Hepatitis E, previously known as “enterically transmitted non-A, non-B, non-C (non-A-C) hepatitis”, is an infectious viral disease with clinical and morphological features of acute hepatitis. The course of disease is mild in most affected people, except pregnant women, for whom the mortality rate can rise to 20% (Aggarwal and Jameel 2011). The etiological agent, first identified in the early 1980s, is the hepatitis E virus (HEV) (Emerson and Purcell 2003). The disease is an important public health concern in developing countries (Southeastern and Central Asia, the Middle East, northern and western areas of Africa and America) where it is frequently epidemic, and is mainly transmitted by the fecal-oral route usually through the consumption of contaminated water or food (Emerson and Purcell 2003; Wibawa et al. 2004; Aggarwal 2011).

Industrialized countries, such as Canada, Europe, Japan and the USA were previously thought to be exempt from HEV, with a limited number of cases reported only in people who had travelled to endemic areas of the world. However, more recent studies have documented a number of sporadic cases in developed areas, including the USA and Europe, among patients who had no history of travelling to hepatitis E endemic countries (Aggarwal and Jameel 2011). Furthermore, a high anti-HEV seroprevalence (in some cases reaching 20%) has been detected in a significant proportion of healthy individuals of non-endemic countries (Aggarwal and Jameel 2011).

Since the early 1990s, serological evidence of HEV infections and in some cases virus detection have been reported in many animal species such as rhesus monkeys, pigs, cattle, sheep, poultry, dogs, cats, goats, rabbits, mongooses, bats and rodents, both in developed and developing countries, suggesting the possibility that these species may become infected with HEV-like viruses (Huang et al. 2002; Emerson and Purcell 2003). However, for some species, such as dogs, goats and cats, the virus has never been recovered or sequenced up to now, and further studies are, therefore, needed to fully understand the significance of seropositivity in these animals. In 1997, a swine HEV strain was identified for the first time in the USA, and named swine hepatitis E virus (swHEV) (Meng et al. 1997).
swine virus was genetically correlated to two human HEV strains isolated in the USA in the same period from patients affected with hepatitis E, who had not travelled to endemic areas (Meng et al. 1997). Since then, swine HEV strains have been isolated across the globe. Frequently, a strict genetic correlation between human and swine strains from the same geographic region has been observed and, during experimental infections, the possibility of cross-species transmission of swine strains to humans and of human strains to non-human primates has been demonstrated (Meng et al. 1998a; Williams et al. 2001; Matsuura et al. 2007). Furthermore, several seroepidemiological studies have reported high antibody prevalence to HEV in people working in direct contact with swine and wild boar (Drobeniuc et al. 2001; Withers et al. 2002; Carpentier et al. 2012). The first direct evidence of a possible zoonotic transmission of HEV was provided in Japan in 2003, when cases of hepatitis E were caused by the ingestion of uncooked meat or organs from pigs, wild boar or deer, a few weeks before the onset of the disease (Tei et al. 2003; Yazaki et al. 2003; Tamada et al. 2004). More recently, a case control study conducted in France using both epidemiological and virological data confirmed that 13 human cases of hepatitis E were conclusively linked to the consumption of raw “figatellu” pig liver sausages (Colson et al. 2010). The disease is now recognized to represent an emerging zoonosis.
2.1 Structure of the Virus and Genome Organization

In 1983, the hepatitis E virus was first identified by immune electron microscopy in the feces of patients with enterically transmitted non-A-C hepatitis. Subsequent establishment of the disease in cynomolgus macaques led to cloning and sequencing of HEV in 1990 (Balayan et al. 1983; Kane et al. 1984).

The hepatitis E virus (HEV) is a small (27–34 nm), icosahedral, non-enveloped single-stranded positive-sense RNA virus.

The HEV genome is approximately 7.2 kb in length, and presents a 7-methylguanosine cap followed by three overlapping open reading frames (ORFs) and a second non-coding region of about 65–74 nucleotides with a 3′ poly A tail. The genome length slightly varies between animal strains; shorter genomes have been detected in rat HEV in Vietnam (6927 nt) (Li et al. 2013), in avian strains (6654 nt) (Huang et al. 2004) and in recently detected bat viruses (6767 nt) (Drexler et al. 2012), although the genome organization seemed to be conserved in all these cases. ORF1 (5073–5124 nt) codes for a non-structural poly-protein of about 1,690 amino acids, which is involved in viral genome replication and viral protein processing. The poly-protein functional domains include a methyltransferase (MeT), flanked by the Y domain, a papain-like cysteine protease (PCP), flanked by the macro domain (X domain), an RNA helicase, and an RNA-dependent RNA polymerase. The N-terminal portion of ORF1 serves as a viral methyltransferase (MeT) that catalyzes the capping of both genomic and subgenomic viral RNAs (Rozanov et al. 1992). Capping of the viral RNA determines its translation and, as recently shown, it is decisive for virus defense from the innate host response, inhibiting interferon cascade activation (Pichlmair et al. 2006). The MeT is followed by the so-called Y domain, which shares significant homology with non-structural proteins of other positive-stranded RNA viruses. This is followed by the papain-like cysteine proteases (PCP) and by the formerly designated X domain, more recently renamed as the “macro domain”, which may be involved in the binding of ADP-ribose and its polymeric form (Neuvonen and Ahola 2009). As for other described PCPs, also in
the HEV genome, the presence of a macro domain following the PCP sequence strengthens its functional homology with proteases of diverse origin, although the specific role of HEV PCP in polyprotein (pORF1) processing still remains undefined (Ahmad et al. 2011). pORF1 is characterized by the presence of a region rich in proline residues and without a predicted secondary structure, which might act as a flexible hinge within the protein. The predicted helicase domain of HEV contains a full complement of conserved helicase motifs (Karpe and Lole 2010), including the seven conserved motifs proposed to contain both the NTPase activity and an RNA binding domain. The HEV helicase possesses an RNA 5′-triphosphatase activity involved in the first step of RNA capping (Karpe and Lole 2010). The C-terminal domain of pORF1 has RNA-dependent RNA polymerase (RdRp) activity (Agrawal et al. 2001); this is an essential enzyme for RNA virus replication through the synthesis of an anti-genomic RNA intermediate. The endoplasmic reticulum was identified as the site of replicase localization, and the intracellular membranes are the possible sites where RNA replication occurs (Rehman et al. 2008). ORF3 (366–369 nt) follows ORF1 and overlaps the N-terminal portion of ORF2, in a different reading frame, and encodes for a small phosphoprotein (pORF3), which is expressed at the intracellular level. The protein contains two hydrophobic and two proline rich domains; these regions contain amino acid motifs involved in signal transduction (Korkaya et al. 2001), and a PSAP motif is present and conserved in all HEV isolates, including avian HEV. pORF3 does not show homology with any other known protein; its role still remains unclear. Recent studies of the biology of HEV replication have shown that pORF3 may be involved in virus release from infected cells (Okamoto 2011), since it is associated with the cytoskeleton and is present on the virion surface (Yamada et al. 2009) (Fig. 2.1). Moreover, pORF3 down-regulates innate host responses through the reduction of the expression of acute phase proteins and promotion of the secretion of α1-microglobulins (Panda et al. 2007).

An additional three ORFs have been described in rat and bat HEV genomes, but their function remains yet unknown. No suggestive similarity of the putative gene products of the internal reading frame to any described protein domain could be detected by BLAST comparison (Johne et al. 2010a; Drexler et al. 2012).

![Fig. 2.1 Genomic organization of HEV, including the three ORFs. Nucleotide positions are referred to a prototype strain (Acc. No. M73218). On the top, functional domains are indicated: MeT methyltransferase; Y domain; PCP protease; X domain; Hel helicase; RdRp RNA dependent RNA polymerase; pORF2 capsid protein; pORF3](image-url)
The viral capsid protein encoded by ORF2 works for particle assembly, binding to host cells, and elicitation of neutralizing antibodies. pORF2 is glycosylated, and three asparagine (Asn) residues for N-linked glycosylation sites have been identified. The virus capsid is made up of subunits containing 30 homodimers of pORF2 (Yamada et al. 2009). The crystal structure of a truncated recombinant pORF2 protein has been obtained, but the real size of the protein in mature virions remains unknown (Yamashita et al. 2009). Among four major mammalian HEV genotypes, sequence identity among the amino acid residues of the capsid protein was over 85%, and many amino acid divergences were found in the N-terminal 111 residues. The N-terminal region of the HEV capsid protein is most likely to represent the shell domain, whereas the C-terminal region of pORF2 is more variable and is considered to be the protruding domain of the HEV capsid protein (Li et al. 2009). The initial contact with host cells in order to initiate viral infection is believed to occur through these protrusions (Pichlmair et al. 2006). Expression of a truncated capsid protein lacking the first 111 amino acids and/or the C-terminal 59 amino acids in insect cells by the baculovirus expression system resulted in self-assembly of the capsid protein and in the production of two types of HEV-like particle (HEV-VLP) with different diameters (Li et al. 1997; Caprioli et al. 2005; Xing et al. 2011), corresponding to different proteolytic cleavages. As demonstrated by protein expression in the baculovirus insect cell expression system, the minimum requirement for assembly was inclusion of amino acid residues 126–601 (Li et al. 2005a). The N-terminal domain followed by the signal sequence (residues 28–101) is an arginine-rich domain resembling the RNA-binding domain of the coat proteins of tombusviruses. The capsid protein binding to Huh-7 liver cells has been studied, and it appeared to be mediated by heparin sulfate proteoglycans (HSPGs), specifically syndecans, as demonstrated using the baculovirus expressed pORF2, assembled in VLPs (Kalia et al. 2009).

Recent studies on HEV particles have provided useful information about the HEV life cycle as well as for the possible development of monovalent or polyvalent vaccines. Indeed, the recombinant HEV capsid protein is currently undergoing clinical trials as a vaccine candidate (Zhu et al. 2010; Zhao et al. 2012), and genotype 2 HEV VLPs have been proposed as a useful carrier for foreign DNA (Takamura et al. 2004) or epitopes into mucosal epithelial cells (Niikura et al. 2002). However, several knowledge gaps still remain concerning the structure of HEV capsid, such as what role pORF3 may possibly have in the virion architecture and function. Further studies will be needed to answer these questions.

### 2.2 Taxonomy and Nomenclature

Because of limitations in allowing it to grow reproducibly and efficiently in vitro, HEV classification has been mainly based on the analysis of the viral RNA by sequencing and phylogenetic techniques (Korkaya et al. 2001).
HEV was initially classified within the *Caliciviridae* family, but the increasing numbers of sequences collected afterwards have clearly unmasked significant differences with other caliciviruses, and since 2004 HEV has been classified as a new genus called *Hepevirus* in the family of *Hepeviridae* (Emerson and Purcell 2003). HEV strains detected in humans and other mammalian species represent the major genus of the *Hepeviridae* (Table 2.1). Although avian HEV strains share only 50–60 % nucleotide identity with mammalian HEV strains (Meng 2010a), specific antibodies are able to cross-react with the capsid protein of both groups of viruses, demonstrating the presence of common epitopes (Haqshenas et al. 2001). Nonetheless, avian HEV strains have never been associated with cases of infection in human beings (Kamar et al. 2012), causing hepatitis-splenomegaly syndrome (HS) only in chickens (Haqshenas et al. 2001). Consequently, it has been proposed to assign avian HEV to a separate genus, consisting of at least three different genotypes (Bilic et al. 2009; Marek et al. 2010).

*Hepeviridae* includes four genotypes of mammalian HEV, which primarily infect humans, domestic pigs, wild boar, deer, and rabbits (Meng et al. 2012). However, genetically distant HEV strains have more recently been identified in the rat (Johne et al. 2010b), ferrets (Raj et al. 2012), wild boar (Takahashi et al. 2011), bats (Drexler et al. 2012), and cutthroat trout (*Oncorhynchus clarkii*) (Batts et al. 2011), suggesting that the *Hepeviridae* family classification should be reviewed. A proposed revision includes introduction of separate clades: one genus would comprise human HEV genotypes and closely related animal viruses, while the others would include viruses from chiropteran (bat), rodent (rat), and avian (chicken) hosts (Drexler et al. 2012). The “cutthroat” hepevirus is genetically the most

<table>
<thead>
<tr>
<th>HEV strains</th>
<th>Natural host</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mammalian HEV</strong></td>
<td></td>
</tr>
<tr>
<td>Genotype 1</td>
<td>Humans</td>
</tr>
<tr>
<td>Genotype 2</td>
<td>Humans</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>Humans, domestic pigs, wild boar, deer, mongooses, rabbits</td>
</tr>
<tr>
<td>Genotype 4</td>
<td>Humans, domestic pigs, wild boar</td>
</tr>
<tr>
<td>Novel unclassified genotype, Rat HEV</td>
<td>Rats</td>
</tr>
<tr>
<td>Novel unclassified genotype, Boar HEV</td>
<td>Wild boar in Japan</td>
</tr>
<tr>
<td>Novel unclassified genotype, Bat HEV</td>
<td>Bats</td>
</tr>
<tr>
<td>Novel unclassified genotype, Ferret HEV</td>
<td>Ferrets</td>
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<tr>
<td><strong>Avian HEV</strong></td>
<td></td>
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<tr>
<td>Genotype 1</td>
<td>Chickens</td>
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<tr>
<td>Genotype 2</td>
<td>Chickens</td>
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<tr>
<td>Genotype 3</td>
<td>Chickens</td>
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<tr>
<td>Genotype ?</td>
<td>Chickens (Hungary)</td>
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<tr>
<td><strong>Trout HEV</strong></td>
<td></td>
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<tr>
<td>Genotype ?</td>
<td>Cutthroat trout (USA)</td>
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</tbody>
</table>
Based on the sequence comparisons of the HEV genome currently available, strains are classified in genotypes and sub-types. The four major genotypes identified in mammalian to date include (Fig. 2.2 and Table 2.1): genotype 1 (Burmese-like Asian strains), genotype 2 (a Mexican strain and some African strains), genotype 3 (strains from humans and animals, worldwide), and genotype 4 (strains from human cases and animal strains, in Asia and Europe) (Meng 2011). Avian HEV, genetically distant from mammalian strains, has been classified separately and comprises at least three different genotypes (Marek et al. 2010), since a putative novel avian HEV genotype has been identified but not yet classified.

**Fig. 2.2** Phylogenetic tree illustrating different genotypes of hepatitis E. The tree is based on full-length sequences of HEV strains of either human or animal origin. The GenBank accession number of each strain used in the tree is indicated.
(Banyai et al. 2012). However, with the recent identification of genetically distinct HEV strains from rats (Johne et al. 2010a), ferrets (Raj et al. 2012), and wild boar (Takahashi et al. 2011), new previously unrecognized genotypes have been proposed.

Despite the knowledge that different HEV genotypes occur, the virus seems otherwise to exist as a single serotype (Aggarwal and Naik 2009).

Most human infections that occur in Asia and Africa are caused by genotype 1, whereas genotype 2 is commonly found in Mexico and West Africa (Nigeria and Chad). In industrialized countries, where until a few years ago hepatitis E was considered non-endemic, autochthonous cases appear to be related to HEV strains belonging to genotypes 3 and 4 (Emerson and Purcell 2003; Okamoto 2007; Kamar et al. 2012; Scobie and Dalton 2013), that are still considered the only zoonotic genotypes. Genotype 3 HEV was first identified in human cases locally acquired in the USA, when the two human strains, called US-1 and US-2, showed only 74–75% nucleotide identity with genotypes 1 and 2, being classified separately (Meng et al. 1997). Since then, genotype 3 has been detected throughout the entire world, associated with sporadic cases and small outbreaks in North America, Europe, Japan and New Zealand (Tsang et al. 2000; Mansuy et al. 2004; Dalton et al. 2007a, b). This genotype is also commonly detected in animals, and a strict genetic correlation has been observed between human and animal strains circulating in the same geographical area. The first animal strain of HEV was identified in swine in the USA in 1997. The virus was shown to belong to genotype 3, and presented a high identity with some human strains (Meng et al. 1997). In particular, the virus shared a 92% nucleotide identity in ORF2 with the two HEV genotype 3 autochthonous human strains (US-1 and US-2) detected in the same period in the USA. Given the strict genetic correlation, the two viruses were classified in the same genotype 3, and since then pigs have been considered a reservoir of HEV (Meng et al. 1997).

Recently, a study conducted in Japan identified a new potential reservoir of genotype 3 HEV virus in the mongoose. As revealed by phylogenetic analysis, the HEV RNAs detected belonged to genotype 3 and were classified into two groups, one of which contains sequences very similar to mongoose HEV previously detected in the same area as well as to HEV identified in a pig (Nidaira et al. 2012).

Genotype 4, the other genotype transmitted by the zoonotic route, is indigenous to Asia, where it has been recovered from both pigs and humans (Wang et al. 2012). Since its first report in China in 1999, genotype 4 has been increasingly described as being endemic in pigs and as the cause of sporadic cases of hepatitis E in humans and infection in the swine in China and Japan, and more recently in Europe (Hakze-van der Honing et al. 2011; Colson et al. 2012; Garbuglia et al. 2013). The question for the recent and increasingly frequent detection of genotype 4 in Europe is of concern for public health, and raises the question whether genotype 4 was somehow introduced into domestic pigs and may be expected to spread further on farms or whether the problem is to be correlated to importation of pig meat of Asian origin into Europe (Colson et al. 2012).
The recent availability of increasing numbers of HEV sequences emphasizes the genome diversity among HEV isolates. According to the nucleotide identity, the four genotypes were further subdivided into subtypes. HEV strains belonging to genotype 1 are more conserved and can be further classified into five subtypes (1a–1e). Genotype 2 sequences are classified into two subtypes (2a and 2b). Genotypes 3 and 4 are extremely diverse and are divided into ten (3a–3j) and seven subtypes (4a–4g), respectively. Thus, in total, at least 24 subtypes of HEV exist in nature (Lu et al. 2006). The diversity of genotypes 3 and 4 appears related to their zoonotic origin from a variety of animals in different parts of the world, whereas the relative conservation of genotypes 1 and 2 is consistent with their primary circulation in humans and a less frequent isolation from animals (Pavio et al. 2010).

Nevertheless, several studies have supported the existence of intra-host quasispecies in both humans (Grandadam et al. 2004) and swine (Bouquet et al. 2012b). A recent study conducted using next generation sequencing (NGS) confirmed the existence of intra-host quasispecies in experimentally infected swine, underlining that the range of quasispecies diversity is lower than for other human viruses, but is in line with other zoonotic viruses (Bouquet et al. 2012b). In human patients undergoing solid-organ transplantation with chronic HEV infection, the quasispecies diversification seems to be related to rapid development and progression of liver fibrosis, since patients had lower quasispecies diversification during the first year than had patients without liver fibrosis progression (Lhomme et al. 2012).

2.3 Viral Replication Cycle

Because cell culture and small animal models to investigate HEV infection was developed only recently, the viral replication and regulation processes involved are poorly understood, being mainly based on HEV genome analysis and homology with other positive-stranded RNA viruses. Nevertheless, the recent identification of various cell lines permissive to HEV replication (hepatic cell lines HuH7, PCL/PRF/5, HepG2; lung carcinoma cell lines A549; human hepatoma-derived cell line, HepaRG; porcine embryonic stem cell-derived cell line, PICM-19) (Okamoto 2011; Rogee et al. 2012) has significantly contributed to clarify some essential steps of the replicative cycle. Although no cellular receptors have been identified up to now, a role of heparin sulfate proteoglycans (HSPGs) in mediating virus attachment has been demonstrated (Kalia et al. 2009). The mechanisms by which the virus is released into the cytoplasm are unknown. Genomic RNA appears to be translated directly into the ORF1 polyprotein, even though it is unclear whether the polyprotein is or is not processed into individual functional units. The RdRp mediates the replication of the positive-sense genomic RNA into negative-sense RNA transcripts, that serve as a template for the synthesis of the full genome, and into the single 2.2 kb subgenomic RNA including the overlapping ORF2 and ORF3 that are translated in the capsid protein pORF2 and in pORF3. pORF2 is assembled in a capsid particle where the RNA genome
is packaged to construct the newly formed virion. Although the role of pORF3 remains largely unclear, this protein mediates virus budding likely based on the interaction of lipid-associated virions with plasma membranes or endomembranes (Ahmad et al. 2011). Binding of the cellular TSG101 (tumor susceptibility gene 101) to pORF3 through amino acid PSAP motif (i.e., amino acids proline, serine, alanine, and proline) has been demonstrated (Surjit et al. 2006). TSG101 has been identified as the critical protein for budding of enveloped viruses, such as the human immunodeficiency virus type-1 (HIV) from the plasma membrane (Martin-Serrano et al. 2001). It is likely that pORF3 mediates virus budding by recruiting the TSG101 (Okamoto 2011).
Hepatitis E virus (HEV) infection is mainly present in developing tropical and sub-tropical countries of most of Asia, North Africa, the Middle East, and Central and South America (Emerson and Purcell 2003; Panda et al. 2007) (Fig. 3.1).

In developed countries, hepatitis E was considered a disease strictly associated with travelling through endemic countries. However, in the last decade sporadic cases or small series of cases in subjects without history of travel abroad have been recorded in the USA, Europe (including the United Kingdom, France, the Netherlands, Austria, Spain, Italy, and Greece), and in developed countries of Asia–Pacific (Japan, Taiwan, Hong Kong, Australia), suggesting the presence of autochthonous reservoirs of hepatitis E virus in these areas.

In highly endemic areas, HEV strains belonging to genotypes 1 and 2 are responsible for most endemic and epidemic cases of hepatitis E. Infection is usually transmitted among humans by the fecal-oral route, and it has proven to cause large outbreaks, particularly when associated with the consumption of contaminated water.

The genotype 1 of HEV is mainly associated with infections in Asia and Africa (Fig. 3.2), whereas genotype 2 is represented by the prototype sequence from an epidemic in Mexico, 1986, and new variants were recently identified from endemic cases in some African countries. There is no known animal reservoir for HEV genotypes 1 and 2 (Scobie and Dalton 2013) except for a genotype 1 reported in horses (Saad et al. 2007).

By contrast, in non-endemic areas infection with HEV normally causes sporadic cases or small outbreaks, and other than in imported cases, it seems to be at least partially associated with zoonotic transmission (Pavio et al. 2010). The autochthonous cases in these areas, corresponding to industrialized countries, are related to genotype 3 and 4 strains (Fig. 3.2). Of these, HEV genotype 3 is more widely distributed globally, from the USA through European countries, to China and Japan. Whereas genotype 3 HEV infects a high number of animal species (pigs, deer, wild boar, mongooses, rodents, and others), genotype 4 seems to affect only humans and pigs in East Asia, apart from recently reported infection in both swine and humans in Europe (Hakze-van der Honing et al. 2011; Garbuglia et al. 2013).
3.1 Epidemiology of Human Infection in Regions with High Disease Endemicity

In these areas, the disease usually occurs with epidemic outbreaks that affect large parts of the population, and are often separated by intervals of a few years. These outbreaks have been observed in the Indian subcontinent, China, Southeast and Central Asia, the Middle East, and the northern and western parts of Africa. In North America (Mexico), two small outbreaks took place between 1986 and 1987, but no further outbreaks have since been reported (Aggarwal and Jameel...
Outbreaks are often large, and some reports account for outbreaks affecting thousands of people, particularly in China, India, Somalia, and Uganda (Zhuang et al. 1991; Naik et al. 1992; Bile et al. 1994; Teshale et al. 2010). Most reported outbreaks have been related to the consumption of drinking water which had been contaminated with human feces, and infection had clearly been transmitted through the classical fecal-oral route. The time course of outbreaks in highly endemic countries varies from a few weeks to more than 1 year, and outbreaks with a more extended duration are likely to be related to a persisting source of water contamination, such as sewage systems intersecting surface water reservoirs, lakes, rivers, or clean water pipelines (Kamar et al. 2012). Particularly, the large outbreaks frequently follow heavy rainfall and floods, which may abruptly determine the discharge of human excreta into the sources of drinking water. Nonetheless, some outbreaks have occurred in hot and dry months, possibly as a result of diminished water flows in rivers that may have led to an increased concentration of fecal contaminants. In Southeast Asia, recurrent epidemics have been shown to be associated with disposal of human excreta into rivers and subsequent use of water from the same river for drinking, cooking, and personal hygiene. Outbreaks of hepatitis E have occurred in underdeveloped urban areas with leaky water pipes passing through the soil, which was found to be contaminated by sewage. Intermittent water supply in these areas leads to negative pressure in pipes during periods with no flow, allowing inward suction of contaminants (Sailaja et al. 2009). Although the dissemination of HEV infection through contamination of food may be possible, few outbreaks related to food-borne transmission have been reported from hepatitis E endemic areas. Less frequent routes of transmission include contaminated food and transfusion of infected blood products (Aggarwal and Jameel 2011). Direct person-to-person transmission seems to be uncommon; however, in a large outbreak recently reported in Uganda household factors (e.g., hygiene measures, storage of drinking water in large-mouthed containers) have been considered to be significant for increasing transmission of infection (Howard et al. 2010).

Overall attack rates during hepatitis E outbreaks have ranged from 1 to 15 % (Aggarwal and Jameel 2011). The rates are higher among young adults (3–30 %), but the reason for this is not clear. Lower attack rates among children may be partially related to a higher proportion of asymptomatic infections at younger ages and not only to an authentic scarceness of infection in young individuals.

However, in most endemic areas, seroprevalence in children below 10 years of age is approximately 5 %, whereas the ratio rises to 10–40 % among adults over the age of 25 years (Emerson and Purcell 2003).

The disease can be long lasting. Men are clinically infected two to five times more frequently than women; this may be due either to their greater risk of exposure to contaminated water, or may indicate that men develop a symptomatic infection more frequently. During hepatitis E outbreaks, pregnant women have a higher disease attack rate, and are more likely to develop fulminant hepatic failure and die. Mortality rates reach 10–25 % in pregnant women (Boccia et al. 2006). However, once acute liver failure appears, the death rate is not different between
pregnant women with hepatitis E and those with severe liver injury of other causes. Immunological factors or hormones may be responsible for this different progression of the disease in pregnant women. In disease endemic areas, HEV infection accounts for a large proportion of cases of acute sporadic hepatitis. These latter patients share similar age distribution, severity and duration of illness, predisposition to worsened prognosis in the case of pregnant women, and absence of chronic sequelae with those involved in HEV outbreaks (Aggarwal and Jameel 2011).

In India, HEV infection is the most common cause of acute sporadic hepatitis, accounting for up to 70 % of all cases among adults. The route of transmission of infection in most patients with sporadic hepatitis E is unclear (Aggarwal and Naik 2009). However, given the low hygienic conditions in underdeveloped areas of India and other Asian countries, the major sources of infection are most likely water and food contaminated with human feces. Asymptomatic infections were estimated to exceed the number of symptomatic cases, by two to four times. HEV genomic sequences were detected in nearly 40 % of sewage specimens collected in a large Indian city throughout the year which clearly indicates the ubiquitous circulation of HEV through the population, even when no disease outbreak or symptomatic cases were recorded.

Unlike several other infections with fecal-oral transmission, person-to-person transmission of HEV from epidemic or sporadic cases is considered to be uncommon (Aggarwal and Jameel 2011). The exact reason for this is unknown, although differences in the minimal infectious dose able to cause overt disease and burden of virus shed in the stools by infected patients, or the environmental resistance of different viruses may play a major role (Emerson and Purcell 2003). The secondary attack rate observed among household contact of patients involved in hepatitis E outbreaks is normally much lower (0.7–2.2 %) than observed among susceptible household contacts of cases for hepatitis A (50–75 %).

The short time lapse normally observed between onset of symptoms in distinct family members during multi-case household outbreaks also supports a reduced person-to-person transmission, rather indicating a common primary source of infection (e.g., water, or food) (Aggarwal and Jameel 2011).

Maternal–fetal transmission of HEV infection has been reported, and the occurrence of HEV viremia among healthy blood donors and transmission of infection to transfusion recipients have been documented in regions endemic for hepatitis E (Haim-Boukobza et al. 2012). However, the impact of parenteral and blood-borne transmission to the overall disease burden remains uncertain.

3.2 Epidemiology of Human Infection in Regions with Low Endemicity

HEV infection is now thought to be endemic also in many industrialized countries. In Europe, the USA, and Japan, increasing reports of sporadic cases have been made in patients who had never travelled to foreign countries. Strains isolated in
these cases were demonstrated to be genetically different from strains isolated in other regions, leading to the hypothesis that they were related to viruses endemic to the specific country (van der Poel et al. 2001; Clemente-Casares et al. 2003; Takahashi et al. 2003). Autochthonous cases of hepatitis E have been detected in all developed countries where they have been searched for, with the exception of Finland (Kantala et al. 2009). Several studies have also reported high HEV seroprevalence rates (5–20 %) among healthy individuals in industrialized countries, suggesting a wide spread infection, which most likely occurs at a subclinical level (Emerson and Purcell 2003; Mansuy et al. 2011). The actual percentage of subjects seropositive to HEV might be proven to be even higher, when further studies are carried out using the more sensitive tests recently developed for anti-HEV antibody detection. Accordingly, the seroprevalence among blood donors in Toulouse (France) was shown to rise from 16 to 52 % using a modern assay with higher sensitivity than previously available assays (Kamar et al. 2012), that may obviously apply to other regions investigated in the past. Since large part of infections occurring in developed countries seem to be asymptomatic, this observation could explain the high seroprevalence rates opposed to the low number of cases reported. Differently than genotypes 1 and 2, infections caused by genotypes 3 and 4 HEV strains appear to cause clinical hepatitis in middle-aged subjects and the elderly. This unusual and peculiar demography also remains unexplained, since exposure to HEV is independent of age and sex, and suggests the presence of host risk factors that may be crucial for clinical manifestation of the disease. Moreover, the high mortality associated with pregnancy and genotype 1 HEV infections has not been reported with either genotypes 3 or 4 strains. Nonetheless, these latter have never been reported in such large outbreaks as genotype 1 HEV strains have, making absolute comparisons difficult with regard to pregnant women infected. In developed countries, the disease appears only as sporadic cases or small outbreaks, and a range of severity of illness has been described, from asymptomatic to acute or subacute liver failure.

In Italy, as an example of a modern country with high hygienic conditions, HEV infection is thought to account for approximately 5–10 % of cases of acute non-A–C viral hepatitis (Zanetti and Dawson 1994), which would lead to estimates of just few hundred cases per year. As in other developed areas, most cases occurring in Italy are associated with travel to endemic areas being reported on return from regions traditionally considered endemic, and the first identification of an autochthonous HEV dates back to 1999, when a genotype 3 virus similar to American strains was identified in a patient who had neither travelled to nor had contact with individuals associated with endemic areas (Schlauder et al. 1999). A recent detailed paper has attributed to HEV the etiology of 134 of 651 (20.6 %) cases of non-A–C hepatitis hospitalized during the period 1994 through 2009 in northern Italy (Romano et al. 2011). Of these patients, 22 (16.4 %) were established as being autochthonous, whereas the other 112 were imported or associated with travelers from Asia or Africa. Although molecular typing of HEV strains was possible for only five and 39 cases, respectively, it is quite remarkable that only the five indigenous strains were genotype 3 HEV whereas the imported cases
were all related with genotype 1 strains. Similar findings have been found in other studies throughout Europe (Kamar et al. 2012). More recently, rare human cases linked to genotype 4 HEV strains, normally endemic in Asia in both humans and pigs (Howard et al. 2010; Wang et al. 2012) have also been reported in Europe (Wichmann et al. 2008; Tesse et al. 2012; Garbuglia et al. 2013), but it was not concluded whether any of these cases may have originated via zoonotic or foodborne transmission. Nonetheless, these findings make it obvious to ask whether genotype 4 may become widespread in Europe, as is presently genotype 3 HEV.

### 3.3 Routes of Transmission

The actual modes of HEV transmission in sporadic cases are still not completely understood; in addition to the ingestion of contaminated water and food, and person-to-person transmission, vertical transmission of virus from mother to infant is known to occur, while there is no evidence of sexual transmission (Kamar et al. 2012; Scobie and Dalton 2013). The possibility of HEV transmission by transfusion of blood or blood products has been documented, but its significance is still not clear (Khuroo et al. 2004; Matsubayashi et al. 2008; Haim-Boukobza et al. 2012). Zoonotic transmission has been assessed, although the extent to which this route impacts with spreading of infection is uncertain. Risk factors can include the direct or indirect contact with infected material from affected animals (professional categories such as veterinary surgeons, farmers, slaughterhouse workers, people assigned to the care of the animals may be at risk), the ingestion of food directly or indirectly contaminated (water, plants, meat products, shellfish), and parenteral transmission (Meng 2003) (Fig. 3.3).

#### 3.3.1 Waterborne Transmission

The knowledge that surface and drinking water are a major vehicle by which HEV can massively diffuse through the population is well consolidated since four water-related epidemics occurred in Kashmir between 1978 and 1982, causing over 50,000 cases of acute liver disease and approximately 1,700 deaths (Khuroo 1991). Reports of water-related hepatitis E have concerned other countries with low sanitary conditions, water quality, and overcrowding, sometimes also in relation with political instability and war situations (Rab et al. 1997; Guthmann et al. 2006; Guerrero-Latorre et al. 2011). Heavy rains and flooding, fecal contamination of surface water reservoirs, wells, and rivers, together with unfitting water pipelines and sewage disposal networks, have all been involved in hepatitis E epidemics. In Southeast Asia, occurrence of hepatitis E outbreaks appeared to be strictly related to the use of river water for drinking, cooking, and personal washing (Razonable et al. 2011). Epidemiological evidence,
therefore, strongly suggests that HEV can persist in environmental water, but no systematic study has been performed to define the kinetics of virus infectivity decay in the environment.

### 3.3.2 Foodborne Transmission

There is already some clear evidence that links onset of hepatitis E to the consumption of contaminated food items, resulting in either sporadic cases or epidemic outbreaks. The first reports regarded endemic Asian countries. In Japan, analysis of risk factors and molecular characterization of HEV from 10 patients with fulminant hepatitis E showed that the patients had eaten grilled or undercooked pig liver 2–8 weeks before onset. Some of the HEV RNA sequences found in clinical specimens were identical or similar to HEV detected in packaged pig liver sold in the market or farm swine samples (Yazaki et al. 2003). This study also showed that approximately 2% of pig livers sold at retail in the area contained detectable viral RNA. Further observations confirming the association between pig liver or grilled pork consumption, wild boar, or deer meat, and hepatitis E were reported in the next few years in Japan (Matsuda et al. 2003, 2005; Takahashi et al. 2004; Li et al. 2005b), and other countries (Gessoni and Manoni 1996; Dalton et al. 2007b; Colson et al. 2010; Widen et al. 2011; Bouquet et al. 2012b).

Presence of HEV in food, primarily pig liver or pork, was confirmed in the USA (Feagins et al. 2007a), the Netherlands (Bouwknecht et al. 2007), UK (Berto
et al. 2012b), Italy, Spain, and the Czech Republic (Di Bartolo et al. 2012), and a recent study performed in France by cell culture techniques confirmed that HEV present in pork liver sausage is infectious, highlighting the actual risk for consumers (Berto et al. 2013a).

In addition to meat from infected animals, the use of HEV containing pig manure or animal or human waste contaminated water for land application and crop field irrigation may lead to contamination of other foodstuff, such as produce or, by run off into rivers and coastal waters, shellfish and eventually cause disease among consumers (Renou et al. 2008; Song et al. 2010; Razonable 2011; Halac et al. 2012). A recent multi-laboratory investigation conducted in three European countries demonstrated that leafy green vegetables intended for the market were contaminated with HEV, finding that 3.4% of 146 samples tested positive by RT-qPCR (Kokkinos et al. 2012). In contrast to norovirus and other enteric pathogens, data on possible association of hepatitis E cases with consumption of vegetables and berry fruits are lacking, which might indicate that residual HEV concentration in fresh produce may be under the limit needed to start productive infection in humans.

### 3.3.3 Professional Exposure Transmission

Human populations with occupational exposure to environmental sources of domestic animal wastes and wild animals (farmers, veterinarians, slaughterhouse personnel) have been shown to present higher anti-HEV serum antibody rates than normal blood donors or normal citizens in several studies particularly associated with the pig industry. For instance, in the USA 26% of veterinarians were found to be seropositive to HEV compared to 18% of regular blood donors (Meng et al. 2002), and HEV antibody prevalence was reported to be 4.5 times higher in subjects exposed to contact with pigs than in normal people (Withers et al. 2002). But similar data are available for several countries worldwide (Karetnyi et al. 1999; Meng et al. 2002; Chang et al. 2009; Masia et al. 2009; Geng et al. 2011b).

A recent investigation on forestry workers in Germany has reported a significantly higher seroprevalence against a recombinant genotype 3 HEV capsid protein fragment that reasonably suggests workers’ exposure during their activity to virus probably shed by wild animals (Dremsek et al. 2012). Interestingly, a few among the forestry workers’ sera also gave a strong reaction against a recombinant capsid protein from the HEV strain recently detected in rats. It is unclear how humans may have come in contact with this novel virus in order to elicit an immune response (Dremsek et al. 2012). Infectious swine hepatitis E virus was demonstrated to be present in pig manure storage facilities in farms in USA, but evidence that this may culminate into contamination of surrounding waters, although reasonable, was lacking in this study (Kasorndorkbua et al. 2005).

Conversely, both swine and human HEV strains have been reported to be present and infectious in raw sewage water in several countries (Jothikumar et al.
In particular, sewage workers were shown to have a significantly higher anti-HEV seroprevalence than normal individuals in India, which increased with the numbers of years spent in that job (Vaidya et al. 2003), highlighting a specific occupational risk.

### 3.3.4 Other Routes of Transmission

As mentioned before, there is no clear demonstration sustaining that direct viral transmission of HEV from person to person is indeed an efficient method for transmitting infection. However, virus spread from infected individuals by fecal shedding in the environment inside confined areas or via contaminated fomites might play a role, especially in conditions of promiscuity and overcrowding. This might explain the difference observed in anti-HEV seroprevalence between northern and southern regions of Italy (Zanetti and Dawson 1994). In fact, the latter routinely face high immigration fluxes from countries where hepatitis E is endemic (Cacopardo et al. 1997; Scotto et al. 2013), and immigrants reside in these areas for several weeks interacting with the local population. However, the habit of eating raw shellfish common in southern Italy could also be considered an additional risk factor, possibly also favored by HEV discharge by infected immigrants into sewage and by following coastal seawater pollution.

A marked gradient of anti-HEV seroprevalence was also shown from north to south France, but in this case the risk factors seemed to be several, including personal water supplies, fresh seafood consumption, and possession of pet pigs (Renou et al. 2008). Nonetheless, HEV circulates largely among pigs in southern France where the habit of consuming a particular fresh liver sausage (figatellu) is particularly marked (Colson et al. 2010), thus particular food eating habits might have at least partially contributed to the observed epidemiological differences.

A mix of transmission modes acting simultaneously may have fed the unique outbreaks involving passengers in a ship returning from a world cruise in 2008, causing overt hepatitis E with jaundice in four patients and IgM seroconversion in 4% of the 789 subjects tested (Said et al. 2009). Overall, 25% of passengers showed anti-HEV IgM and/or IgG, indicating both recent and past infections. The virus detected in patients was a single genotype 3 HEV similar to strains circulating in Europe, suggesting a common source of infection, and seafood consumption was a risk factor. However, from the experience built from norovirus outbreaks aboard cruise ships, it cannot be excluded that virus transmission was favored by other routes, such as water or environmental contamination within common areas.

Differently than other systemic viral infections (such as HIV, HBV and HCV), diverging data exist on the association between HEV transmission and injection drug use (IDU). Specific seroprevalence ranged from 2 to over 60% in different countries, although significant differences between IDU subjects and healthy blood donors have not been observed in all cases (Gessoni and Manoni 1996;
Thomas et al. 1997; Kaba et al. 2010a). This might be related to either low viral load in the blood or transient viremic status in the case of HEV, which might explain the higher impact of blood transfusion or organ transplantation as a risk factor for hepatitis E (Thomas et al. 1997; Khuroo et al. 2004; Boxall et al. 2006; Colson et al. 2007; Razonable 2011; Halac et al. 2012).

Finally, vertical transmission from mother to fetus has been reported frequently resulting in the death of the fetus (Khuroo et al. 2009; Aggarwal 2011).

3.4 Evidence of Zoonotic Transmission

Since the early 1990s, HEV antibodies have been detected in the sera of a variety of animals, such as monkeys, pigs, rodents, cattle, sheep, poultry, dogs, and cats, in both developed and developing countries (Wang et al. 2002; Chang et al. 2009; Meng 2010a; Wang and Ma 2010). Early after the discovery of HEV-like viruses in pigs in 1997, and a few years later in rodents (Favorov et al. 2000; Peralta et al. 2009b), the existence of endemic animal reservoirs was questioned with respect to the sporadic cases reported in humans in industrialized countries. Therefore, the possibility that the viruses causing human hepatitis E might be similar to animal strains and infect other species started being investigated by comparative molecular characterization of strains of different origin.

Presently, it is known that persons working in close contact with animals of species susceptible to HEV infection frequently result positive at testing for anti-HEV antibodies (Karetnyi et al. 1999; Meng et al. 2002; Chang et al. 2009; Masia et al. 2009). The ability of genotype 3 HEV strains to cross species barriers has been supported largely (Meng 2010a), and the foodborne transmission of animal viruses from pork, wild boar, and deer has been confirmed by a series of different evidence, as addressed in detail above.

Today, cross-species passage is thought to represent an important mode of transmission for zoonotic genotype 3 HEV, and may in fact be the main source for autochthonous HEV infection cases in North America and Europe (Meng 2010a; Pavio et al. 2010).

The ability of HEV to cross the species barrier has been confirmed by experimental infections of SPF (Specific Pathogen Free) piglets with a human genotype 3 virus, and by similar demonstration that swine genotypes 3 and 4 strains are able to infect non-human primates (Meng et al. 1998b; Arankalle et al. 2006; Ji et al. 2008), in support of the observations that genotype 3 strains typically detected in swine can naturally infect humans (Pavio et al. 2010). These viruses apparently cause mild or subclinical infection in primates, but also considering the high genetic variability within HEV, the possibility that specific strains within animal genotypes 3 and 4 are more virulent than others cannot be excluded. Since the molecular basis for host-pathogen interaction in HEV is still largely unknown, strains that normally present lower virulence might lead to a more severe course of disease in particular host conditions (Bouquet et al. 2012a).