludge from sewage plants treating domestic or urban waste waters.

SGMSF: Steering Group on the Microbiological Safety of Food.

SMALL ROUND STRUCTURED VIRUSES: the viral agents most commonly associated with foodborne viral infections. Distinguished from other viruses by their distinctive ragged surface morphology.

SOAEFD: Scottish Office Agriculture, Environment and Fisheries Department.

SOLID PHASE IMMUNE ELECTRON MICROSCOPY (SPIEM): electron microscopic examination of viruses which have been captured onto a solid grid by specific antibodies.

SOMATIC: refers to the body or main part of a cell. Thus, eg, a somatic antigen is a molecule which forms part of the body of a cell, usually at the cell surface, rather than one which occurs, eg, in a capsule or flagellum.

SPECIES: a classification of organisms within a genus which have similarities and can be further sub-divided into sub-species.

SPIEM: see solid phase immune electron microscopy.

SPORADIC CASE: a single case of disease apparently unrelated to other cases.

SRSVs: see small round structured viruses.

STRAIN: a population of organisms within a species or sub-species distinguished by sub-typing.

SUB-SPECIES: a classification of organisms within a species which have similarities.

SUBSTRATE: a specific compound acted upon by an enzyme.

SUB-TYPING: any method used to distinguish between species or sub-species.

SUSCEPTIBLE INDIVIDUAL: an individual who has no pre-existing immunity or resistance to infection, and who is therefore liable to become infected.

SYSTEMIC INFECTION: an infection disseminated widely through the body (ie. not localised).

TAXONOMY: the science of classification.

TITRATION: a procedure to determine the amount of a component (in a solution) by measuring the volume of a known concentration of reagent required to complete a reaction with that solution.

TITRE: the amount of the standard reagent necessary to produce a certain result in a titration.

TYPING: any method used to distinguish between closely related microorganisms.

UV: ultra violet.

VECTOR: any living organism which effects the transmission of a parasite from one individual (man, animal or plant) to another.

VENEPUNCTURE: the puncturing of a vein for any therapeutic purpose.

VIRULENCE: virulence is defined broadly in terms of the severity of the symptoms in the host. Thus, a highly virulent strain may cause severe symptoms in a susceptible individual, while a less virulent strain would produce relatively less severe symptoms in the same individual.

VIRUS: a sub-microscopic organism which is only capable of replication within living cells.

VISCERA: the organs within the body cavities, especially those of the abdominal cavities.

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* NB: these are not small-round viruses

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