

# Minimum Infective Dose of the Major Human Respiratory and Enteric Viruses Transmitted Through Food and the Environment

Saber Yezli · Jonathan A. Otter

Received: 5 January 2011 / Accepted: 26 February 2011 / Published online: 16 March 2011  
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**Abstract** Viruses are a significant cause of morbidity and mortality around the world. Determining the minimum dose of virus particles that can initiate infection, termed the minimum infective dose (MID), is important for the development of risk assessment models in the fields of food and water treatment and the implementation of appropriate infection control strategies in healthcare settings. Both respiratory and enteric viruses can be shed at high titers from infected individuals even when the infection is asymptomatic. Presence of pre-existing antibodies has been shown to affect the infectious dose and to be protective against reinfection for many, but not all viruses. Most respiratory viruses appear to be as infective in humans as in tissue culture. Doses of  $<1$  TCID<sub>50</sub> of influenza virus, rhinovirus, and adenovirus were reported to infect 50% of the tested population. Similarly, low doses of the enteric viruses, norovirus, rotavirus, echovirus, poliovirus, and hepatitis A virus, caused infection in at least some of the volunteers tested. A number of factors may influence viruses' infectivity in experimentally infected human volunteers. These include host and pathogen factors as well as the experimental methodology. As a result, the reported infective doses of human viruses have to be interpreted with caution.

**Keywords** Minimum infectious dose · Respiratory viruses · Enteric viruses · Infection

## Introduction

Viruses are the most abundant biological entities on the planet. There are an estimated  $10^{31}$  viruses on earth, most of which are bacteriophages (Breitbart and Rohwer 2005). Humans have been infected by viruses throughout their evolutionary history and it seems likely that viruses have played a role in human evolution (Van Blerkom 2003). Viruses are a significant cause of morbidity and mortality around the world and can be transmitted via air, food, water, or by direct contact with contaminated body fluids. Viruses can enter the body through various sites including the respiratory and enteric tracts by aerosolized droplets, droplet nuclei, or the fecal–oral route. Understanding the epidemiology and pathogenesis of viral infections, and the hosts' immune response to such infections are key to the control and prevention of viral diseases and to the development of vaccines. Determining the minimum dose of virus particles that can initiate infection, termed the minimum infective dose (MID), and the factors influencing this dose are important for the development of risk assessment models in the fields of food and water treatment and the implementation of appropriate infection control strategies to prevent viral transmission in healthcare settings.

As obligate intracellular parasites, viruses must invade host cells to initiate infection whether in cultured tissues or in the body of the host. Infections in humans normally require extensive viral replication in order to be detected due to the limited sensitivity of diagnostic methods. Typically direct detection of infectious progeny viruses in body products such as nasal secretions, blood and feces, or host responses such as antibody production have been used to monitor viral infections in experimentally infected humans. The doses of virus administered are usually determined from cell culture infectivity assays where the presence of

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S. Yezli (✉) · J. A. Otter  
Bioquell UK Ltd, 52 Royce Close, West Portway,  
Andover, Hampshire SP10 3TS, UK  
e-mail: saber.yezli@bioquell.com

the infectious virus is detected by its ability to cause changes in cell appearance or even cell destruction throughout a monolayer of cells (cytopathic effect) or in restricted regions of the monolayer (plaque formation). These viral doses are then expressed either as the dilution of virus sufficient to cause cytopathic effect in 50% of the inoculated culture (TCID<sub>50</sub>) or as plaque-forming units (pfu) (Ward et al. 1984a).

Investigations into the MID of human viruses have typically involved the experimental administration of the virus and the use of dose response data to determine the viral concentration required to infect 50% of the population (Human ID<sub>50</sub>; HID<sub>50</sub>) or concentrations required to infect lesser percentages of the populations such as HID<sub>10</sub> and HID<sub>1</sub>. Since determining the HID<sub>10</sub> and HID<sub>1</sub> requires a large number of test subjects to achieve statistically significant values, making it a time-consuming and expensive process, most human MIDs have been expressed as HID<sub>50</sub>. Since the HID<sub>50</sub> represents the viral concentration required to infect 50% of the population, it follows that this value is always greater than the minimum dose required to cause infection (Ward et al. 1984a). Furthermore, since the titer of virus used to challenge a volunteer is often expressed as a TCID<sub>50</sub>, the actual number of virus particles involved in infection is often unknown.

A number of factors may influence the virus dose response data obtained from studies of experimentally infected human volunteers. These include host factors such as age, health status, and previous exposure to the virus; pathogen factors such as virulence of the viral strain and passage in cell culture; and experimental factors such as the route of inoculation and the sensitivity of the assay used to determine the viral dose administered. Since MID studies typically use young, healthy volunteers, and single laboratory adapted or attenuated virus strains, they are, therefore, of limited value in assessing the hazard from wild-type strains for the general population and even less so for individuals with particular risk factors.

The use of mathematical models to extrapolate dose-response experimental data to extremely low exposure levels combined with the likely underestimation of the MID through the use of HID<sub>50</sub> to express the MID and the experimental limitations outlined above, are some of the reasons why the concept of MID as a threshold below which infection cannot occur has received criticism. As consequence, the notion that a single pathogen may be capable of causing infection (single-hit model) has steadily gained support especially in relation to viruses (Haas 1983; Haas et al. 1993; Teunis and Havelaar 2000).

A thorough review of MID of human viruses has not been published since the article by Ward et al. (1984a) published decades ago. Here, we review the infectious doses reported for the major human respiratory and enteric

viruses determined from experimental infections of human volunteers.

## Respiratory Viruses

The respiratory and the gastrointestinal tracts are the two most important portals of entry of viruses in humans (Evans 1982). A large proportion of viral MID studies have focused on respiratory viruses (Ward et al. 1984a). Following the interest in respiratory virology in the 1960s, species of rhinoviruses, coronaviruses, enteroviruses, adenoviruses, parainfluenza viruses, and respiratory syncytial virus (RSV) were added to influenza and measles viruses as causes of respiratory tract infections. In restricted patient groups, such as the immunocompromised, members of the family of herpesviruses including herpes simplex, cytomegalovirus, varicella-zoster virus, Epstein-Barr virus, and human herpes virus 6 have also been associated with respiratory disease (Mackie 2003). This list of pathogens was extended in more recent years with the discovery of novel respiratory viruses such as the human metapneumovirus and the human bocavirus (Kahn 2007).

Respiratory viruses most often infect through the airborne route although contact with contaminated surfaces is also an important mode of their transmission (Brankston et al. 2007; Couch et al. 1966; Falsey and Walsh 2000; Gwaltney and Hendley 1982; Nicas et al. 2005). More than 200 antigenically distinct viruses have been documented as causes of sporadic or epidemic respiratory infections in infants, children, and adults (Mackie 2003; Nichols et al. 2008). However, this diverse group can be organized into a small number of distinct families. The major respiratory RNA viruses belong to the following families: the *Orthomyxoviridae* (including influenza virus), the *Paramyxoviridae* (including parainfluenza virus and RSV), the *Picornaviridae* (including the rhinoviruses and the enteroviruses such as coxsackievirus and numbered enteroviruses), and the *Coronaviridae* (including human coronavirus (HCoV) 229E, HCoVOC43 and the severe acute respiratory syndrome-associated CoV (SARS-CoV)). Important respiratory DNA viruses belong to the families *Adenoviridae*, *Parvoviridae*, and *Herpesviridae*.

Although viral respiratory infections can be classified by the causative virus, they are generally classified clinically according to the syndrome they produce (e.g., the common cold, bronchiolitis, pneumonia, and croup). Infection of the respiratory tissues is normally initiated in the upper respiratory tract, and this is also the site where viruses come into contact with the immune system. Certain respiratory viruses such as the rhinoviruses, most adenoviruses, and coronaviruses are limited to infection of the upper respiratory tract, where they cause generally self-limiting common

cold-like illnesses. Some viruses, however, are capable of penetrating the initial defenses in the upper respiratory tract to cause more serious and potentially life-threatening lower respiratory tract infections. These viruses, the most important of which are influenza virus, RSV, and parainfluenza virus type 3 (PI-3V), cause the greatest burden of respiratory disease in humans (Adair 2009).

Experimental human infections with respiratory viruses have mainly been conducted by administration of the virus via nasal drops or by aerosols. The drops promote infection of the upper respiratory tract while aerosols allow infection of both the upper and lower regions of this tract. Although some of the viruses discussed in this section are enteric viruses (coxsackieviruses and adenoviruses), they are known to infect both the respiratory and intestinal tracts and hence are included in this section.

### Influenza Virus

Influenza virus infection is a highly contagious respiratory disease that can spread easily and is responsible for considerable morbidity and mortality each year (Kawaoka 2006; Nicholson et al. 2003). Influenza is caused by a RNA virus of the family *Orthomyxoviridae* and is classified into three types, influenza A, B, and C. Influenza A are essentially avian viruses that periodically transmit to other species including mammals. However, they are the most virulent human pathogens among the three influenza types and cause the most severe disease. Furthermore, influenza A viruses comprise a large variety of antigenically distinct subtypes that replicate asymptotically in the intestine of birds and constitute a large reservoir of potentially pandemic viruses (Hay et al. 2001). Influenza C infects humans and some other animals such as pigs (Guo et al. 1983; Matsuzaki et al. 2000) while influenza B almost exclusively infects humans (Hay et al. 2001). Three different modes of influenza transmission have been identified: droplet, airborne (droplet nuclei), and contact transmission (Brankston et al. 2007; Garner 1996; Nicas et al. 2005; Tellier 2006). Which of the three modes is responsible for most influenza infections remains highly controversial (Brankston et al. 2007; Tang and Li 2007; Tellier 2006; Weber and Stilianakis 2008). Numerous studies reported the infectious dose of the influenza virus in human volunteers (Table 1) using various strains of the influenza A or B virus administered either by nasal drops or aerosols. The results of these studies suggest that the nasal infectious dose of influenza virus A is several orders of magnitude higher than that of airborne infection (Weber and Stilianakis 2008).

Many of the published infectious doses of influenza virus come from studies into the prophylactic or

therapeutic effect of various compounds and investigations into their role in preventing or treating experimentally induced influenza infection in human volunteers. These studies have often used high doses of virus inoculated intranasally to produce disease in as many subjects as possible. For example, approximately  $10^7$  TCID<sub>50</sub> of influenza B strain B/Yamagata/16/88 infected over 80% of inoculated subjects but produced illness in only a few (Barroso et al. 2005; Hayden et al. 2000). A similar dose of influenza B/Panama/45/90 infected 55% of the 11 inoculated subjects, four of whom developed illness (Treanor et al. 1999). Doses between  $10^5$  and  $10^7$  TCID<sub>50</sub> of the H<sub>1</sub>N<sub>1</sub> influenza A (A/Texas/36/91), infected most intranasally inoculated volunteers and caused illness in the majority of subjects in some (Brankston et al. 2007; Murphy et al. 1998) but not all studies (Barroso et al. 2005; Hayden et al. 1999; Treanor et al. 1999). Another H<sub>1</sub>N<sub>1</sub> influenza A strain, A/Kawasaki/9/86, infected most subjects and caused disease in about half of them when administered intranasally at a dose of  $10^7$  TCID<sub>50</sub> (Doyle et al. 1998; Gentile et al. 1998; Hayden et al. 1994). The H<sub>3</sub>N<sub>2</sub> influenza strain A/England/42/72 infected and caused disease in just over half of the inoculated population when a  $3.5 \times 10^3$  TCID<sub>50</sub> or a 1,000 times higher doses were used (Arroyo et al. 1975; Douglas et al. 1975). The infection rate with  $1.2 \times 10^4$  TCID<sub>50</sub> of the A/England/40/83 strain was reported to be over 90% and this dose caused illness in 40% of the subjects (Al-Nakib et al. 1986). The two H<sub>3</sub>N<sub>2</sub> influenza strains, A/University of Maryland/1/70 and A/University of Maryland/2/74, caused illness in a majority (>86%) of volunteers inoculated with about  $10^4$  TCID<sub>50</sub>. A  $6.4 \times 10^4$  TCID<sub>50</sub> of the A<sub>2</sub>/Rockville/1/65 virus delivered to the nasopharynx infected most inoculated subjects with approximately half of them developing influenza symptoms (Bloomfield et al. 1970; Togo et al. 1968). A ten times higher dose ( $6.3 \times 10^5$  TCID<sub>50</sub>) of the same strain administered in a similar manner to the nasopharynx of adult male volunteers did not show a higher attack rate (Mann et al. 1968).

The HID<sub>50</sub> of human influenza virus has been determined mainly for attenuated vaccine strains using dose response data. These attenuated cold-adapted or avian-human reassortant influenza virus vaccines are commonly derived from mating of a wild-type human influenza virus with the cold-adapted H<sub>2</sub>N<sub>2</sub> (*ca*) A/Ann Arbor/6/60 or B/Ann Arbor/1/66 donors or the H<sub>2</sub>N<sub>2</sub> avian influenza A/Mallard/New York/6750/78 virus. The HID<sub>50</sub> determined in this manner is not always an accurate representation of the HID<sub>50</sub> of the wild-type virus, which may be more virulent than the attenuated vaccine strain (Clements et al. 1983; Snyder et al. 1986a). For example, Snyder et al. (1986a) reported that a dose of  $3.1 \times 10^4$  TCID<sub>50</sub> of the A/California/10/78 wild-type virus infected 93% of the

**Table 1** Calculated or actual infectious doses of influenza virus from studies on human volunteers

Influenza strain	Target of virus delivery	Dose	% Infected <sup>a</sup>	% Ill	References
A/Alaska/6/77 (H <sub>3</sub> N <sub>2</sub> )	Nose	$1.5 \times 10^4$ TCID <sub>50</sub>	100 (8/8)	50 (4/8)	Clements et al. (1983)
A/California/10/78 (H <sub>1</sub> N <sub>1</sub> )	Nose	$3.1 \times 10^4$ TCID <sub>50</sub>	93 (14/14)	43 (6/14)	Snyder et al. (1986a)
A/England/42/72 (H <sub>3</sub> N <sub>2</sub> )	Nose	$3.5 \times 10^3$ TCID <sub>50</sub> <sup>b</sup>	50 (5/10)	70 (7/10)	Douglas et al. (1975)
A/England/42/72 (H <sub>3</sub> N <sub>2</sub> )	Nose	$3.1 \times 10^6$ TCID <sub>50</sub>	65 (13/20)	65 (13/20)	Arroyo et al. (1975)
A/England/40/83 (H <sub>3</sub> N <sub>2</sub> )	Nose	$1.2 \times 10^4$ TCID <sub>50</sub>	92 (63/68)	40 (27/68)	Al-Nakib et al. (1986)
A <sub>2</sub> /Bethesda/10/63 (H <sub>2</sub> N <sub>2</sub> )	Nose + throat	$4.0 \times 10^1$ TCID <sub>50</sub>	50 (15/30)	33 (10/30)	Jao et al. (1970)
A <sub>2</sub> /Bethesda/10/63 (H <sub>2</sub> N <sub>2</sub> )	–	$8.0 \times 10^4$ – $1.8 \times 10^5$ TCID <sub>50</sub>	100 (8/8)	50 (4/8)	Alford et al. (1967b)
A <sub>2</sub> /Bethesda/10/63 (H <sub>2</sub> N <sub>2</sub> )	Nose	$5.0 \times 10^0$ TCID <sub>50</sub>	44 (4/9)	33 (3/9)	Alford et al. (1966)
A/Equi 2/Miami/1/63 (H <sub>3</sub> N <sub>8</sub> )	Nose + throat	$4.0 \times 10^4$ – $2.0 \times 10^5$ TCID <sub>50</sub>	63 (21/33)	12 (4/33)	Alford et al. (1967a)
A/Equi 2/Miami/1/63 (H <sub>3</sub> N <sub>8</sub> )	Nose + oropharynx	$7.9 \times 10^4$ TCID <sub>50</sub>	100 (5/5)	20 (1/5)	Kasel et al. (1965a)
A <sub>2</sub> /Hong Kong/1/68 (H <sub>3</sub> N <sub>2</sub> )	Nose	$1.0 \times 10^3$ TCID <sub>50</sub>	78 (11/14)	42 (6/14)	Couch et al. (1971)
A/Kawasaki/9/86 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^7$ TCID <sub>50</sub>	70 (12/17)	–	Gentile et al. (1998)
A/Kawasaki/9/86 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^7$ TCID <sub>50</sub>	100 (16/16)	69 (11/16)	Hayden et al. (1994)
A/Kawasaki/9/86 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^7$ TCID <sub>50</sub>	92 (49/53)	49 (26/53)	Doyle et al. (1998)
A/Korea/1/82 (H <sub>3</sub> N <sub>2</sub> )	Nose	$1.5 \times 10^6$ TCID <sub>50</sub>	100 (14/14)	50 (7/14)	Snyder et al. (1986a)
A/Nederland/37/57	Nose + throat	$1.0 \times 10^3$ EID <sub>50</sub> <sup>c</sup>	40 (2/5)	40 (2/5)	Isaacs et al. (1957)
A <sub>2</sub> /Rockville/1/65	Nose + throat	$6.3 \times 10^5$ TCID <sub>50</sub>	75 (9/12)	25 (3/12)	Mann et al. (1968)
A <sub>2</sub> /Rockville/1/65	Nose + throat	$6.4 \times 10^4$ TCID <sub>50</sub>	86 (6/7)	57 (4/7)	Togo et al. (1968)
A <sub>2</sub> /Rockville/1/65	Nose + throat	$6.4 \times 10^4$ TCID <sub>50</sub>	88 (8/9)	44 (4/9)	Bloomfield et al. (1970)
A/Shangdong/9/93 (H <sub>3</sub> N <sub>2</sub> )	Nose	$1.0 \times 10^7$ TCID <sub>50</sub>	50 (4/8)	50 (4/8)	Treanor et al. (1999)
A/Texas/36/91 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^3$ TCID <sub>50</sub>	50 (9/18)	–	Hayden et al. (1996)
A/Texas/36/91 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^6$ TCID <sub>50</sub>	100 (14/14)	71 (10/14)	Murphy et al. (1998)
A/Texas/36/91 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^5$ TCID <sub>50</sub>	100 (8/8)	100 (8/8)	Calfee et al. (1999)
A/Texas/36/91 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^7$ TCID <sub>50</sub>	58 (7/12)	50 (6/12)	Treanor et al. (1999)
A/Texas/36/91 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^6$ TCID <sub>50</sub>	67 (8/12)	33 (4/12)	Hayden et al. (1999)
A/Texas/36/91 (H <sub>1</sub> N <sub>1</sub> )	Nose	$1.0 \times 10^7$ TCID <sub>50</sub>	94 (17/18)	24 (4/17)	Barroso et al. (2005)
A/Texas/1/85 (H <sub>1</sub> N <sub>1</sub> )	Nose	$5.0 \times 10^6$ TCID <sub>50</sub>	91 (20/22)	41 (9/22)	Sears and Clements (1987)
A/University of Maryland/1/70 (H <sub>3</sub> N <sub>2</sub> )	Nose + throat	$6.4 \times 10^4$ TCID <sub>50</sub>	100 (7/7)	86 (6/7)	Togo et al. (1972)
A/University of Maryland/2/74 (H <sub>3</sub> N <sub>2</sub> )	–	$2.0 \times 10^4$ TCID <sub>50</sub>	–	88 (8/9)	Cohen et al. (1976)
A/Victoria/3/75 (H <sub>3</sub> N <sub>2</sub> )	Nose	$2.5 \times 10^3$ TCID <sub>50</sub>	80 (12/15)	80 (12/15)	Magnussen et al. (1977)
B/Georgia/26/74	–	$6.4 \times 10^4$ TCID <sub>50</sub>	–	66 (10/15)	Togo and McCracken (1976)
B/Panama/45/90	Nose	$1.0 \times 10^7$ TCID <sub>50</sub>	55 (6/11)	36 (4/11)	Treanor et al. (1999)
B/Yamagata/16/88	Nose	$0.5 \times 10^7$ TCID <sub>50</sub>	84 (16/19)	21 (4/19)	Hayden et al. (2000)
B/Yamagata/16/88	Nose	$3.1 \times 10^7$ TCID <sub>50</sub>	95 (18/19)	0 (0/19)	Barroso et al. (2005)

<sup>a</sup> As determined by virus shedding and/or antibody response

<sup>b</sup> 50% Tissue culture infective dose

<sup>c</sup> 50% Egg infective dose

inoculated subjects and 43% of them became ill, while  $1.5 \times 10^6$  TCID<sub>50</sub> of the A/Korea/1/82 wild-type virus infected all volunteers and caused illness in half of them. The HID<sub>50</sub> of the avian influenza A/California/10/78 (H<sub>1</sub>N<sub>1</sub>) and A/Korea/1/82 (H<sub>3</sub>N<sub>2</sub>) reassortant vaccines were  $8.0 \times 10^4$  TCID<sub>50</sub> and  $2.5 \times 10^5$  TCID<sub>50</sub>, respectively. These values are comparable to the reported HID<sub>50</sub> of the A/Mallard/New York/6750/78 × A/Washington/897/80 (H<sub>3</sub>N<sub>2</sub>) avian human reassortant virus ( $8.0 \times 10^5$  TCID<sub>50</sub>) (Murphy et al. 1985) or the cold-adapted reassortant virus from the same wild-type strain (HID<sub>50</sub> =  $4.5 \times 10^5$

TCID<sub>50</sub>) (Clements et al. 1984). The HID<sub>50</sub> of the avian influenza A/Kawasaki/9/86 (H<sub>1</sub>N<sub>1</sub>), A/Texas/1/85 (H<sub>1</sub>N<sub>1</sub>), and A/Bethesda/1/85 (H<sub>3</sub>N<sub>2</sub>) reassortant vaccines were reported to be  $8.0 \times 10^2$  TCID<sub>50</sub>,  $2.5 \times 10^5$  TCID<sub>50</sub> and  $3.1 \times 10^6$  TCID<sub>50</sub>, respectively, in seronegative volunteers (Sears et al. 1988; Steinhoff et al. 1991). A higher HID<sub>50</sub> ( $4.0 \times 10^4$  TCID<sub>50</sub>) of the avian influenza A/Bethesda/1/85 (H<sub>3</sub>N<sub>2</sub>) reassortant vaccine in seronegative young children was reported by others (Steinhoff et al. 1990).

Clements et al. (1983) determined the HID<sub>50</sub> of the A/Alaska/6/77 *ca* virus in seronegative adults to be

$3.1 \times 10^5$  TCID<sub>50</sub>. The attenuated vaccine was derived from the A/Ann Arbor/6/60 (H<sub>2</sub>N<sub>2</sub>) cold-adapted (*ca*) donor virus and the A/Alaska/6/77 (H<sub>3</sub>N<sub>2</sub>) wild-type virus. Ten and one hundred HID<sub>50</sub> infected 73 and 83% of those vaccinated, respectively, and approximately 75% developed immunological response at these doses. A  $1.5 \times 10^4$  TCID<sub>50</sub> dose of the wild-type infected all eight volunteers and caused illness in half of them. The A/Alaska/6/77 *ca* virus was only slightly less infectious for seronegative adults than was the A/Hong Kong/123/77 (H<sub>1</sub>N<sub>1</sub>) *ca* virus which had a HID<sub>50</sub> of  $10^5$  TCID<sub>50</sub> in persons vaccinated who had not been previously infected with an H<sub>1</sub>N<sub>1</sub> virus (Murphy et al. 1980). In two separate studies, the HID<sub>50</sub> of the B/Texas/1/84 (H<sub>1</sub>N<sub>1</sub>) vaccine virus (CRB 87), resulting from the crossing of influenza B/Ann Arbor/1/66 *ca* virus with the wild-type influenza B/Texas/1/84, was  $3.1 \times 10^4$  and  $2.5 \times 10^5$  TCID<sub>50</sub> (Anderson et al. 1992; Keitel et al. 1990). The HID<sub>50</sub> of the wild-type B/Texas/1/84 virus was less than  $8.0 \times 10^3$  TCID<sub>50</sub> as all volunteers given  $8.0 \times 10^3$ – $1.2 \times 10^7$  TCID<sub>50</sub> of the wild-type virus were infected (Keitel et al. 1990). A comparable HID<sub>50</sub> ( $8.0 \times 10^4$  TCID<sub>50</sub>) of the A/Ann Arbor/6/60 × A/Texas/1/85 (H<sub>1</sub>N<sub>1</sub>) cold-adapted reassortant virus was reported in another study in seronegative adult volunteers (Sears et al. 1988). The HID<sub>50</sub> of the A/Bethesda/1/85 (H<sub>3</sub>N<sub>2</sub>) cold-adapted reassortant vaccine was reported to be  $2.5 \times 10^4$  TCID<sub>50</sub> by Steinhoff et al. (1990) and  $2.5 \times 10^6$  TCID<sub>50</sub> by Sears et al. (1988). A similar HID<sub>50</sub> ( $2.5 \times 10^6$  TCID<sub>50</sub>) was determined for an influenza B cold-adapted vaccine derived from the influenza B/Ann Arbor/1/86 wild-type virus and the influenza B/Ann Arbor/1/66 *ca* virus (Clements et al. 1990). The HID<sub>50</sub> of the cold-adapted influenza A/Kawasaki/9/86 (H<sub>1</sub>N<sub>1</sub>) *ca* virus was low in seronegative children (HID<sub>50</sub> =  $4.0 \times 10^2$  TCID<sub>50</sub>), which is similar to that of the avian human A/Kawasaki/9/86 reassortant vaccine (HID<sub>50</sub> =  $8.0 \times 10^2$  TCID<sub>50</sub>) (Steinhoff et al. 1991).

Aerosolized influenza infection has been documented in mouse models, squirrel monkey models, and human volunteers (Alford et al. 1966; Hood 1963; Snyder et al. 1986b). The HID<sub>50</sub> of Asian influenza virus, A<sub>2</sub>/Bethesda/10/63 (H<sub>2</sub>N<sub>2</sub>) was reported to be 0.6–3.0 TCID<sub>50</sub> when administered in small particle aerosols to serum antibody-free volunteers, if one assumes a retention of 60% of the inhaled particles (Alford et al. 1966). This infectious dose was comparable to that found by other investigators when the same strain of virus was given to mice (Hood 1963). Of the nine volunteers given 5 TCID<sub>50</sub> of influenza A<sub>2</sub>/Bethesda/10/63 in small particle aerosols, 44% seroconverted and 33% became ill while 1 TCID<sub>50</sub> of the same virus was sufficient to cause disease in the one inoculated subject. Moreover, low levels of serum-neutralizing antibody were not completely effective in preventing infection

and illness (Alford et al. 1966). The illness produced in the study was of severity equal to that produced previously by administration of  $8.0 \times 10^4$ – $1.8 \times 10^5$  TCID<sub>50</sub> of the same strain nasopharyngeally (Knight et al. 1965). Jao et al. (1970) reported a low HID<sub>50</sub> of 40 TCID<sub>50</sub> of influenza A<sub>2</sub>/Bethesda/10/63 when delivered to the nose and throat of healthy susceptible volunteers. Illness occurred in over 30% of the inoculated subjects. The same strain infected all inoculated subjects when doses between  $8.0 \times 10^4$  and  $1.8 \times 10^5$  TCID<sub>50</sub> were used (Alford et al. 1967b). Hayden et al. (1996) reported that intranasal inoculation of approximately  $10^3$ ,  $10^5$ , and  $10^7$  TCID<sub>50</sub> of the influenza virus strain A/Texas/36/91 (H<sub>1</sub>N<sub>1</sub>) caused infection in 50, 75, and 80% of susceptible adults, respectively. A comparable nasal HID<sub>50</sub> was reported for the A/England/42/72 (H<sub>3</sub>N<sub>2</sub>) strain (Douglas et al. 1975). A dose of  $7.9 \times 10^4$  TCID<sub>50</sub> of the Equine influenza A/Equi 2/Miami/1/63 (H<sub>2</sub>N<sub>2</sub>) virus administered to five volunteers through the nose and the oropharynx infected all individuals but caused illness in only one of them (Kasel et al. 1965a). The absence of illness in the remaining volunteers was suspected to be a result of loss of the virus virulence as a result of egg passage of the virus inoculum. Loss of virulence of human influenza strains after passage in chick embryo has been reported (Isaacs et al. 1957). A similar observation was reported when a comparable dose of the same strain was used to inoculate human volunteers and was found to infect 63% of the subjects but cause illness in only 12% of them (Alford et al. 1967a).

Correlation of 1 TCID<sub>50</sub> value to the number of infectious influenza virions is, however, not clear. Ratios of TCID<sub>50</sub> to number of virions of 1:100, 1:400, and 1:650 have all been documented (Weber and Stilianakis 2008). Using real-time quantitative PCR, van Elden et al. (2001) reported that 13 copies of viral RNA of influenza A and 11 copies of viral RNA for influenza B equaled 0.02 (1:650) and 0.06 (1:183) TCID<sub>50</sub>, respectively. Using a field flow fractionation and multi-angle light scattering method optimized for the analysis of size distribution and total particle counts, the ratio of TCID<sub>50</sub> to the total virus count was in the range of 1:100–1:1,000 (Wei et al. 2007), a value not unusual for influenza virus preparations (Bancroft and Parslow 2002; Enami et al. 1991). The evidence suggests that many natural influenza infections occur by the aerosol route and that the lower respiratory tract may be the preferred site of initiation of the infection (Atkinson and Wein 2008; Tellier 2006). When patients acutely infected with influenza A sneeze or cough, their respiratory secretions containing high virus titer will be aerosolized. The viral titer measured in nasopharyngeal washes culminates on approximately day 2 or 3 after infection and can reach up to  $10^7$  TCID<sub>50</sub>/ml (Douglas 1975; Murphy et al. 1973). It is thought that



between  $10^3$  and  $10^7$  virions fit into aerosolized influenza droplets with diameters between 1 and 10  $\mu\text{m}$  (Weber and Stilianakis 2008). Considering that the airborne infectious dose of influenza is approximately 0.67 TCID<sub>50</sub> for virus reaching the respiratory epithelium (Atkinson and Wein 2008), this shows that the influenza HID<sub>50</sub> could easily fit into one aerosolized droplet (Weber and Stilianakis 2008).

In summary, influenza viruses cause a highly contagious respiratory disease that can spread easily and is responsible for considerable morbidity and mortality worldwide. Of the three types of influenza, influenza A viruses are the most virulent human pathogens and cause the most severe disease. Three different modes of influenza transmission have been identified: droplet, airborne (droplet nuclei), and contact transmission, all of which may play a role in the transmission of infection. The HID<sub>50</sub> of influenza A (H<sub>2</sub>N<sub>2</sub>) was reported to be 0.6–3.0 TCID<sub>50</sub> when administered in small particle aerosols to serum antibody-free volunteers. Studies suggest that the nasal infectious dose of influenza virus A is several orders of magnitude higher than that of airborne infection.

## Rhinovirus

Human rhinoviruses (Jackson and Muldoon 1973) are the most common cause of acute respiratory tract illness globally including the common cold (Makela et al. 1998; Rotbart and Hayden 2000). They infect both upper and lower respiratory tract tissues (Savolainen et al. 2003), and are a major factor in exacerbation of asthma (Johnston et al. 1995; Nicholson et al. 1993) and chronic obstructive pulmonary disease (Johnston 2005). These viruses are also associated with other severe diseases including otitis media (Arola et al. 1988), sinusitis (Pitkaranta et al. 1997), and pneumonia (Abzug et al. 1990). Human rhinoviruses have been classified into distinct serotypes, more than a 100 of which have been officially characterized (Savolainen et al. 2003). Rhinoviruses are shed from both infected and ill individuals (Cate et al. 1964; Mufson et al. 1963). Rhinovirus type 15 was detected in nasopharyngeal washings from experimentally infected volunteers up to 15 days after inoculation with a maximal virus titer of  $10^{4.2}$  TCID<sub>50</sub>/ml (Cate et al. 1965). Jarjour et al. (2000) reported a peak nasal virus titer in rhinovirus type 16 infected asthmatic volunteers of  $10^{5.5}$  TCID<sub>50</sub>/ml. Rhinoviruses are transmitted via contact or airborne routes (Couch et al. 1966; Gwaltney and Hendley 1982). The latter is a transfer of infection via particle aerosols while contact transmission occurs by physical contact between infected and susceptible subjects or indirectly from contaminated environment surfaces.

Experimental infections by rhinoviruses have been conducted in both animals and human volunteers. Successful infection of chimpanzees with type 14 and 43, and gibbons with type 1a, 2, and 14 have all been reported (Dick 1968; Dick and Dick 1968; Pinto and Haff 1969). Rhinoviruses normally induce illness after inoculation of the nasal mucosa (Drake et al. 2000; Holmes et al. 1976; Perkins et al. 1969; Peterson et al. 2009) but produced little, if any, illness when inoculated through the mouth (Bynoe et al. 1961; Hendley et al. 1973). The infective doses of rhinoviruses in the nose and eyes are thought to be comparable because the virus does not infect the eyes but appears to travel from the eyes to the nasal mucosa via the tear duct (Bynoe et al. 1961; Winther et al. 1986). Bynoe et al. (1961) found that colds could be produced almost as readily by applying virus by nasal and conjunctival swabs as by giving nasal drops to volunteers, and that the throat was relatively resistant to infection. D'Alessio et al. (1984) reported that the HID<sub>50</sub> of rhinovirus type 16 in susceptible human volunteers inoculated in the mouth was 8,000-fold higher than the HID<sub>50</sub> in the nose. This may explain the difficulty of direct oral transmission of rhinoviruses.

Numerous other studies have reported infective doses of various rhinovirus serotypes in human volunteers (Table 2). Doses ranging from 10 to  $10^{5.5}$  TCID<sub>50</sub> administered intranasally through nasal drops or via aerosols, infected up to 100% of the inoculated subjects. The infectious dose of rhinoviruses appears to be lower in the nose compared to other sites of inoculation. D'Alessio et al. (1984) found that when susceptible adult volunteers were inoculated with rhinovirus type 16 in the nose, the inside nares, the tongue or the outside nares, the HID<sub>50</sub> was 0.28 TCID<sub>50</sub>, 1.39 TCID<sub>50</sub>, 2.260 TCID<sub>50</sub>, and  $1.1 \times 10^4$  TCID<sub>50</sub>, respectively. In addition, the infectious dose of rhinoviruses appears to be also lower when given by nasal drops than in aerosols. Couch et al. (1966) reported a 20-fold difference between HID<sub>50</sub> of rhinovirus type 15 (strain NIH 1734) when administered by nasal drops (HID<sub>50</sub> = 0.032 TCID<sub>50</sub>) and by aerosols (HID<sub>50</sub> = 0.68 TCID<sub>50</sub>). They found that when virus aerosol particles (0.3–2.5  $\mu\text{m}$ ) were inhaled, a dose of 2 TCID<sub>50</sub> failed to infect all volunteers and that none of the three who inhaled 0.06 TCID<sub>50</sub> became infected. In contrast, all volunteers who received 0.1 TCID<sub>50</sub> by nasal drops became infected, although none became infected with lower doses. These are some of the lowest reported values for rhinoviruses infection in human volunteers. Low HID<sub>50</sub> values of 5.7 and 0.4 TCID<sub>50</sub> for rhinovirus serotypes 14 and 39, respectively, have been reported when the viruses were administered by nasal drops to antibody-free volunteers following two passages in diploid human embryonic lung cells (Hendley et al. 1972). Passage of rhinoviruses in tissue culture has been shown to reduce virulence. Douglas and Couch

**Table 2** Infectivity of rhinovirus in humans

Rhinovirus (RV) serotype	Volunteers (antibody titer)	Dose (TCID <sub>50</sub> <sup>a</sup> )	% Infected <sup>b</sup>	% Ill	References
ECHO-28 (NIH52992)	Adult males ( $\leq 1:4$ )	$1.0 \times 10^4$	100 (5/5)	40 (2/5)	Mufson et al. (1963)
RV13 (NIH353)	Adult males ( $\leq 1:4$ )	$3.1 \times 10^3$ – $1.0 \times 10^4$	100 (16/16)	81 (13/16)	
RV39	Healthy adults ( $\leq 1:4$ )	$1.0 \times 10^2$	85 (88/103)	66 (58/103)	Turner et al. (2005)
RV39 (SF299)	Adults ( $< 1:2$ )	$5.0 \times 10^{-2}$ – $5.0 \times 10^1$	83 (44/53)	80 (35/44)	Hendley et al. (1972)
RV14 (SF765)		$5.0 \times 10^{-1}$ – $3.0 \times 10^2$	64 (27/42)	–	
RV16	Adults with COPD <sup>c</sup> ( $< 1:2$ )	$1.0 \times 10^1$	100 (4/4)	100 (4/4)	Mallia et al. (2006)
RV16	Healthy + asthmatic adults ( $\leq 1:2$ )	$1.0 \times 10^2$ – $1.5 \times 10^2$	100 (15/15)	100 (15/15)	Zambrano et al. (2003)
RV16	Adult asthmatics ( $\leq 1:2$ )	$1.2 \times 10^3$ – $1.2 \times 10^4$	100 (8/8)	100 (8/8)	Jarjour et al. (2000)
RV16	Adults ( $< 1:3$ )	$2.8 \times 10^{-1}$	50 (14/38)	–	D'Alessio et al. (1984)
RV23	Adults ( $\leq 1:4$ )	$1.0 \times 10^2$	–	33 (7/21)	Drake et al. (2000)
RV23	Adults ( $\leq 1:4$ )	$1.0 \times 10^2$ – $3.0 \times 10^2$	57 (24/42)	33 (14/24)	Turner et al. (2000)
RV13 (NIH353)	Adult males ( $< 1:2$ )	$6.3 \times 10^4$ – $1.6 \times 10^5$	100 (5/5)	80 (4/5)	Cate et al. (1964)
RV15 (NIH1734)		$6.3 \times 10^2$ – $3.1 \times 10^5$	100 (13/13)	77 (10/13)	
RV16 (NIH11757)		$1.0 \times 10^5$ – $6.3 \times 10^5$	100 (3/3)	100 (3/3)	
RV13	Adult males ( $< 1:4$ )	$1.0 \times 10^2$	95 (22/23)	78 (18/23)	Perkins et al. (1969)
RV44	Healthy adult males ( $\leq 1:2$ )	$1.0 \times 10^2$	88 (8/9)	55 (5/9)	Pachuta et al. (1974)
RV32		$1.0 \times 10^2$	100 (9/9)	66 (6/9)	
RV15 (NIH1734)	Healthy adult males ( $< 1:2$ )	$3.2 \times 10^{-2}$ – $6.8 \times 10^{-1}$	50 (22/43)	–	Couch et al. (1966)
RV15 (NIH1734)	Healthy adult males ( $< 1:2$ )	$1.6 \times 10^1$ – $6.6 \times 10^1$	100 (8/8)	100 (8/8)	Cate et al. (1965)
RV2 DP29	Adults ( $\leq 1:8$ )	$1.0 \times 10^5$	85 (6/7)	28 (2/7)	Holmes et al. (1976)
RV2 DP29		$1.0 \times 10^2$ – $5.0 \times 10^3$	40 (4/10)	0 (0/10)	
RV2 Hu <sub>4</sub>		$1.0 \times 10^2$ – $1.2 \times 10^2$	80 (4/5)	66 (4/6)	
RV39	Healthy adults ( $\leq 1:2$ )	$1.0 \times 10^3$	92 (22/24)	42 (10/24)	Peterson et al. (2009)
Hank's		$1.0 \times 10^3$	93 (31/33)	58 (19/33)	

<sup>a</sup> 50% Tissue culture infective dose

<sup>b</sup> As determined by virus shedding and/or increase in antibody titer

<sup>c</sup> Chronic obstructive pulmonary disease

(1969) reported that three passages of rhinovirus type 15 in human embryonic lung fibroblasts (WI-26) attenuated the virus strain. Attenuation was indicated by a 30-fold decrease in infectivity to humans, failure to produce illness, and decreased frequency of virus shedding. Three passages of the virus in WI-26 cells rather than two passages, increased its HID<sub>50</sub> from 0.032 to 1 TCID<sub>50</sub> and decreased its illness rate in infected volunteers from 88 to 0%.

The presence of serum antibodies against rhinoviruses was also linked to an altered infectious dose for these viruses and decreased frequency and severity of illness (Cate et al. 1964; Hendley et al. 1972). Rhinovirus type 13 caused illness in 87, 50, and 39% of inoculated volunteers with neutralizing antibody titers of  $< 1:2$ , 1:8 to 1:16, and 1:32 to 1:64, respectively (Mufson et al. 1963). The HID<sub>50</sub> of rhinovirus 14 increased from 5.7 TCID<sub>50</sub> in antibody-free volunteers to 33 TCID<sub>50</sub> in those with antibody titer 1:2 to 1:32 (Hendley et al. 1972). A 50 TCID<sub>50</sub> dose of rhinovirus 39 which has a calculated intranasal HID<sub>50</sub> of 0.4 TCID<sub>50</sub> in antibody-free volunteers, did not infect any of the eight inoculated subjects with antibody titers of 1:64.

The HID<sub>50</sub> increased from 0.4 TCID<sub>50</sub> to 6.5 TCID<sub>50</sub> in volunteers with antibody titer of 1:8 to 1:32 (Hendley et al. 1972).

In summary, human rhinoviruses are the most common cause of acute respiratory tract illness globally including the common cold. Presence of pre-existing antibodies as well as passage in tissue culture has been shown to reduce virulence, increase the HID<sub>50</sub> and decrease illness rate in infected volunteers. Rhinovirus 15 was shown to have greater infectivity in man than in culture, with a HID<sub>50</sub> of 0.032 TCID<sub>50</sub>. Rhinovirus is more infectious when given as nasal droplets than as an aerosol spray, and it has a lower infectious dose in the nose compared to other sites of inoculation such as the mouth.

### Coxsackievirus

The coxsackieviruses are extremely small (Huebner et al. 1950; Quigley 1949) single-stranded RNA viruses first reported in 1948 by Dalldorf and Sickles (1948). They are

members of the family *Picornaviridae* in the genus *Enterovirus* which also includes the poliovirus. These viruses are divided into group A and group B based on the early observations of their pathogenicity in mice (Carpenter and Boak 1952). Many coxsackievirus serotypes have been identified and the coxsackievirus A21 in particular has been used in experimental infections of human volunteers (Couch et al. 1965, 1966; Spickard et al. 1963). Although classified as an enteric virus culturable from rectal swabs and feces of naturally infected individuals, recovery from pharynx of such individuals is more common (Johnson et al. 1962). Coxsackievirus A21 has been shown to cause respiratory illness in both natural (Bloom et al. 1962; Johnson et al. 1962) and experimental infections (Couch et al. 1965; Spickard et al. 1963).

Airborne transmission of coxsackievirus A21 has also been reported by Couch et al. (1970). In this study, 39 antibody-free volunteers were quartered in barracks separated in the center by a double-wire barrier. Ten volunteers on one side were inoculated with the virus by small particle aerosol while 10 on the same side and 19 on the opposite side received placebo inoculation. Contact between men on the two sides was prevented and contact with individuals outside the barracks was minimized. A dose of 71 TCID<sub>50</sub> of the virus caused infection in all of the ten inoculated volunteers and illness in eight of them. All the remaining volunteers were infected with coxsackievirus A21 during the 26-day study and 12 of these became ill. The virus was recovered from airborne particles in cough and sneeze samples produced by the inoculated volunteers at levels of up to  $1.5 \times 10^4$  TCID<sub>50</sub> for sneeze samples and  $9.0 \times 10^3$  TCID<sub>50</sub> for cough sample. The virus was also recovered from room air samples at levels of 300–700 TCID<sub>50</sub> per sample. Although direct correlation between 1 TCID<sub>50</sub> and coxsackievirus A21 virus particle number has rarely been determined, a 2.3 particle to TCID<sub>50</sub> ratio for a viral preparation has been reported (Ward et al. 1984a).

Couch et al. (1966) reported that the HID<sub>50</sub> of coxsackievirus A21 strain 49889 passaged once in human embryonic kidney cells, when administered to antibody-free volunteers by particle aerosol was 28 TCID<sub>50</sub>. Only two of the infected subjects failed to develop illness, indicating that the HID<sub>50</sub> and the 50% illness dose are nearly the same. In a previous study (Spickard et al. 1963), all antibody-free volunteers inoculated in the nasopharynx by coarse spray and drops with  $3.0 \times 10^3$  TCID<sub>50</sub> of the same strain, became infected and eight of these developed upper respiratory disease. The virus was isolated frequently from throat swabs of infected subjects for up to 6 weeks. In the same study, the immunologic status of the volunteers was found to be critical in determining the biological and clinical sequelae to viral administration. High antibody titer (>1:128) gave resistance to infection with none of the

$3.0 \times 10^3$  TCID<sub>50</sub>-inoculated volunteers developing illness and with the virus rarely recovered. Other studies have also reported that subjects with detectable antibody levels exhibited milder illness and less viral shedding when inoculated with coxsackievirus A21 (Couch et al. 1965, 1966).

When antibody-free volunteers were inoculated with coxsackievirus A21 strain 48654 passaged twice in human embryonic lung fibroblasts (WI-26) via particle aerosols, the HID<sub>50</sub> was approximately 30–34 TCID<sub>50</sub> and nearly all infected subjects developed illness (Couch et al. 1965, 1966). However, the above aerosol HID<sub>50</sub> estimations were based on inhaled doses of which only 50 to 70% was retained, hence the actual HID<sub>50</sub> values were probably considerably less than estimated. Nevertheless, when the above strain of the virus was administered to antibody-free volunteers by nasal drops, there was 5-fold decrease in the calculated HID<sub>50</sub> (HID<sub>50</sub> = 6 TCID<sub>50</sub>) with five of the seven infected subjects developing illness (Couch et al. 1966). The above results demonstrate that coxsackievirus A21 strain 48654 passaged once or twice in cell culture had similar HID<sub>50</sub>. The same strain obtained from naturally occurring cases of illness but not passaged in tissue culture given to antibody-free volunteers resulted in similar degree of infectivity as one or two passages (Couch et al. 1965). Lang et al. (1965) reported that inoculation of 20 antibody-free volunteers with 100–1,600 TCID<sub>50</sub> of coxsackievirus A21 strain 48560 passaged nine times in primary human embryonic kidney tissue culture by the nasopharyngeal route infected all subjects and caused illness in 85% of them. The same strain passaged two more times in human embryonic lung (WI-26) tissue and administered via aerosols in a dose of 160 TCID<sub>50</sub> caused illness in 90% of the inoculated subjects.

Intestinal administration of coxsackievirus A21 to volunteers strongly indicated that the intestine is not the primary site of multiplication of this virus in human adults (Spickard et al. 1963). Antibody-free volunteers given 320 TCID<sub>50</sub> of coxsackievirus A21 in coated capsules showed no symptom of illness. The virus was not recovered from rectal or oropharyngeal specimens and no neutralizing antibodies were detected 4 weeks after feeding. Moreover, inoculation of the intestinal tract of volunteers with a larger virus dose ( $3.2 \times 10^5$  TCID<sub>50</sub>) through a Rehffuss tube or in enteric-coated capsules resulted in no illness, no positive throat cultures, and only transient intestinal infection as judged by cultures of stool. Furthermore, there were no detectable antibodies 35 days after inoculation of these subjects. In contrast, inoculation of  $3.2 \times 10^5$  TCID<sub>50</sub> and even  $3.0 \times 10^3$  TCID<sub>50</sub> of the same virus by the respiratory route caused illness in volunteers followed by an increase in neutralizing antibody titer and recovery of the virus from their pharynx (Spickard et al. 1963).



In summary, although classified as an enteric virus, coxsackievirus A12 is an important cause of respiratory illness in humans. The presence of pre-existing antibodies has been shown to provide protection against infection by the virus and to lead to milder illness and less viral shedding. Unusually, passaging of the virus once or twice in cell culture did not affect its infectivity. Coxsackievirus A21 is more infectious when given as nasal droplets ( $\text{HID}_{50} = 6 \text{ TCID}_{50}$ ) than as particle aerosols ( $\text{HID}_{50} = 28\text{--}34 \text{ TCID}_{50}$ ) in the respiratory tract, and shows poor infectivity in the gastrointestinal tract.

## Adenovirus

Adenoviruses are a group of non-enveloped icosahedral DNA viruses that infect a broad range of vertebrate species (Davison et al. 2003). Human adenoviruses were first isolated in the early 1950s from adenoid tissue (Hilleman and Werner 1954; Rowe et al. 1953) and are highly prevalent in the human population (Vogels et al. 2003). These viruses cause mainly respiratory, gastrointestinal and urinary tract, and eye infections, and occasionally can lead to more severe diseases affecting the brain, heart, kidney, or liver especially in immunodeficient individuals (Goncalves and de Vries 2006; Kojaoghlanian et al. 2003). Human adenoviruses are included in the genus *Mastadenovirus* of the family *Adenoviridae* and comprise more than 50 serotypes (Goncalves and de Vries 2006).

Early experimental infection of volunteers with adenoviruses (Bell et al. 1956) reported that intranasal instillation of adenoviruses type 1, 2, 3, 4, 5, or 6 and the swabbing of the oropharynx with type 4 virus have produced infection as demonstrated by a complement-fixing antibody response. Occasionally, minor respiratory illness followed such inoculations but could not be attributed to infection with these viruses. On the other hand, both infection and illness were readily produced in susceptible volunteers by swabbing the lower palpebral conjunctiva with adenoviruses type 1, 3, 4, or 5. Swabbing viruses onto conjunctiva produced a higher frequency of conjunctivitis than dropping of virus into the conjunctival fornix. These studies, however, did not quantify the amount of virus in the inocula administered to the volunteers. Experiments in which adenoviruses were given parenterally by the intramuscular, intracutaneous, intratumor, and intravenous routes (Hilleman et al. 1955; Huebner et al. 1954, 1956; Southam et al. 1956) presented evidence of viral infection but without significant illness. Hilleman et al. (1957), however, reported that intramuscular injection of volunteers with a pool of type 3, 4, and 7 adenovirus propagated in tissue cultures of human embryo intestine and with infectivity titer of  $10^{-3}$ , caused acute respiratory illness.

Chaproniere et al. (1956) used the adenovirus type 1 strain APC obtained by direct passage of the virus from a culture of human adenoid tissue into cultures of embryonic human kidney tissue, to inoculate a group of 11 volunteers by nasal instillation. After inoculation with  $1.6 \times 10^4 \text{ TCID}_{50}$ , type 1 virus was recovered from four of the antibody-free volunteers, half of whom developed acute pharyngitis. The  $\text{HID}_{50}$  of adenovirus type 21 vaccine was reported to be  $4.0 \times 10^4 \text{ TCID}_{50}$  (Dudding et al. 1972). The virus strain was originally isolated in human embryonic kidney cells from throat washings of a patient with pharyngitis. The strain was passaged four times in human embryonic kidney cells then 11 times in human diploid fibroblast cultures (WI-38) and given to antibody-free volunteers in enteric-coated capsules. The virus was recovered from stools of 54% of the volunteers given an inoculum of  $6.3 \times 10^6 \text{ TCID}_{50}$  but from none given lower doses (Dudding et al. 1972). Vaccination of volunteers with strains of adenovirus serotypes 3, 4, or 7 which had been isolated and passaged several times in human embryo lung diploid fibroblast cultures caused infection accompanied by increase in serum-neutralizing antibody levels not only against the serotype inoculated but also in varying degrees against other serotypes. The virus was administered to the volunteers in 0.5 ml volume of material containing 100  $\text{TCID}_{50}/\text{ml}$  sprayed into the nose with a nebulizer (Selivanov et al. 1972).

Studies on the MID of adenovirus type 4 suggested that a greater dose of the virus is required to initiate infection in the lower intestinal tract and nasopharynx than in the lower respiratory tract (Couch et al. 1966, 1969; Gutekunst et al. 1967). In a study to determine the smallest adenovirus type 4 dose capable of causing infection in the lower intestinal tract, antibody-free volunteers were given the virus in enteric-coated capsules. The  $\text{HID}_{50}$  of the virus given in this manner was 10–500  $\text{TCID}_{50}$  (Gutekunst et al. 1967). The  $\text{HID}_{50}$  of adenovirus type 4 by nasal inoculation was reported to be 35  $\text{TCID}_{50}$  but only 0.5  $\text{TCID}_{50}$  when administered by small particle aerosol. Doses ranging from 0.1 to 171  $\text{TCID}_{50}$  caused infection in 16 and illness in 15 of the 21 antibody-free volunteers inoculated by aerosol. In contrast, an approximately 70-fold greater dose was required to cause infection in susceptible volunteers inoculated by nasal drops and illness occurred infrequently (Couch et al. 1969).

Others reported the  $\text{HID}_{50}$  of adenovirus type 4 suspension with particle to  $\text{TCID}_{50}$  ratio of 1:13.2 administered by nasal drops to antibody-free volunteers to be 9  $\text{TCID}_{50}$  (Hamory et al. 1972). Although no comprehensive infectious dose studies were reported with adenovirus type 4 given in large particle aerosols, administration of  $10^3 \text{ TCID}_{50}$  by this route infected all six antibody-free volunteers inoculated but caused illness in only half of them while a dose of 5  $\text{TCID}_{50}$  given via small aerosol particles

(0.3–2.5  $\mu\text{m}$ ) caused infection and illness in all volunteers inoculated (Couch et al. 1966). In the same study, it was reported that none of the three volunteers with pre-existing antibody titer of 1:32 to 1:64 became infected with 6 TCID<sub>50</sub> given by small particle aerosol. This indicates that presence of serum antibody may cause resistance to infection with adenoviruses, an observation which has also been reported by others (Bell et al. 1956).

In view of the low infectious dose of adenovirus type 4 given by small particle aerosol, attempts have been made to characterize the inocula in terms of the number of viral particles necessary to cause infection. Couch et al. (1969) reported that an average of 6.6 viral particles by aerosol were sufficient to infect 50% of susceptible volunteers while the HID<sub>50</sub> by nasal drops corresponded to 462 particles. The authors noted that taking into account that a portion of the dose taken by aerosol will be exhaled, and that two thirds of the viral particles were single virions, the infectious dose of adenovirus for man by small aerosol is exceedingly small.

In summary, adenoviruses are highly prevalent in the human population, comprising more than 90 serotypes and cause a wide range of infections. The presence of pre-existing antibodies has been shown to be protective against infection with adenovirus type 4. Doses of 0.5 TCID<sub>50</sub>, or 6.6 adenovirus type 4 particles were reported to infect 50% of the tested population. Studies suggest that a higher dose of this virus is required to initiate infection in the lower intestinal tract and nasopharynx than in the lower respiratory tract. A higher dose of adenovirus type 4 was also required to cause infection when administered by nasal drops than when administered via aerosols.

### Respiratory Syncytial Virus

In 1956, a novel virus was recovered from a chimpanzee with respiratory symptoms and designated chimpanzee coryza agent (Blount et al. 1956). In the ensuing decade, the virus was renamed respiratory syncytial virus (RSV) to reflect the giant syncytia which formed in tissue cultures, and epidemiological studies clearly established RSV as one of the most important causes of severe respiratory tract infection in infants and young children as well as elderly persons and adults with underlying cardiopulmonary diseases (Falsey and Walsh 2000; Hall 2001; Thompson et al. 2003). Human RSV is an enveloped RNA virus and is a member of the genus *Pneumovirus*, classified within the family *Paramyxoviridae*. The virus is highly contagious and believed to spread primarily by large droplets and fomites and can survive on non-porous surfaces, skin, and gloves for many hours. Hence, close person-to-person contact or contact with contaminated environmental

surfaces and autoinoculation are required for transmission (Falsey and Walsh 2000). RSV is shed in high titers from infants hospitalized for lower respiratory tract disease for up to 21 days and with a mean maximal nasal wash titer of  $2.2 \times 10^4$  TCID<sub>50</sub>/ml (Hall et al. 1976). In adult challenge studies, volunteers excreted the virus for up to 8 days with peak virus titer of up to  $10^5$  TCID<sub>50</sub>/ml of nasal wash (Lee et al. 2004).

An isolate of RSV passaged twice in rhesus monkey kidney cells was administered by intraoral and intranasal spray to adult volunteers with serum antibody titers of 1:16 or higher (Johnson et al. 1961; Kravetz et al. 1961). A dose ranging between 160 and 640 TCID<sub>50</sub> caused illness in 20 of the 41 volunteers inoculated with an additional 14 subjects shedding the virus or developing serological evidence of infection. This study suggested that the HID<sub>50</sub> of the virus is less than 640 TCID<sub>50</sub>. When 32 healthy, susceptible adult volunteers were inoculated intranasally with a dose of approximately  $10^6$  pfu of a safety-tested clinical isolate of RSV type B, 18 subjects (56%) became infected as determined by either viral shedding (47%) or nasal antigen detection (41%) or a 4-fold rise in virus-specific antibody titer (34%) (Buchman et al. 2002). Higgins et al. (1990) reported that intranasal inoculation of 10 healthy adult volunteers with  $6.3 \times 10^4$  TCID<sub>50</sub> of a bacteriologically sterile MRC<sub>5</sub> tissue culture fluid of the RSS-2 strain of RSV of the 11th passage containing  $3.1 \times 10^5$  TCID<sub>50</sub>/ml (McKay et al. 1988) failed to produce symptoms in any of the volunteers. Out of the 19 volunteers challenged with 0.5 ml of the above fluid to each nostril, 14 showed laboratory evidence of infection as shown by viral isolation and/or antibody raise, and seven developed colds (Higgins et al. 1990).

A number of studies reported the infectious doses of RSV A2. The safety-tested pool of the virus used for inoculation had undergone six passages in human embryonic kidney cells, ten passages in calf kidney cells, and several additional passages in bovine embryonic kidney cells. In one study (Mills et al. 1971),  $5.0 \times 10^2$  pfu of RSV A2 administered as intranasal drops to male volunteers with varying levels of serum and nasal antibodies, infected 100% (16/16) of the challenged subjects but produced no illness. Interestingly, a higher dose of  $10^5$  pfu infected only 53% (9/17) of the volunteers but caused illness in six of them. The discrepancy in infection rates was thought to be due to variation in the sensitivity of the assay system used for detection of the virus (Mills et al. 1971). Noah and Becker (2000) nasally inoculated 10 healthy, non-smoking young adults with approximately  $10^3$  pfu of the RSV A2. They reported that 30% (3/10) developed clinical symptoms of upper respiratory infection and also shed the virus.

In another study (Lee et al. 2004), healthy adult subjects with varying antibody titers were inoculated intranasally by

a challenge virus pool of an RSV A2 strain. The virus was cloned and passaged several times in three different cell lines to contain  $10^5$  TCID<sub>50</sub>/ml. A dose of  $5.0 \times 10^4$  TCID<sub>50</sub> infected 8 of the 14 challenged subjects, as determined by virus shedding (7/14) and 4-fold rise in serum antibodies (8/14), and caused illness in 38% of these (3/8). A lower dose ( $5.0 \times 10^3$  TCID<sub>50</sub>) infected 35% (5/14) of inoculated volunteers with 21% shedding the virus and 28% having a 4-fold raise in serum antibody titer. A relationship between pre-challenge serum-neutralizing antibody titer of the volunteers and infectivity of the virus was established. The highest infection rate was achieved using the high inoculum dose in subjects with low pre-inoculation serum-neutralizing antibody titer. This observation was confirmed when 92% (12/13) of subjects with pre-inoculation serum-neutralizing antibody titer of  $\leq 1:660$  challenged with  $5.0 \times 10^4$  TCID<sub>50</sub> of the virus, became infected (Lee et al. 2004).

Hall et al. (1981) investigated the infectivity of RSV A2 strain administered by nose, eye, and mouth in adult volunteers. They reported that the virus may infect by eye or nose and both routes appear to be equally sensitive. A dose of  $1.6 \times 10^5$  TCID<sub>50</sub> infected three of the four volunteers given either into the eyes or nose while only one out of the eight were infected via mouth inoculation, and this was thought to be due to secondary spread of the virus. With an inoculum of  $1.6 \times 10^3$  TCID<sub>50</sub>, the proportion of subjects infected by either route was 25% (1/4), while a dose of  $1.6 \times 10^2$  TCID<sub>50</sub> caused no infection (0/4). The HID for the wild strain of RSV most likely would be less than those reported for multiply passaged strains because a second passage of wild strain RSV strain infected 83% of seropositive adult volunteers when administered in doses of 160 to 640 TCID<sub>50</sub> (Kravetz et al. 1961). A trial of a highly attenuated RSV vaccine (*ts-2*) administered intranasally to seropositive children have shown that a dose of  $2.0 \times 10^6$  TCID<sub>50</sub> did not cause infection (Wright et al. 1982). However, others reported that a dose of  $10^4$  TCID<sub>50</sub> of RSV attenuated vaccine (*ts-1*) infected all 32 seropositive and seronegative infants and children challenged as documented by recovery of virus from the throat and/or significant rise in nasal secretion antibody (Kim et al. 1973). Moreover, one of three seronegative infants administered a much lower dose (30–40 TCID<sub>50</sub>) of the *ts-1* mutant RSV vaccine became infected (Parrott et al. 1975). The above results suggest that it is likely that the dose of wild RSV required to infect an infant would be even less (Hall et al. 1981).

In summary, RSV is a major cause of severe respiratory tract infection in infants and young children. Experimental infection studies report a relationship between pre-challenge serum-neutralizing antibody titer of the volunteers and infectivity of the virus. RSV A2 had poor infectivity

when administered via the mouth but was shown to infect by eye and nose and both routes appear to be equally sensitive to the virus. A low dose of 30–40 TCID<sub>50</sub> of the *ts-1* mutant RSV vaccine caused infection in an infant. However, because RSV infection studies rely on the use of attenuated vaccine strains, passaged several times in tissue culture, the MID of wild RSV is probably less than the above.

## Enteric Viruses

Enteric viruses represent a wide spectrum of viral genera that invade and replicate the mucosa of the intestinal tract. These viruses are characterized by their small size and are transmitted primarily via the fecal–oral route. They are important agents of gastroenteritis, hepatitis, neurological diseases, and other illnesses worldwide (Bishop and Kirkwood 2008; Sair et al. 2002; Vasickova et al. 2005). Enteric viruses are spread across a wide range of taxonomic genera including both DNA and RNA viruses. Important RNA enteric viruses include the *Caliciviridae* (including *Norovirus* and *Saprovirus*), the *Picornaviridae* (including *Hepatovirus* such as hepatitis A virus (HAV) and the enteroviruses such as polioviruses, coxsackieviruses, echoviruses, and enteroviruses), the *Reoviridae* (including rotaviruses), and the *Astroviridae* (including *Astrovirus*). Important DNA enteric viruses include the *Adenoviridae* (including adenoviruses) and the *Parvoviridae* (including parvoviruses).

Experimental infection studies with enteric viruses have been conducted in humans primarily with epidemiologically important viruses such as norovirus, polioviruses, echoviruses, and rotaviruses. These studies will be discussed in the following section. Other enteric viruses which are known to infect the respiratory system (coxsackieviruses and adenoviruses) have been discussed in the earlier section.

## Rotavirus

Rotaviruses are recognized as a major etiologic agent of gastroenteritis in human infants and young children and in the young of most species of domesticated and laboratory animals (Cukor and Blacklow 1984; Fulton et al. 1981; Hoshino et al. 1982; Kraft 1957; Sato et al. 1982; Schoub 1981; Woode 1976). Because of safety and medical ethics of performing live virus inoculation studies in susceptible human volunteers (children), most of experimental rotavirus infection studies were performed in young animals to simulate infection of infants. Aich et al. (2007) reported that the injection of  $1.7 \times 10^5$  pfu of bovine rotavirus into the intestinal loops of 1-day old calves was sufficient to

induce consistent fluid accumulation and visible histological changes in the intestinal villi. In another study (Ramig 1988), 7-day old mice, born to seronegative dams, were orally inoculated with  $10^5$  pfu of a number of animal and human rotaviruses. It was found that simian (SA11), rhesus (RRV), and bovine (B223) rotaviruses replicated and caused severe diseases. Canine (K9), bovine (B641), and human (Wa) rotaviruses either replicated minimally and caused minimal disease (K9, B641) or failed to replicate or cause disease (Wa). Dose response studies using the Simian (SA11) virus showed that a dose as low as  $10^2$  pfu induced virus replication and disease in mice, although both the intestinal virus titer and the severity of disease increased in parallel with virus dose. An earlier study, however, reported that although inoculation of 7-day old mice with  $5.0 \times 10^6$  pfu of the same virus strain caused illness in 90% of the mice, doses less than  $5.0 \times 10^6$  pfu failed to produce clinical symptoms (Offit et al. 1984). However, rotavirus-specific immune response was observed in mice inoculated with doses as low as  $5.0 \times 10^3$  pfu (Offit et al. 1984).

Miniature piglets are highly susceptible to infection with  $10^7$  pfu of porcine rotavirus (Graham et al. 1984). Payment and Morin (1990) reported that intragastric inoculation of rotaviruses-specific antibody-free piglets with low doses of the OUS strain of porcine rotavirus also caused infection. As low as 90 rotavirus particles, equivalent to 0.006 TCID<sub>50</sub> or 0.04 mpniu (most probable number of infectious units), was sufficient to induce infection as defined by the development of clinical symptoms with or without the excretion of viral particles in stools. Porcine gastrointestinal tract and digestive physiology are very similar to that of man and pigs have been suggested as an appropriate model for enteric infections in humans (Cliver 1981). The study was hence an appropriate simulation of infection in infants. An earlier investigation using the same porcine rotavirus strain found that the lowest dose for inducing clinical illness or to demonstrate viral replication in highly susceptible (colostrums deprived, caesarean derived) newborn miniature piglets, was 1 pfu (Graham et al. 1987). Investigators also found that the tissue culture-passaged virus was much less virulent, an observation which has been reported by others (Bohl et al. 1984; Kapikian et al. 1983). Interestingly, passage of rotavirus in primary cells both increased virus infectivity and adapted the viruses for growth in continuous cell lines (Ward et al. 1984b).

One of the few human volunteer rotavirus infection studies was conducted by Ward et al. (1986) using an unpassaged, safety-tested strain (CJN) of human rotavirus obtained from a stool specimen of a hospitalized child with acute gastroenteritis. Adult healthy volunteers with low (<1:30) titers of serum-neutralizing antibody to the challenge virus ingested  $9.0 \times 10^3$  to  $9.0 \times 10^4$  ffu (focus

forming units) of the virus to determine the dose required to produce infection with or without illness. The rotavirus preparation was characterized by a particle/ffu ratio of  $1.56 \times 10^4$ . Although safety concerns regarding this study have been raised (Graham 1987), the investigation revealed that the HID<sub>50</sub> of the virus was approximately 10 ffu, and that 1 ffu should infect nearly 25% of susceptible adult subjects. The dose required to cause infection was comparable to that needed to produce illness (Ward et al. 1986). A rotavirus-infected subject can shed  $>10^{12}$  virus particles/g of fecal matter (Bishop 1996; Flewett 1983; Gratacap-Cavallier et al. 2000; Ward et al. 1984b) and the virus can survive for days under environmental conditions (Moe and Shirley 1982). As little as 1 µg of infectious material could therefore contain several times the MID of the virus, and contact with such material represents a risk of infection especially for susceptible individuals such as children and infants. The risk of infection through such contact increases as the contaminating dose increases. A few microliters of infected material still represent a tiny drop, but that drop may contain sufficient number of viruses to cause infection. A less obvious, but an important health hazard is the consequence of asymptomatic viral infections of less susceptible hosts. Ward et al. (1986) reported that only 17 of the 30 healthy adult human volunteers experimentally infected with rotavirus experienced illness, and that many of these were shedding the virus. Such asymptomatic infected individuals may amplify the virus and serve as virus shedders or reservoirs of infection for transmission to highly susceptible young children.

In summary, rotaviruses are recognized as a major cause of gastroenteritis in infants and young children. They are shed at high titer, even if the infection is asymptomatic, which plays a role in their transmission. Unlike many viruses, passage of rotavirus, in fecal specimens, in primary cells did not decrease its infectivity but increased its virulence. Low doses of rotavirus were shown to cause infection with less than 1 ffu of strain CJN causing infection in adult volunteers. However, because of safety and medical ethics experimental infections with rotavirus were conducted in young animals or adult human volunteers while the most susceptible humans to rotavirus infection are infants and young children.

## Poliovirus

Poliomyelitis is an acute central nervous system viral disease affecting motor neurons within the brainstem and spinal cord (Racaniello 2006). The causative agent of this disease, the poliomyelitis virus (later shortened to poliovirus) was identified in 1908 (Landsteiner and Popper 1908). Human poliovirus is a small, single strand RNA



virus, a member of the genus *Enterovirus* classified under the family *Picornaviridae*. All three serotypes of poliovirus cause paralytic disease and transmission of the virus is thought to occur by close personal contact, mostly via the fecal–oral route. Polioviruses are excreted by the majority of infected, previously unvaccinated infants and young children, for up to 4 weeks. The duration of viral shedding is shorter among previously vaccinated children, those with pre-existing antibodies to the infecting serotype or those who had previous intestinal infection with a homologous poliovirus (Alexander et al. 1997). The development of live attenuated (vaccine) strains of polioviruses in the 1950s provided the opportunity to conduct infectious dose studies in human subjects including infants and children which appear to be the most susceptible to natural infection.

One of the earliest studies in this field was conducted by Koprowski et al. (1956) who administered the SM strain of poliovirus type 1 in gelatin capsules to susceptible human volunteers with no antibodies for the virus. The SM strain had been attenuated by rodent adaptation followed by successful passages of the virus in chick embryo and monkey kidney tissue culture. The results (Table 3) showed that a calculated dose of 20 pfp (plaque-forming particles) infected all four test subjects as determined by virus shedding and antibody response. Two of the three children were infected with a dose of 2 pfp, whereas infection did not occur in two subjects who received 0.2 pfp. In the same study, another rodent-adapted attenuated poliovirus type 2 strain (TN) was administered to subjects in liquid form in milk suspensions. The laboratory determination of the infectivity of the inocula was done in mice because of the non-cytopathogenic character of the virus strain. The method is much less exact than that employed for type 1 virus. The results (Table 3) revealed that an individual was found susceptible to a dose as small as 300 PD<sub>50</sub> (50% mouse paralytic dose), although few subjects were not infected with 10 times or greater doses. No infection occurred with a dose of approximately 30 PD<sub>50</sub>, and unlike the SM strain, the TN strain was rarely excreted from infected individuals. Flack et al. (1956) administered the same two live attenuated strains to 24 infants less than 6 months old by the oral route. Most of the subjects had antibodies acquired from their mother to the challenge virus. Sixteen received the SM (type 1) virus alone at concentrations ranging from  $6.3 \times 10^2$  to  $3.1 \times 10^5$  TCID<sub>50</sub> and two received the TN (type 2) at a concentration of  $6.3 \times 10^2$  PD<sub>50</sub>. All infants developed active immunity after inapparent alimentary infection demonstrated by isolating the virus from the feces.

The P712 strain of poliovirus used by Sabin (1957) was infective in several volunteers at a dose of 100 TCID<sub>50</sub>. Plotkin et al. (1959) reported that oral doses of 30 to 80

TCID<sub>50</sub> of type 3 Fox strain of poliovirus infected seven of the nine infants tested and that the HID<sub>50</sub> of type 1 CHAT strain was  $10^4$  and  $8.0 \times 10^4$  TCID<sub>50</sub>. In a latter study (Katz and Plotkin 1967), the poliovirus type 3 Fox strain was administered directly to the stomach by gavage tube to 22 premature infants. Delivery of the virus directly into the stomach precludes any inaccuracy due to loss in the mouth or regurgitation. At the lowest dose delivered (1 TCID<sub>50</sub>), 30% (3/10) of the subjects were infected. Doses of 2.5 TCID<sub>50</sub> and 10 TCID<sub>50</sub> infected 33% (3/9) and 67% (2/3) of the infants, respectively. Based on these data, the calculated HID<sub>50</sub> for this strain was 4 TCID<sub>50</sub>. It was predicted that 10% infection in premature infants (HID<sub>10</sub>) would result from administration of 0.3 TCID<sub>50</sub>. The authors pointed out that the calculated HID<sub>50</sub> for the infants tested may have been influenced by the apparent resistance of some new born infants to any dose of attenuated poliovirus (Warren et al. 1964). This, along with the assumption that virulent polioviruses and other wild enteroviruses are at least as infective as the attenuated virus used, the authors concluded that contamination with a small quantity of a potentially pathogenic virus may be of great consequence for some individuals, even if the proportion of affected individuals in the community remains quite low (Katz and Plotkin 1967).

Minor et al. (1981) calculated the HID<sub>50</sub>, HID<sub>10</sub>, and HID<sub>1</sub> of live poliovirus type 1 Sabin vaccine strain administered orally to 32 2-month old infants to be 72, 39, and 20 TCID<sub>50</sub>, respectively. Although a small number of subjects were included in the study, the presence of maternal antibodies against poliovirus type 1 did not appear to be a major influencing factor on the estimated HID<sub>50</sub>. When a  $10^6$  TCID<sub>50</sub> dose of the same Sabin vaccine strain was administered in enteric capsules to three adult volunteers with no detectable antibodies for the virus, infection, as determined by virus recovery from rectal swabs, feces and rise in neutralizing antibody titer, occurred in all subjects (Spickard et al. 1963). In another study (Horstmann et al. 1957), an attenuated strain of poliovirus type 3 (Leon KP-34) (Sabin et al. 1954) was administered orally in milk suspension to subjects possessing homotypic antibody at titers of 1:8 to 1:64, in either large ( $3.1 \times 10^7$  TCID<sub>50</sub>) or smaller ( $3.1 \times 10^4$  TCID<sub>50</sub>) doses. Both individuals who were fed the large dose became infected, both secreted the virus in the throat and feces and had rise in antibody titer. Two of the three subjects fed the smaller dose became infected, one with natural antibodies and the other with acquired type 3 antibodies as a result of vaccination with formalinized vaccine.

In summary, poliovirus is the causative agent of poliomyelitis, an acute disease of the central nervous system. The development of live attenuated vaccine strains of the

**Table 3** Response of human volunteers to different doses of attenuated poliovirus

Virus type (strain)	Subject	Pre-feeding antibodies	Route	Dose	Infected (%) <sup>a</sup>	References
3 (Leon KP-34)	Adults + 9 year old girl	Present	In milk fed by tablespoon	$3.1 \times 10^7$ TCID <sub>50</sub> <sup>b</sup> $3.1 \times 10^4$ TCID <sub>50</sub>	2/2 (100) 2/3 (67)	Horstmann et al. (1957)
1 (Sabin)	Adults	Absent	In enteric capsule	$1.0 \times 10^6$ TCID <sub>50</sub>	3/3 (100)	Spickard et al. (1963)
1 (SM)	Children	Absent	In gelatin capsule	$2.0 \times 10^2$ pfp <sup>c</sup> $2.0 \times 10^1$ pfp $2.0 \times 10^0$ pfp $2.0 \times 10^{-1}$ pfp	4/4 (100) 4/4 (100) 2/3 (67) 0/2 (0)	Koprowski et al. (1956)
2 (TN)	Children	Absent	In milk suspension	$3.1 \times 10^4$ PD <sub>50</sub> <sup>d</sup> $3.1 \times 10^3$ PD <sub>50</sub> $3.1 \times 10^2$ PD <sub>50</sub> $3.1 \times 10^1$ PD <sub>50</sub>	2/3 (67) 0/3 (0) 1/2 (50) 0/2 (0)	
1 (SM)	Infants under 6 months old	Present	In formula	$6.3 \times 10^2$ – $3.1 \times 10^5$ TCID <sub>50</sub>	16/16 (100)	Flack et al. (1956)
2 (TN)	Infants 10–27 days old	Present	In formula	$6.3 \times 10^2$ PD <sub>50</sub>	2/2 (100)	
1 (Sabin)	Infants 2 months old	Absent	In aqueous suspension using 1-ml syringe	$9.0 \times 10^1$ TCID <sub>50</sub> $6.5 \times 10^1$ TCID <sub>50</sub> $5.5 \times 10^1$ TCID <sub>50</sub> $5.0 \times 10^1$ TCID <sub>50</sub> $1.6 \times 10^1$ TCID <sub>50</sub>	1/2 (50) 0/3 (0) 1/1 (100) 2/4 (50) 0/1 (0)	Minor et al. (1981)
		Present		$2.1 \times 10^2$ TCID <sub>50</sub> $1.6 \times 10^2$ TCID <sub>50</sub> $9.0 \times 10^1$ TCID <sub>50</sub> $8.0 \times 10^1$ TCID <sub>50</sub> $6.5 \times 10^1$ TCID <sub>50</sub> $5.5 \times 10^1$ TCID <sub>50</sub> $4.2 \times 10^1$ TCID <sub>50</sub> $1.6 \times 10^1$ TCID <sub>50</sub>	2/2 (100) 3/3 (100) 2/2 (100) 1/1 (100) 0/3 (0) 0/2 (0) 0/1 (0) 0/1 (0)	
3 (Fox)	Premature infants	–	In suspension via gavage tube	$1.0 \times 10^1$ TCID <sub>50</sub> $2.5 \times 10^0$ TCID <sub>50</sub> $1.0 \times 10^0$ TCID <sub>50</sub>	2/3 (67) 3/9 (33) 3/10 (30)	Katz and Plotkin (1967)

<sup>a</sup> As determined by virus shedding and/or increase in antibody titer

<sup>b</sup> 50% Tissue culture infective dose

<sup>c</sup> Plaque-forming particles

<sup>d</sup> 50% Mouse paralytic dose

virus provided the opportunity to conduct infectious dose studies in human subjects including infants and children. Various live attenuated strains have been used and doses of 2 pfp, 16 TCID<sub>50</sub>, 310 TCID<sub>50</sub>, and 1 TCID<sub>50</sub> of poliovirus type 1 SM, type 1 Sabin, type 2 TN, and type 3 Fox strains, respectively, were all shown to cause infection in at least some of the inoculated subjects. Because of the use of attenuated vaccine strains, the MID of wild-type polioviruses, which unvaccinated infants may encounter in real life, may be lower than the above values.

## Norovirus

Non-bacterial gastroenteritis is a very common illness that frequently occurs in epidemics (Caul 1996a, b). Since they were first recognized as an agent of viral gastroenteritis (Adler and Zickl 1969), Noroviruses are frequently the cause of sporadic cases and outbreaks of acute gastroenteritis in children and adults (Blacklow and Greenberg 1991; Caul 1996b) particularly in semi-closed environments such as schools, cruise ships, hospitals, and

residential homes (Green et al. 1998; Lopman et al. 2002; Vipond 2001). These viruses are currently recognized as the cause of almost all (>96%) outbreaks of non-bacterial gastroenteritis in adults (Mead et al. 1999), particularly in Europe and Australia where there is active surveillance (Lopman et al. 2002). In the US alone, noroviruses have been estimated to cause 23,000,000 infections each year, resulting in 50,000 hospitalizations and 310 fatalities (Mead et al. 1999).

Once evocatively called “winter vomiting disease”, the pathogen’s name has changed alongside improved scientific understanding. It was first called Norwalk virus (or Norwalk-like virus) in reference to the outbreak of winter vomiting disease in 1968 at an elementary school in Norwalk, Ohio in the USA (Adler and Zickl 1969), then “Small Round Structured Virus” based on its appearance under the electron microscope (Dolin et al. 1972; Kapikian et al. 1972). The International Committee on Taxonomy of Viruses has now settled on “Norovirus”, a member of the *Caliciviridae*, based on morphology and phylogeny. The norovirus genome is single positive-strand RNA enclosed in a non-enveloped protein coat with distinct cup-shaped depressions. The diversity among noroviruses is great, and human strains are classified into three genogroups (GI, GII, and GIV), at least 25 genotypes, and numerous subgroups, with the prototype Norwalk virus designated as GI.1 (i.e., genogroup I, genotype 1). Despite this diversity, in recent years only a few strains, primarily those of genogroup II, genotype 4 (II.4), have been responsible for a majority of the cases and outbreaks (Glass et al. 2009). For the purpose of this review, the current name norovirus will be used to refer to viruses referred to as other names such as Norwalk virus in previous publications.

Norovirus is present in feces and vomitus of infected people and is shed at high concentrations by both routes. Atmar et al. (2008) experimentally infected human volunteers with doses between 4.8 and 48,000 RT-PCR units of norovirus prepared from liquid feces from individuals who participated in a previous norovirus challenge study (Graham et al. 1994). All inoculated subjects (16/16) became infected, of these 69% (11/16) met the predefined definition of viral gastroenteritis. Norovirus was detected in fecal samples for median of 4 weeks and for up to 8 weeks after virus inoculation. The peak virus titer as measured by RT-PCR had a median of approximately  $10^{11}$  copies/g feces and was most commonly found in fecal samples collected after resolution of the symptoms. One sample had a virus titer of  $>10^{12}$  copies/g feces. Chan et al. (2006) described patients who shed  $>10^{10}$  norovirus copies/g feces, whereas the peak fecal virus titer observed by Ozawa et al. (2007) in infected food handlers was about 10-fold lower. The peak median titer of  $10^{11}$  copies/g feces is

higher than would be expected from electronic microscopic studies (Atmar and Estes 2001; Thornhill et al. 1975).

Norovirus has been detected in vomitus (Greenberg et al. 1979) and an infected patient can vomit  $>10^7$  virus particles assuming a vomit lobus volume of 20–30 ml and the fact that  $10^6$  particles/ml need to be present for detection by electron microscopy (Caul 1994; Reid et al. 1988). The distribution of more than  $10^7$  virus particles as an aerosol from projectile vomiting associated with norovirus infection suggests that the airborne inhalation route may be important in the transmission of infection. From the widespread environmental contamination resulting from norovirus infection, the virus environmental robustness and its low estimated infective dose of 10–100 particles, Caul (1994) also concludes that in addition to possible aerosol inhalation, hands and surfaces also play an important part in facilitating transfer of norovirus infection, either by direct fecal–oral transfer or by transfer to foods that are eaten without further cooking. Barker et al. (2004) investigated the transfer of norovirus from contaminated fecal material via fingers and clothes to other hand-contact surfaces using a PCR assay. They reported that norovirus was consistently transferred via contaminated fingers to other surfaces and that contaminated fingers could sequentially transfer virus to up to seven clean surfaces. Decontamination of contaminated surfaces with bleach/detergent formulation, without prior cleaning, was sufficient to prevent transfer.

Norovirus has remained fastidious and noncultivable in cell cultures and in readily available animal models which has hampered studies of pathogenicity of the virus. However, in vitro replication systems for the virus have recently been described (Guix et al. 2007; Katayama et al. 2006). Using the only available small-animal model of norovirus infection, the murine norovirus 1 (MNV-1), Liu et al. (2009) reported the first unequivocal estimation of MID of human norovirus. They experimentally inoculated groups of 129SvEv mice with doses of  $10^1$  to  $10^7$  pfu of MNV-1. They noted that doses higher than  $10^3$  pfu initiated infection in the majority of mice while lower doses ( $10^1$  and  $10^2$ ) caused infection in only a minority of the inoculated mice. They calculated the MID of MNV-1 to be 800 pfu for intestinal infection, 250 pfu for mesenteric lymph nodes infection and 400 pfu for splenic infection. The minimum MNV-1 dose required for seroconversion was found to be between 1 and 100 pfu (Liu et al. 2009).

Norovirus-like particles (VLPs), made from recombinant virus capsid protein are a promising vaccine and have been used in experimental inoculation of human volunteers. Three of the five (60%) seropositive volunteers given oral doses of 100  $\mu$ g of norovirus VLPs developed a rise in serum antibody. After 250  $\mu$ g of VLPs with bicarbonate buffer, 15 (100%) of 15 volunteers developed a 4-fold rise

in enzyme-linked immunosorbent assay (ELISA) antibody to norovirus protein (Ball et al. 1999). Tacket et al. (2003) investigated the hormonal, mucosal, and cellular immune responses to oral norovirus VLPs in volunteers. Thirty healthy adult volunteers received 250, 500, or 2,000  $\mu\text{g}$  of orally administered norovirus VLPs. All vaccines developed a significant rise in IgA anti-VLP antibody-secreting cells and 30–40% of these developed mucosal anti-VLP IgA. Ninety percent of those who received 250  $\mu\text{g}$  developed raised in serum anti-VLP IgG. Neither the rates of seroconversion nor geometric mean titers increased at higher doses. In an earlier study (Tacket et al. 2000), 20 volunteers ingested 215–751  $\mu\text{g}$  of norovirus VLPs contained in transgenic potatoes, of whom 19 developed an immune response of some kind. Nineteen (90%) developed a significant increase in the number of specific IgA antibody-secreting cells, four (20%) developed specific serum IgG, and six (30%) developed specific stool IgA.

Early studies of norovirus infectivity in humans reported that infection could be experimentally transmitted when volunteers were administered oral stool filtrates from diseased patients. However, these studies did not report the virus doses administered as titration of infectious virus particles in the inocula was not possible. Dolin et al. (1971) showed that a stool filtrate (8FIIa) from an affected adult in the original outbreak in Norwalk, Ohio could reproduce the disease when administered orally to healthy adult volunteers. In a subsequent study (Wyatt et al. 1974), this filtrate produced illness in 19 (53%) of the 36 volunteers inoculated. Illness was characterized primarily by vomiting and/or diarrhea. Using the same 8FIIa filtrate, Keswick et al. (1985) successfully infected 14 (88%) of the 16 volunteers as determined by rise in antibody titer and 11 (69%) of the volunteers became ill. The titer of the 8FIIa inoculum used (2% stool filtrate) could not be measured. However, the norovirus virus antigen in this inoculum has been detected at dilutions of up to 1:125 (Greenberg et al. 1978) and the dilution in the Keswick et al. study (Keswick et al. 1985) was 1:2,000.

The development of RT-PCR for detection of norovirus RNA provided an alternative method for norovirus enumeration and a number of experimental infections in volunteers reported infectious doses of the virus in terms of genome copies (Lindesmith et al. 2003; Teunis et al. 2008). Teunis et al. (2008) reported the first quantitative estimation of norovirus infectivity in human volunteers using RT-PCR and dose response models. A primary virus inoculum was prepared from the original norovirus isolate 8FIIa (Dolin et al. 1971) and used to challenge volunteers. A secondary inoculum designated 8FIIB was prepared from a stool sample of an infected subject from the first experiment, and was used to challenge other volunteers. Comparison of results from primary and secondary inocula

showed that passage through a human host did not change the norovirus infectivity. The virus was reported to be at least as infectious as rotavirus, and the estimated average probability of infection for a single norovirus particle was close to 0.5, exceeding that reported for any other virus studied up to that date. The dose response relation for the aggregated norovirus inoculum had a  $\text{HID}_{50}$  of 1,015 genome copies, approximately equivalent to 2.6 aggregated particles. The dose response relation for completely disaggregated virus lead to an estimated  $\text{HID}_{50}$  of 18 viruses (Teunis et al. 2008).

An unusual pattern of immunity is seen in norovirus infections. Pre-existing serum antibody to norovirus is not associated with protective immunity, and persons with higher levels of pre-existing antibody are in fact more likely to experience symptomatic disease in most (Blacklow et al. 1979; Johnson et al. 1990) but not all studies (Madore et al. 1990). Graham et al. (1994) challenged 50 adult volunteers with the 8FIIa inoculum and reported 82% of them became infected and 68% had symptoms of illness. They reported that the proportion of subjects infected was similar for those with and without pre-existing antibodies. Similarly, Erdman et al. (1989) found that the presence of pre-existing serum IgA did not appear to be associated with resistance to infection or lessening in severity of symptoms. A number of other studies, however, demonstrate short-term immunity to norovirus (Dolin et al. 1972; Wyatt et al. 1974). For instance, five volunteers infected with norovirus were all protected from subsequent norovirus challenge 6–14 weeks later (Wyatt et al. 1974). Long-term immunity to norovirus has been difficult to prove as the same volunteers initially susceptible to norovirus infection were re-infected 27–42 months latter (Parrino et al. 1977).

Norovirus challenge studies found that not all individuals are susceptible to norovirus infection and disease symptoms (Blacklow et al. 1979; Graham et al. 1994; Parrino et al. 1977). These observations led to the hypothesis that there was a genetic resistance or susceptibility factor missing or present in some people. Hutson et al. (2005) reported that ABO histo-blood group type and secretor status are two genetically determinate factors that contribute to resistance and susceptibility to norovirus. An increased risk of infection was associated with blood group O, and norovirus VLPs bound to gastroduodenal epithelial cells from individuals who were secretors (Se+), but not to cells from non-secretors (Se-). This observation was in agreement with an earlier study (Lindesmith et al. 2003) which reported that 34 (62%) of the 55 Se+ volunteers challenged with doses of norovirus inocula ranging from  $10^1$  to  $10^8$  PRC-detectable units developed an infection. At each norovirus dose level, 50–90% of Se+ volunteers became infected. However, even at the highest dose, only 68% of the Se+ subjects became infected, suggesting the



existence of additional mechanisms that prevent norovirus infection. None of the Se- volunteers became infected at all of the challenge doses.

In summary, norovirus is the major cause of outbreaks of non-bacterial gastroenteritis worldwide. The virus is present in feces and vomitus of infected people and is shed at high concentrations by both routes causing considerable environmental contamination. The virus is thought to have a low infective dose of 1–100 particles and recent work suggests that the  $HID_{50}$  of the virus is 18 particles. An unusual pattern of immunity is seen in norovirus infections. A number of studies demonstrated short-term immunity to the virus, however, long-term immunity has been difficult to prove. Pre-existing serum antibodies were not associated with protective immunity, and persons with higher levels of pre-existing antibodies were found to be more likely to experience symptomatic disease in most, but not all, studies. It was shown that some genetic factors contribute to resistance and susceptibility to norovirus.

## Echovirus

Echoviruses (Enteric Cytopathogenic Human Orphan viruses) (Committee on the ECHO viruses 1955) are small non-enveloped icosahedral RNA viruses classified within the family *Picornaviridae* under the genus *Enterovirus*. Although echoviruses are organisms of the gastrointestinal tract, they cause a wide spectrum of disease some of which can be severe such as aseptic meningitis, respiratory infections, encephalitis, and myocarditis (Hill 1996; Wenner 1982). Outbreaks of disease caused by echoviruses demonstrate their ability to cause significant morbidity and mortality world-wide (Hill 1996) especially among infants and children (Arnon et al. 1991; Krous et al. 1973; Ventura et al. 2001). A number of studies reported experimental infection with echoviruses in animals (Pindak and Clapper 1965; Vasilenko et al. 1967; Vasilenko and Atsev 1965) and few used human volunteers (Buckland et al. 1959; Kasel et al. 1965b; Philipson 1958; Saliba et al. 1968; Schiff et al. 1984b).

A strain of echovirus 11, U-virus, closely resembling modern enteroviruses in its physio-chemical properties (Philipson and Wesslen 1958) was successfully transmitted via the nasal route to adult volunteers and passage in monkey kidney cells was shown to significantly attenuate the virus when compared to short-term passage in human embryo lung culture (Buckland et al. 1959). In a preliminary experiment, up to  $10^5$  TCID<sub>50</sub> of the virus passaged 22 times in monkey kidney tissue failed to cause illness in the four volunteers inoculated by nasal drops. However, the virus was recovered from throat swabs or feces from three of the four challenged subjects. In a

subsequent test, nine volunteers were inoculated with 1 ml of U-virus culture fluid containing  $10^5$  TCID<sub>50</sub> of virus passaged three times in human embryo lung cells. All subjects were infected as determined by virus isolation from their throat and feces and a rise in their antibody titer, and eight of them developed a mild but definite illness 1–3 days after inoculation. A further eight volunteers were inoculated with  $10^5$  TCID<sub>50</sub> of the virus passaged 11 times in monkey kidney cells. Although all subjects became infected, none of them developed any definite illness. Virus titers in throat swabs and fecal specimens of infected volunteers were  $8.0 \times 10^1$ – $6.3 \times 10^2$  TCID<sub>50</sub> and  $1.2 \times 10^3$ – $8.0 \times 10^3$  TCID<sub>50</sub>, respectively (Buckland et al. 1959).

Saliba et al. (1968) reported the infectivity of echovirus type 11 in man by experimentally infecting healthy adult volunteers with either high ( $10^6$  TCID<sub>50</sub>) or low ( $10^2$ ,  $10^3$ , and  $10^4$  TCID<sub>50</sub>) doses of the virus. The volunteers had varying levels of antibody titers against echovirus 11 including some with 1:20 or greater. The source of the challenge virus was from either direct nasal secretion of naturally infected individuals at a titer of  $10^2$  TCID<sub>50</sub> or from a 10th tissue culture passage ( $10^3$ ,  $10^4$  and,  $10^6$  TCID<sub>50</sub>). The  $10^4$  TCID<sub>50</sub> dose was administered via oral capsule and the rest were administered by nasal drops. Notwithstanding the presence of antibodies, all 32 volunteers given high titer virus ( $10^6$  TCID<sub>50</sub>) intranasally became infected, developed significant increase in serum-neutralizing antibodies and shed the virus from both the respiratory (66%) and enteric tracts (74%). Low titer challenge doses were infectious for 43% (30/70) of the subjects and caused illness in 12% of them. These low dose inocula, however, failed to elicit a significant antibody response in 93% of the subjects or immunity to reinfection upon rechallenge. The calculated  $HID_{50}$  for the virus by either the respiratory or enteric route was  $10^4$  TCID<sub>50</sub> and the MID for man was estimated to be less than  $10^{-3}$  TCID<sub>50</sub>. This demonstrated that echovirus appears to be equally infective in respiratory and intestinal tracts. Taking that the nasal secretion of naturally acquired echovirus type 11 infection has a virus titer of  $10^2$  TCID<sub>50</sub>/ml, nasal discharges during infections therefore contained about  $10^5$  MID for man per milliliter.

Schiff et al. (1984b) conducted a comprehensive study to determine the MID of echovirus type 12 in human volunteers. The study differed from most previous studies using enteric viruses in that it used an unattenuated “wild” virus strain administered by the natural route to a large number of susceptible individuals under carefully controlled conditions in an isolation facility. The echovirus type 12 strain had been isolated from an 8-year old girl with erythema infectiosum and was passaged twice in primary monkey kidney cells and safety tested prior to use. The titer of the viral preparation was  $5.2 \times 10^7$  pfu/ml as

determined on RD (human rhabdomyosarcoma) cells and had a particle/pfu ratio of 1:41. Healthy adult male volunteers with no serological evidence of previous echovirus type 12 infection were administered doses ranging from 0 to  $3.3 \times 10^5$  pfu of virus in 100 ml of non-chlorinated water. No volunteer developed significant illness; hence infection data were based on viral shedding and seroconversion.

The results (Table 4) indicate that the  $HID_{50}$  and  $HID_1$  of echovirus type 12 was 919 and 17 pfu, respectively. In a previous report of the results of this study, when a 33-fold less sensitive plaque assay (LLC-MK<sub>2</sub> cells) was used, the calculated  $HID_{50}$  was 30 pfu and  $HID_1$  was about 1 pfu (Schiff et al. 1984a). A  $1.5 \times 10^3$  pfu dose of the virus which caused infection in 60% of healthy adults with no detectable neutralizing antibody, caused infection in 72% (13/18) of previously infected individuals (Schiff et al. 1984b). The presence and concentration of serum antibody caused no significant change in the rate of infection or duration of viral shedding. These results indicated that previous infection with echovirus type 12 does not provide lasting protection against reinfection. Although under the above study conditions, the infectivity of echovirus type 12 was much less in susceptible healthy adults than in sensitive culture cells, the fact that the original viral isolate was passaged twice in primary monkey kidney calls could have reduced its infectivity in man. Furthermore, the use of other subjects such as infants who may have been more easily infected, and better conditions for infection such as the addition of food to buffer stomach acids maybe also have increased the infectivity of the virus for man (Ward et al. 1984).

In summary, echoviruses are organisms of the gastrointestinal tract but can cause a wide spectrum of disease in humans. Echovirus was found to be equally infectious to the upper respiratory and intestinal tracts. Exposure to echovirus 12 did not provide lasting immunity against

reinfection. The presence and concentration of serum antibodies caused no significant change in rate of echovirus 12 infection or duration of its shedding. Similarly, the main effect of antibody was to decrease virus excretion and to shorten illness but did not prevent infection with echovirus 11. Low doses of echovirus were shown to cause infection in at least some of the volunteers tested. Less than  $10^{-3}$  TCID<sub>50</sub> of echovirus 11 and 17 pfu of echovirus 12 infected at least 1% of the inoculated individuals.

## Hepatitis A Virus

Hepatitis A (formally infectious hepatitis) is caused by the hepatitis A virus (HAV), a small (27 nm in diameter), icosahedral, non-enveloped, single-stranded RNA virus, belonging to the family *Picornaviridae*, and the only member of the genus *Hepatovirus* (Hollinger and Emerson 2001). There is only one HAV serotype, and immunity after infection is lifelong (Lemon et al. 1992). The virus is a significant cause of morbidity globally with outbreaks have been reported from all over the world (Gust 1992). Clinical manifestations of symptomatic HAV infection vary from mild, anicteric illness, to fulminant hepatitis. The liver is the site of HAV replication but the virus is mainly excreted in stool (Dienstag 1981; Tjon et al. 2006). Prolonged HAV fecal excretion, up to 6 months after infection, and presence in blood of persons with natural or experimental infections have been reported (Rosenblum et al. 1991; Tjon et al. 2006). The concentration of HAV range from  $10^6$  copies/ml in serum to more than  $10^8$  copies/ml in stool (Tjon et al. 2006).

The infectious dose of HAV is unknown, and although Grabow (1997) suggested that one virion can cause infection, the infectious dose is presumed to be of the order of 10 to 100 virions (Venter et al. 2007). HAV is primarily

**Table 4** Infectivity of echovirus type 12 in adult healthy volunteers as determined by intestinal shedding of the virus and seroconversion (Schiff et al. 1984b)

Dose (PFU) <sup>a</sup>	No of subjects in indicated group			
	Total	Shedding of the virus (%)	Seroconversion (%) <sup>b</sup>	Infection (%)
0.0	34	0 (0)	0 (0)	0 (0)
$3.3 \times 10^2$	50	14 (28)	7 (14)	15 (30)
$1.0 \times 10^3$	20	8 (40)	3 (15)	9 (45)
$3.3 \times 10^3$	26	18 (69)	11 (42)	19 (73)
$1.0 \times 10^4$	12	11 (92)	3 (25)	12 (100)
$3.3 \times 10^4$	4	2 (50)	1 (25)	2 (50)
$3.3 \times 10^5$	3	2 (67)	1 (25)	2 (67)

<sup>a</sup> Plaque-forming unit

<sup>b</sup> A 4-fold or greater increase in antibody titer

transmitted by the fecal–oral route, either by person-to-person contact or by ingestion of contaminated food or water. Transmission can also occur after exposure to HAV-contaminated blood or blood products, but not by exposure to saliva or urine (Fiore 2004). A wide variety of vehicles have been implicated in hepatitis A outbreaks, including recreational and drinking water, raw milk, orange juice, salads, cold meat, hamburgers, pasties, and seafood such as shellfish (Dienstag 1981; Gust 1992; Venter et al. 2007). The spread of the virus is enhanced by its environmental robustness. HAV has been shown to be resistant to concentrations of free residual chlorine of 0.5–1.5  $\mu\text{g/ml}$  for 1 h, to withstand temperatures of 60–80°C for 1 h, freeze-thawing, low relative humidity ( $\pm 25\%$  for 7 days), and pH values as low as pH 1 (Dienstag 1981; Venter et al. 2007). HAV has been shown to survive for months in experimentally contaminated fresh water, seawater, marine sediments, wastewater, soils, and oysters (Venter et al. 2007).

Animal studies have shown that stool filtrates of patients or animals infected with HAV inoculated orally or intravenously, were infectious (Dienstag et al. 1975; LeDuc et al. 1983; Purcell et al. 2002). Surprisingly, for a virus that is normally transmitted by the fecal–oral route, the wild-type HAV was shown to be  $3.1 \times 10^4$ -fold less infectious by the oral, compared with the intravenous, route in tamarins and chimpanzees. This observation has already been demonstrated in young adult volunteers experimentally infected with the live attenuated strain HM-175 of HAV (Sjogren et al. 1992). However, few of the experiments in animals determined the virus titer in their inocula. In a recent study, Amado et al. (2010) used a 0.5 ml of a stool suspension of the HAV strain HAF-203, recovered from a child with sporadic hepatitis, to infect cynomolgus monkeys. The stool suspension was titered by ELISA (1:320) and real-time PCR ( $3 \times 10^5$  copies/ml) and administered to the animals intravenously. None of the animals exhibited clinical manifestations related to hepatitis A infection but all showed histological and biomedical signs of hepatitis. A similar result was obtained in an earlier investigation where 0.5 ml of the same stool suspension was orally administered to marmosets by applying it to the posterior pharynx of the animals (Pinto et al. 2002). Hornei et al. (2001) used two tissue culture-adapted variants of the HAV strain HM-175 to transmit infection to guinea pigs. Animals inoculated with  $10^5$  TCID<sub>50</sub> of the virus intraperitoneally or orally developed an active, asymptomatic infection with specific histopathological changes in the liver.

Voegt (1942) was the first to report the transmission of HAV to volunteers by feeding duodenal fluid and blood obtained from patients in the acute phase of disease. Since then, numerous attempts to infect volunteers with feces, serum, nasopharyngeal washings and urine from infected cases were reported (Havens 1948). These studies showed

that doses in the range of 1 g of feces or 1–4 ml of fecal suspensions from infected patients administered via the oral, parenteral, or the nasopharyngeal routes, were infectious (Drake et al. 1950; Havens 1946b; Maccallum and Bradley 1944). Krugman and Ward (1958) found that 0.1, 1, 2, and 4 g fecal doses from patients within the first 8 days of the onset of jaundice produced the disease in 25% (2/8), 45% (5/11), 80% (4/5), and 92% (12/13) of the orally inoculated individuals, respectively. The MID and the estimated HID<sub>50</sub> of the virus pool was 0.1 g and approximately 1–2 g, respectively. In 1943, Cameron (1943) described the transmission of hepatitis A to six volunteers by the intramuscular injection of blood obtained from a patient early in disease. Further work by Drake et al. (1950) and Krugman and Giles (1970) showed that small volumes of sera (0.05–4 ml) from hepatitis A patients were sufficient to initiate infection when inoculated orally, subcutaneously, or intramuscularly to volunteers. Krugman et al. (1962) found that one of eleven individuals given intramuscular injections of 0.0025 ml of serum from hepatitis A patients acquired anicteric hepatitis. In this study, the HID<sub>50</sub> of the serum preparation was calculated to be 0.025 ml. Work by Havens (1946a, b) showed that hepatitis with jaundice occurred in volunteer subjects after parenteral administration of only 0.01 ml of serum. The infectivity of both urine and nasopharyngeal washings has been incompletely investigated. Contradictory results have followed administration of urine to volunteers by the oral or the nasopharyngeal routes (Findlay and Willcox 1945; Havens 1946b; Maccallum and Bradley 1944; Neeffe and Strokes 1945; Voegt 1942). The results of testing nasopharyngeal washings have been negative (Havens 1946b; Neeffe and Strokes 1945) with one possible exception (Maccallum and Bradley 1944).

Some evidence for the infectious dose of HAV comes from experiments using the HAV vaccine since the late 1980s. The most important feature of the vaccine is the appearance of neutralizing antibodies to HAV, which can be measured by ELISA and the virus titer is expressed in terms of ELISA units (ELU). Studies showed that a 0.5 ml intramuscular dose of a vaccine prepared from the HM-175 strain of HAV containing 720 ELU of hepatitis A antigen had a seroconversion rate of 44% in infants (De et al. 2006), 100% in children (Findor et al. 1996) and 88–100% in adults (Andre et al. 1992; Clemens et al. 1995; Davidson et al. 1992; Goubau et al. 1992; Westblom et al. 1994), after 1 month of inoculation. Experiments with other doses showed that seroconversion rates after 1 month inoculation of the vaccine containing 360 ELU of hepatitis A antigen was 95% in children (Clemens et al. 1995) and 85–93% in adults (Goubau et al. 1992). Doses of 1440 and 180 ELU seroconverted 100 and 71% of adults vaccinated, respectively, after 1 month of inoculation (Goubau et al. 1992;

Westblom et al. 1994). In another study (Sjogren et al. 1992), none of the eight volunteers who received doses of  $10^4$ ,  $10^5$ ,  $10^6$ , and  $10^7$  TCID<sub>50</sub> of the similar vaccine orally had an antibody response. Volunteers who received similar doses by the intramuscular route developed antibody to hepatitis A 3 weeks after immunization with  $10^6$  and  $10^7$  TCID<sub>50</sub>. Doses of 160 and 80 ELU of vaccine derived from the GBM strain of HAV were highly immunogenic in seronegative adults and children, respectively (Fisch et al. 1996; Lagos et al. 2003). A vaccine derived from the live attenuated F' variant of the CR-326F strain of HAV showed 97% rate of seroconversion within 4 weeks after a 25 ELU dose (Innis et al. 1994). Doses of 25, 50, and 100 ELU of the same vaccine seroconverted 65, 89, and 93% of adults  $\geq 30$  years old and weight  $\geq 77$  kg after 4 weeks (Bertino et al. 1998). In another study,  $1.3 \times 10^4$ ,  $1.6 \times 10^5$ ,  $1.3 \times 10^6$ , and  $2.0 \times 10^7$  TCID<sub>50</sub> of a similar vaccine seroconverted 20, 40, 60, and 100% of recipients, respectively (Midthun et al. 1991). Wang et al. (2007) reported that approximately 81% of children administered a dose of  $10^7$  TCID<sub>50</sub> of vaccine derived from the H<sub>2</sub> strain of HAV seroconverted a month after inoculation. Ren et al. (2002) showed that doses of 500, 1000, and 1,440 ELU of vaccine derived from the TZ-84 strain of HAV seroconverted 50, 70, and 87% of seronegative adult volunteers a month after vaccination.

In summary HAV causes hepatitis A, a significant cause of morbidity globally, with lifelong immunity after infection. The virus replicates in the liver but can also be detected in high concentrations in stool and blood of infected individuals. HAV is primarily transmitted by the fecal–oral route, either by person-to-person contact or by ingestion of contaminated food or water. Transmission also occurs after exposure to HAV-contaminated blood or blood products, but not by exposure to saliva or urine. The virus was shown to be less infectious by the oral, compared with the intravenous, route. There is little quantitative data on the MID in humans. A 0.5 ml stool suspension containing  $3.0 \times 10^5$  RNA copies/ml of the HAV strain HAF-203 administered orally or intravenously to animals caused infection. Similarly an oral dose of 0.1 g of stool and an intramuscular dose of 0.0025 ml of blood from patients with hepatitis A were infectious in volunteers. Vaccine studies using attenuated HAV strains showed that over 70% seroconversion was achieved by intramuscular inoculation with doses of 25, 80, and 180 ELU of vaccines derived from CR326F, GBM, and HM-175 strains of HAV, respectively.

### Concluding Remarks

Most of the reviewed studies investigated infection in susceptible volunteers free of antibodies against the

administered virus. In some reports, however, the subjects either had pre-existing antibodies for the test virus or their immune status was not known. Presence of pre-existing antibodies has been shown to affect the infectious dose and to be protective against infection for many viruses including rhinovirus, coxsackievirus, RSV, and adenovirus. However, unusual patterns of immunity diverging from the above trend have also been reported. Exposure to norovirus or echovirus 12 did not provide lasting immunity against reinfection. The presence and concentration of serum antibodies caused no significant change in rate of echovirus 12 infection or duration of its shedding. Similarly, the presence of pre-existing serum antibody against norovirus was not associated with protective immunity and persons with higher levels of antibodies were found to be more likely to experience symptomatic diseases in most but not all studies.

Because of ethical and safety concerns surrounding experimental infection of human volunteers with live wild-type viruses, many studies rely on the use of animal models to simulate infection in man or the use of young healthy adult human volunteers. The results of such investigations have to be interpreted with caution as it is not always possible to correlate data generated from animal studies with human subjects, and by design, some of the human studies may have excluded subjects who are most susceptible to viral infections. For instance, experimental infections with rotavirus were mainly conducted in young animals or adult human volunteers while the most susceptible humans to rotavirus infection are infants and young children. Similarly, with some exceptions, most viral infection studies were conducted in healthy individuals, excluding a large population of immunocompromised and diseased individuals who may be much more susceptible to infections.

In addition to the use of adult healthy volunteers that may not accurately reflect at-risk populations, investigations have commonly used lab-adapted or -attenuated strains potentially useful as live vaccines. These strains which have been passaged through cell cultures are less virulent than the wild-type strain. Infectious doses determined using these attenuated strains of viruses may not be an accurate reflection of doses required to cause infection by the wild-type strain which may be significantly lower. This is evident in studies which used polio vaccine strains to determine infectious doses in children and infants. While infants and children may be the most suitable subjects for testing infectivity of poliovirus, vaccine strains are attenuated and are less virulent than wild-type strains which unvaccinated infants may encounter in real life. It is worth noting, however, that virus passage through cell cultures was not always associated with reduced virulence. Some reports demonstrated that passage in cell cultures or



through human hosts not only did not affect virulence but also, in some cases, increased virus infectivity. Coxsackievirus A21 strains passaged once or twice in cell culture were as infectious as unpassaged strain obtained from naturally occurring cases of illness. Passage of norovirus through human host did not change the virus infectivity and passage of rotavirus, in fecal specimens, in primary cells, increased its virulence.

The method of virus administration is an important factor for consideration when interpreting infectious doses of viruses in experimentally infected volunteers. This is particularly relevant in relation to respiratory viruses most of which are able to infect both the upper and lower respiratory tract regions or to viruses capable of infecting both respiratory and gastrointestinal tracts. The viral dose required to cause an infection varies depending on the virus and the preferred site of infection for each virus type. This is expressed by the differences in the viral doses required to cause infection when delivered by nasal drops, which promotes infection of the upper respiratory tract, or by aerosols, which allows infection of upper and lower regions of the tract. For instance, rhinovirus 15 and coxsackievirus A21 were more infectious when given as nasal droplets than as an aerosol spray, while adenovirus 4 and influenza required higher virus doses to cause infection when administered by nasal drops than when administered via aerosols. For viruses that can multiply both in the gastrointestinal and respiratory tracts such as coxsackievirus and adenovirus, higher doses of viruses were required to initiate infection in the gastrointestinal tract compared with the respiratory tract. The  $HID_{50}$  of adenovirus type 4 was 10–500  $TCID_{50}$  when given in enteric-coated capsules, 35  $TCID_{50}$  by nasal inoculation and only 0.5  $TCID_{50}$  when administered by small particle aerosol. Similarly, when  $3.0 \times 10^5$   $TCID_{50}$  of coxsackievirus were delivered to the nasopharynx by drops and to the intestine by either enteric-coated capsules or Rehfuss tube, illness was induced only after inoculation of the respiratory tract. Echovirus, however, was found to be equally infectious to the upper respiratory and intestinal tracts.

Respiratory viruses can be shed at high titers from infected individuals and transmitted by various routes including from contaminated environmental surfaces and via aerosols. Most of these viruses appear to be as infective in humans as in tissue culture. Depending on the delivery method, some of these viruses such as rhinovirus were shown to have greater infectivity in man than in culture. Enteric viruses are also shed at high concentrations from diseased individuals and can cause considerable

environmental contamination from vomit and feces of infected subjects. They are transmitted mainly via the oral–fecal route from contact with contaminated surfaces and eating or drinking contaminated material. As demonstrated in most experimental virus inoculations, infection and shedding of the virus may occur without development of illness. These asymptomatic infections are an important health hazard because infected individuals with no signs of illness may amplify the virus and serve as shedders or reservoirs of diseases for transmission to susceptible individuals.

For many of the respiratory and enteric viruses, the minimal dose that caused infection in humans reported in the literature (Table 5) appears to be small especially for highly susceptible subjects such as infants. Doses less than 1  $TCID_{50}$  of influenza virus, rhinovirus, and adenovirus were reported to infect 50% of the tested population. Similarly, low doses of the enteric viruses, norovirus, rotavirus, echovirus, and poliovirus, caused infection in at least some of the volunteers tested. In the case of norovirus and HAV, it is possible that a single virus particle is able to initiate infection. It is, however, important to note that relatively few investigations reported the infective dose in the form of number of infective particles. A high percentage of morphologically identical viral particles in a sample, as determined by electron microscopy, will typically be non-infectious for any known cell system. In fact, the particle/infectivity ratio is rarely equal for any virus assay system. Moreover, few studies have determined this ratio or the ratio of infective particles/ $TCID_{50}$ . It is also worth noting that very few recent data regarding MID of human viruses have been published and many of the studies reviewed in this article were carried out many decades ago. Hence, the reported infective doses of human viruses may also change if more up to date studies are conducted aided with our improved understanding of viral epidemiology, microbiology, and infection, and utilizing more sensitive virus assay, cultivation, and quantification techniques.

In summary, many studies have been conducted that provide information on the MID of human viruses. However, due to differences in the epidemiology and culture methods for each virus and differences and limitations of experimental procedures, estimations of the MID should be interpreted with caution. Notwithstanding these limitations, the MID of respiratory and enteric viruses appears to be low and should be viewed in relation to the likely host characteristics of the at-risk population of interest.

**Table 5** Minimum infectious dose of human viruses

Virus	Type/Strain	Dose	% Infected <sup>a</sup>	Method of delivery	References
<b>Respiratory</b>					
Influenza virus	Asian influenza A2	0.6–3 TCID <sub>50</sub> <sup>b</sup>	50	Small particle aerosol	Alford et al. (1966)
Rhinovirus	RV15	0.032 TCID <sub>50</sub>	50	Nasal drops	Couch et al. (1966)
Coxsackievirus	A21-48654	6 TCID <sub>50</sub>	50	Nasal drops	Couch et al. (1966)
Adenovirus	Type 4	0.5 TCID <sub>50</sub>	50	Small particle aerosol	(Couch et al. 1969)
		6.6 virus particles	50		
RSV	ts-1	30–40 TCID <sub>50</sub>	33	Nasal drops/coarse spray	Parrott et al. (1975)
	A2	501 pfu <sup>c</sup>	100	Nasal drops	Mills et al. (1971)
<b>Enteric</b>					
Rotavirus	CJN	0.9 ffu <sup>d</sup>	14	Oral ingestion	Ward et al. (1986)
Poliovirus	Type 1 SM	2 pfp <sup>e</sup>	61	Gelatin capsule	Katz and Plotkin (1967)
	Type 3 Fox	1 TCID <sub>50</sub>	30	Gavage tube	
Norovirus	8FIIa	18 viruses	50	Oral suspension	Teunis et al. (2008)
Echovirus	Type 11	>10 <sup>-3</sup> TCID <sub>50</sub>	>1	Gelatin capsule	Saliba et al. (1968)
	Type 12	17 pfu	1	Oral suspension	Schiff et al. (1984)

<sup>a</sup> As determined by virus shedding and/or increase in antibody titer

<sup>b</sup> 50% Tissue culture infective dose

<sup>c</sup> Plaque-forming units

<sup>d</sup> Focus forming units

<sup>e</sup> Plaque-forming particles

**Conflict of interest** S.Y. and J.A.O are employed by Bioquell (UK) Ltd.

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