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1 INTRODUCTION

Many viruses are excreted by humans and animals. Some of these are present in very large numbers but cannot be grown, others can only be grown with difficulty in cell cultures. Enteroviruses, hepatitis A virus, rotaviruses, parvovirus-like viruses, astroviruses, caliciviruses, adenoviruses and coronaviruses may be present in human excreta. Rotaviruses are the commonest cause of acute non-bacterial gastroenteritis in infancy and childhood. In children aged 6 months to 10 years, infection by rotaviruses occurs more frequently during winter months in temperate countries. In tropical countries, there is considerable variation in the incidence of rotavirus infections among children.

Enteroviruses include the polio, coxsackie A and B and echoviruses. These belong to the family of Picornaviridae. They contain single-stranded RNA. Poliomyelitis is rare in developed countries, as a result of the widespread use of oral polio vaccines, but continues to circulate in developing countries. Similarly, hepatitis A is an RNA virus and has the physicochemical characteristics of a typical enterovirus. It is transmitted from person to person by the faeco-oral route. Outbreaks may originate from viral contamination of food, water, milk and shellfish. Rotaviruses and parvovirus-like viruses cause gastroenteritis. The role of adenoviruses, astroviruses, caliciviruses and coronaviruses is not well-established. However, it is now recognized that these viruses can cause outbreaks of acute diarrhoeal disease. Enteric adenoviruses represent a separate serotype distinct from adenoviruses associated with acute respiratory infection and have been associated with acute diarrhoeal disease.

Besides parvovirus-like viruses, other small rounded viruses have been detected in faecal samples from patients with acute diarrhoeal disease. These include:

1. astroviruses, encountered in outbreaks in children’s wards (Kurtz et al., 1977), stools of lamb with diarrhoea and calf faeces
2. caliciviruses, detected in stools in children in winter and staff with diarrhoea and also asymptomatic children (McSwiggan et al., 1978)
3. coronaviruses, which cause severe diarrhoea in pigs and calves; there is no conclusive evidence that these viruses cause diarrhoea in humans.

2 VIRUSES IN WASTEWATER

The types and numbers of human enteric viruses in raw wastewater depend on the origin and nature of the wastewater. Enteroviruses, including hepatitis A virus, adenovirus and rotaviruses, are frequently present in domestic wastewater. These viruses reflect those circulating in the community and depend on a number of factors, which include population, climate, seasonal and diurnal fluctuations and the presence of chemical effluents. Raw wastewater contains viral numbers which vary widely from country to country. For example,
in raw wastewater in Israel, higher numbers are generally present than in the USA, although the numbers reported depend on the detection and concentration methods used.

Animal viruses may also be present in raw wastewater from the faeces of household pets and effluents from farms, abattoirs and stormwater. The isolation of animal viruses from wastewater, especially abattoir wastes, has been reported by several workers. However, more emphasis has been on human viruses due to their potential for causing human diseases.

Viruses may be categorized or divided into three main groups:

1. viruses associated with humans
2. viruses associated with higher plants and animals
3. viruses associated with the microbial flora (bacteriophages)

Over a hundred different types of viruses are known (WHO, 1979). Excreta is the commonest source, but by no means the only source. Nose and throat secretions also contribute some viruses to wastewater, and viruses from skin lesions and blood (which may find its way through baths and toilets during acute stages of infection giving rise to viraemia) can also be found in wastewater. These sources, although they do not contribute much, may in certain situations be important.

Viruses associated with plants and animals may be important for economic reasons, especially in countries where the reuse of wastewater for crop irrigation is practised. Some of these viruses come from the wastewater itself, whereas others may enter the sewer via wastewater from farms, slaughterhouses, food-processing factories and stormwater. Faecal materials from birds, dogs and other animals will be washed from roofs and roads by rain and find their way ultimately into the wastewater.

The third group comprises the bacteriophages, which are viruses that parasitize bacteria and, because of the huge variety of bacteria in wastewater, which may all have their own phages, phage numbers in wastewater can be very high.

3 VIRUS RECOVERY FROM WASTEWATERS

One of the major problems encountered in the enumeration of viruses in water and wastewater before and, particularly, after treatment is the large quantity of sample that has to be examined in order to detect and count them. This is made even more difficult by the fact that the enumeration technique usually employed for rotavirus assays utilizes only 25–200 μl of sample. For enterovirus assays, 0.1–1 ml can usually be assayed conveniently. The consequent dilution of viruses in faeces when discharged into receiving waters or sewage means the sample must be concentrated in order to analyse them. Various techniques are available for concentrating viruses (see Oragui and Mara, 1996).

One of the difficulties encountered with concentrated wastewater samples is the toxic effects that they have on the cell lines used for virus propagation and enumeration. Concentrates of raw wastewater and, in particular those obtained from anaerobic waste stabilization ponds, are very toxic to the cells used for rotavirus assay. Toxic effects are indicated by the destruction or detachment of cells during rotavirus assay by the indirect immunofluorescence technique. A simple method for the detoxification of concentrate has been developed and is described by Oragui and Mara (1989).

4 VIRUS REMOVAL AND SURVIVAL IN WASTEWATER TREATMENT

Conventional wastewater treatment plants are not specifically designed to reduce the number of excreted pathogens, including excreted viruses. However, there is usually some incidental removal of viruses – primary sedimentation ranges from 30 to 65% (Berg, 1973; Rao et al., 1977); in activated sludge from 80 to >90% (Clarke et al., 1961; Malina, 1976); and in trickling filters from 10 to 20% (Sherman et al., 1975), although Berg (1973) reported removals of 15–100%. Disinfection processes, principally chlorination and ozonation, for the removal of faecal bacteria, have not been
widely adopted other than in a few countries, notably the USA, Israel and South Africa, because of the detrimental effects on the natural fauna of receiving waters and the production and discharge of toxic and carcinogenic chlorination by-products.

In a study on rotavirus removal in a series of five 3 m deep waste stabilization ponds in northeast Brazil, Oragui and Mara (1996) found four log unit removals of both rotaviruses and faecal coliform bacteria (Table 29.1). The highest rotavirus removal was found in the anaerobic pond (95%), with lower removal in the facultative and maturation ponds (76–88%).

### 4.1 Factors affecting rotavirus removal in ponds

Several factors may explain viral inactivation in waste stabilization ponds, including solar radiation, temperature, pH, adsorption onto solids, heavy metals, algal and bacterial activity, and the action of certain chemicals, notably ammonia and sulphide.

The unionized ammonia (dissolved NH₃ gas) is toxic to bacteria (Pearson et al., 1987), with toxicity increasing with increasing pH. The effect of ammonia toxicity on rotavirus inactivation has also been shown to be pH dependent (Oragui and Mara, 1996): at pH 9.0 there was a reduction of just over half a log unit at all ammonia concentrations (20–80 mg N/l), whereas at pH of 6.9, the log reduction ranged from 1.3 to 2.2. In contrast to the effect of ammonia on bacteria, ammonia is more toxic to rotaviruses at pH 6.9, when it is essentially all present as the ammonium ion, than at pH 9.

Sulphide is one of the main toxicants present in raw wastewater and anaerobic pond effluents. Sulphide was found to exert the most effect on simian rotaviruses at pH 6.9, with log reductions of 0.1–3.9; at pH 8 reduction was 0.04–0.09, but at pH 9 it was 0.03–0.46. In contrast to ammonia, increasing sulphide concentrations in the range 4–16 mg S/l increased the degree of virus inactivation.

In 2 m deep facultative ponds in-pond rotavirus numbers decreased principally in the top 1 m of the pond, with little difference found in rotavirus numbers in the 1–2 m lower layer. This indicates that, as the surface layers experience algae-induced pH changes, the toxic effects of ammonia and sulphide are likely to be the main mechanism of removal of rotaviruses, and possibly of all excreted virus, although adsorption on to the algae and subsequent settlement when the algae die may be an important secondary mechanism.

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