

Hepatitis E virus: an emerging zoonotic agent

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Summary

Hepatitis E is an infectious viral disease with clinical and morphological features of acute hepatitis. The aetiological agent is the hepatitis E virus (HEV). The disease represents an important public health problem in developing countries where it is frequently epidemic and is primarily transmitted by the faecal-oral route. In recent years, a number of sporadic cases have also been described in industrialised countries, Italy included. Swine HEV was first identified in 1997 and is now considered a ubiquitous virus. Human and swine strains from the same region have been shown to have a high level of nucleotide homology and, in experimental infections, the possibility of cross-species transmission of swine strains to humans and of human strains to non-human primates has been demonstrated. Furthermore, some seroepidemiological studies have demonstrated that people working in contact with swine have a higher risk of infection than regular blood donors. Recently, cases of HEV hepatitis in Japan were directly associated with the ingestion of uncooked meat from pigs, wild boar or deer and today the disease is considered an emerging zoonosis. The authors summarise current virological and epidemiological knowledge on HEV infections so as to stimulate interest in a virus that has not received much attention in veterinary medicine, but that could become an important zoonotic agent.

Keywords

Animal diseases – Hepatitis E – Pigs – Public health – Viruses – Zoonoses.

Introduction

Hepatitis E, previously known as enterically transmitted non-A, non-B, non-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 who can suffer mortality rates reaching 20% (1). The aetiological agent, identified only in the early 1980s, is the hepatitis E virus (HEV) (14). The disease is an important public health concern in developing countries (South-East and Central Asia, the Middle East, northern and western areas of Africa and North America) where it is frequently epidemic and is mainly transmitted by the faecal-oral route usually through contaminated water or food (1, 14, 58). Previously, industrialised countries and areas, such as Canada, Europe, Japan and the USA were thought to be HEV-free, with few cases reported only in people who had travelled to endemic areas. However, more recent studies have documented a number of sporadic cases in developed countries, including Italy, among patients who had not travelled to endemic areas (1, 14, 37, 63). Furthermore, a high anti-HEV seroprevalence (in some cases reaching 20%), has been detected in a significant proportion of healthy individuals of those countries once thought to be HEV-free (1, 14, 58). In the veterinary field, since the early 1990s, HEV antibodies have been detected in the sera of many

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animals, such as monkeys, pigs, cattle, sheep, poultry, dogs, cats and rodents, both in developed and developing countries, suggesting the possibility that these species may become infected by HEV-like viruses (1, 2, 14, 25, 37). At that time, the possibility of animals acting as a reservoir of infection was raised, together with the hypothesis that some of the sporadic cases reported in the literature may have been of zoonotic origin (37).

In 1997, a swine HEV virus was identified for the first time. The virus was named swine hepatitis E virus (swine HEV) (37). This swine virus was demonstrated to be genetically and phylogenetically correlated to two HEV human strains isolated in the USA from patients affected by hepatitis E, but who had not travelled to endemic areas (37). Since then, swine HEV strains have been isolated across the globe. Frequently, a strict nucleotide homology 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 (3, 22, 35, 36, 37, 38, 59). Furthermore, some seroepidemiological surveys have reported high HEV antibody prevalence in people working in direct contact with swine (12, 34, 45, 60). The direct evidence of the possibility of zoonotic transmission of this virus was provided by 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 days before the onset of the clinical signs (33, 51, 52, 61). The disease is now considered an emerging zoonosis.

The virus

The HEV is a small (27-34 nm), icosahedral non-enveloped single-strand positive-sense RNA virus (1). The virus was first identified in 1983 and between 1988 and 1998 was classified in the

Caliciviridae family. Recently, studies of non-structural regions have shown significant differences with other caliciviruses and for this reason the virus has been removed from that family and is now classified as a new genus called *Hepevirus* (14, 58). The HEV genome is of approximately 7.5 kb, containing a short non-coding region 5' (27-35 nucleotides) 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. ORF1 (5073-5124 nucleotides) encode for a non-structural polyprotein of about 1690 amino acids that is involved in viral genome replication and viral protein processing. In addition, ORF1 has two regions called Y and X domains, the functions of which remain unknown. ORF2 (1977-1980 nucleotides) encode for a capsid protein of 72 kDa containing 660 amino acids, while ORF3 (366-369 nucleotides) encode for a small 123 amino acid protein (pORF3) which is expressed at the intracellular level. Recent studies of the biology of HEV replication have now shown that pORF3 may be capable of associating with the liver cell cytoskeleton, serving as an anchor site (58).

To date, HEV has not been grown reproducibly or efficiently *in vitro*, so diagnostic techniques and information on characterisation of the strains have been mainly based on the analyses of the viral RNA using biomolecular technologies (14). Detected HEV strains are currently genetically characterised on the basis of ORF regions. Four major genotypes have been identified to date, although the picture is constantly evolving and the consensus on this classification is not unanimous (58). Despite the diversity of the HEV genotypes, the virus seems to exist as a single serotype (14, 58).

The majority of infections that occur in Asia and Africa are caused by genotype 1, while in Mexico

and Nigeria genotype 2 prevails. In industrialised countries, where until a few years ago the infection was considered non-endemic, only strains of genotypes 3 and 4 have been described (1, 14, 15, 58, 62). Genotype 3 prevails in the USA while genotype 4 is prevalent in the People's Republic of China and Taiwan (2, 26, 58). Genetically heterogeneous isolates from several European countries have been designated as new genotypes, but should probably be grouped with the US isolates into a large, heterogeneous group (58).

The first HEV animal strain was identified and characterised in the Midwestern regions of the USA in 1997. The virus, named swine hepatitis E virus or swine HEV, belonged to genotype 3 and presented a high homology with some human strains (37). In particular, the virus shared a 92% nucleotide homology and a 97% amino acid homology in ORF2 with two HEV US autochthonous human strains (US-1 and US-2 strains). Given this significant homology, the two viruses have been considered part of the same family and pigs are now considered an alternative animal model for studies on human HEV (37). Since 1997, other swine HEV strains, all belonging to genotype 3 or 4, have been identified in many industrialised countries and they have often proved to be closely related genetically to strains causing sporadic disease outbreaks in humans (3, 5, 9, 15, 18, 23, 26, 37, 38, 41, 48, 49, 50, 57). As with the human strain, those of swine are usually genetically different from region to region (10, 14, 38, 49, 50, 62). Recently, HEV viruses have also been isolated from rodents and chickens (24, 25). In Nepal, phylogenetic analysis of murine HEV isolates have demonstrated the existence of a strict correlation between HEV viruses of human and rodents, each having a 95-98% nucleotide homology and a 98% amino acid homology (25). In contrast, avian hepatitis E virus is genetically related but distinct from the other

HEV strains and has been associated with the chicken hepatitis-splenomegaly syndrome (HS) (24). HS syndrome was first reported in 1991 in western Canada and then in the USA. The disease is characterised by increased rates of mortality among broilers and laying chickens and was responsible for decreases in egg production of up to 20%. Regressive ovaries, red fluid in the abdomen and enlarged liver and spleen with histological changes of hepatic necrosis and haemorrhaging were often reported in infected chickens (47). The avian virus is also experimentally infectious for turkeys but, unlike swine HEV, cannot be transmitted to monkeys (27, 47). Avian HEV shares approximately 40-60% nucleotide sequence identities with known human and swine HEV strains although specific antibodies are able to cross-react with the capsid protein of both viruses, demonstrating the presence of common epitopes (23, 24, 27, 47). At present, it is not clear if this agent represents a new HEV genotype or should be considered a more distant parent of human and swine viruses.

Human HEV infections

Epidemiology

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 (1, 14, 37, 58). In these areas, the disease usually occurs with epidemic outbreaks affecting large parts of the population. The disease can be long-lasting with overall attack rates ranging from 1% to 15%, with the higher rates occurring among adults (1). In most endemic areas, seroprevalence in children below 10 years of age is approximately 5%; this ratio rises to 10-40% among adults over the age of 25 (1, 14). HEV outbreaks frequently follow heavy rains and floods, when water sources became contaminated (1, 14). Person-to-person transmission appears to be

uncommon, with secondary attack rates among household members being less than 5% which is much lower when compared to those of hepatitis A virus (50-75%). This could be due to differences in the number of viral particles required to cause disease, differences of the virus titres in stools of infected patients, or variability of the virus to persist in the environment (1, 14).

HEV infection is now thought also to be endemic in many industrialised countries (28, 34). In Europe, Japan and the USA increasing reports of sporadic cases have also been made in patients who had never travelled to foreign countries (8, 10, 14, 15, 37, 48, 56, 58, 61, 63). Strains isolated in these cases were demonstrated to be genetically different from strains isolated in other regions, leading to the hypothesis that these cases had to be due to viruses endemic to the territory (1, 7, 14, 26, 37, 38, 44, 58, 62). Many seroepidemiological studies have also reported high anti-HEV seroprevalence (5-20%) in a significant proportion of healthy individuals of industrialised countries suggesting well spread infection, although generally at a subclinical level (1, 2, 14, 37, 41, 44, 48, 58). The high level of hygiene in these regions probably prevents the occurrence of epidemics observed in developed countries and for this reason the disease appears only sporadically. Modes of 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 from mother to infant is known to occur, while there is no evidence of sexual transmission (1, 14). The possibility of transmission by transfusion of blood or blood products has been documented, but its significance is still not clear (1, 14, 17). Zoonotic transmission has now been assessed (52). 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 could be at risk), the ingestion of directly or indirectly contaminated food (water, plants, meat products, shellfish) and xenotransplantation (37).

In Italy, HEV infection accounts for approximately 10% of the cases of acute non-A, non-B, non-C viral hepatitis (63). Most of the cases are associated with travel to endemic areas and are reported in travellers returning from areas traditionally considered endemic. However, in 1999, a new HEV variant strain was identified in the faeces of a patient who had never travelled to or had contact with individuals associated with endemic areas (63). This strain was genetically different from those isolated in other countries, showing a relative nucleotide homology only with American strains of genotype 3 (44, 63) and was consequently considered an autochthonous Italian strain.

Serologically, the presence of anti-HEV antibodies have been detected in many different regions of Italy (4, 6, 11, 17, 19, 20, 40, 43, 46, 64), with prevalences ranging from 1% to 5%.

Higher seropositivity rates have been found among haemodialysed patients, intravenous drug users and people positive to other post-transfusion viral hepatitis markers. A gradient of positivity between northern and southern regions has also been detected (63).

The higher prevalence in southern Italy is probably due to the proximity with countries in which the disease is endemic and to the high immigration levels from these areas; the common habit in southern Italy of eating raw shellfish could also be considered an additional risk factor (4).

Pathogenesis

Several elements of pathogenesis have been outlined on the basis of data from experimentally infected non-human primates. Infection can be transmitted by either the intravenous or oral routes; the latter,

which in most cases is the natural infectious route, requires an infectivity titre 10 000 higher than the intravenous route (1, 14). The incubation period after oral exposure is about four to five weeks. The route and mechanism by which the virus reaches the liver from the intestinal tract remain unknown. Once in the liver, the virus replicates in the cytoplasm of hepatocytes, accumulates in the bile and is subsequently shed in the faeces. Viraemia commences when the virus is already detectable in the liver (1). It is not known whether there are extra hepatic sites of replication and if the entire virus in the faeces originates in the liver or if the virus also replicates in the intestinal tract. Viraemia and faecal shedding are detected prior to liver abnormalities which normally appear in conjunction with humoral immune response and are characterised by an elevation of transaminases (1). The HEV can be detected in stool commencing approximately one week before the onset of illness and can persist for as long as two weeks thereafter. Virus-RNA can be detected in the serum of most patients with acute hepatitis E for approximately two weeks but, in some cases, for as long as 16 weeks after the onset of disease (1). The mechanism that causes liver damage is not clear, but is probably more immune-mediated than a result of direct action of the virus which is not considered cytopathogenic. This hypothesis is supported by the fact that, during the course of the disease, infiltrating lymphocytes in the liver have been found to have mainly a cytotoxic/suppressor immune phenotype (1). IgM anti-HEV appears during the early phase of clinical illness and disappears rapidly over four to five months. During an epidemic, IgM have been detected in more than 90% of serum samples obtained from patients within one week to two months after the onset of illness. The IgG response appears shortly after the IgM response, and its titre increases throughout the acute phase into the

convalescent phase, remaining high from one to four years after the acute phase of illness. The exact duration and persistence of anti-HEV antibodies is not known, but IgG have been detected up to 14 years post infection (pi) (1, 14).

Clinical features

Clinical features of hepatitis E can be very different. Acute icteric hepatitis, the most common recognisable form of illness, has an initial prodromal phase lasting a few days, with a variable combination of flu-like symptoms, fever, mild chills, anorexia, nausea, abdominal pain, vomiting, diarrhoea, arthralgia, asthenia and a transient macular skin rash. These symptoms are followed within a few days by darkening of the urine, lightening of the stool colour and the appearance of jaundice. With the onset of jaundice, fever and other prodromal symptoms tend to diminish rapidly and soon disappear entirely. Laboratory test abnormalities include bilirubinuria, variable degrees in the rise of serum bilirubin (predominantly conjugated) and a marked elevation in hepatic enzymes. As the illness subsides, this is usually self-limiting and typically lasts between one and four weeks, the haematochemical abnormalities start receding, gradually reaching normal values (1). Hepatitis E is an acute disease; no evidence of chronic hepatitis or cirrhosis has been reported to date. However, a few patients can suffer «prolonged» clinical illness with cholestasis, persistent jaundice and prominent itching. In these cases, laboratory tests show a rise in alkaline phosphatase and a persistent bilirubin rise even after transaminase levels have returned to normal. The prognosis is good as jaundice finally resolves spontaneously after two to six months (1, 14). However, a small proportion of patients have a more severe disease with fulminant or subacute hepatic failure, sometimes leading to death of the subject. In contrast, many infected individuals exhibit a milder clinical course and develop only

flu-like symptoms. In these patients, liver involvement is recognised only if laboratory studies are performed (1).

The case-fatality rate in many reports has ranged from 0.07% to 2%, which is slightly higher than that of hepatitis A. Pregnant women, particularly those in the second and third trimesters, are more frequently affected during hepatitis E outbreaks and the outcome is worse, with mortality rates ranging between 15% and 25%. Abortions, stillbirths and neonatal deaths have also been reported. The reason for particularly severe liver damage in pregnant women with hepatitis E is not known (1). This does not appear to be related to the poor health conditions frequently observed in developing countries, as the lethality rate in pregnant women in industrialised countries is equally high.

In its most benign form, HEV infection is entirely unapparent and asymptomatic and passes unnoticed. The precise frequency of asymptomatic infections is not known, but probably far exceeds that of icteric disease as, in endemic areas, a large proportion of individuals who test positive for anti-HEV antibodies do not recall having had jaundice (1, 14).

From a pathological point of view, the most common form is a cholestatic-type of hepatitis, which is characterised by canalicular bile stasis and a gland-like transformation of parenchymal cells. In other patients, changes resemble those of other forms of acute hepatitis with regressive disseminated alterations of the hepatocytes (apoptosis, presence of ballooned hepatocytes and acidophilic cells, steatosis, focal hepatocyte necrosis), marked cholestasi with or without proliferation of bile ducts, Kupffer cells hypertrophy with accumulation of bile, inflammation of portal tracts with infiltration of neutrophil, macrophages and lymphocytes (1, 14).

Specific therapy against HEV is not currently available and a vaccine does not exist, although studies to develop one are currently in progress.

For these reasons, prevention is all the more important (58).

Swine hepatitis E infections

Epidemiology

Swine HEV infections are spread both in industrialised and developing countries where for many years the disease has been considered endemic in humans (28, 34). Since the disease was first identified in 1997, several other swine strains have been isolated in North and Central America, Asia, Europe, New Zealand and Australia (2, 5, 8, 9, 10, 18, 26, 37, 41, 42, 49, 50, 56, 57, 62). These isolates always belong to genotype 3 or 4 and, as for human strains, show a high degree of nucleotide and phylogenetic divergence from region to region. Swine strains, particularly those isolated in industrialised countries, have often been related to human cases of disease in which the source of infection was unknown (3, 8, 16, 23, 37, 38, 49). In countries where the virus has been identified and serological studies have been performed, most pigs over the age of three to four months have been shown to possess HEV-antibodies (2, 5, 8, 9, 10, 18, 26, 34, 37, 41, 42, 49, 50, 56, 57, 62). Seroprevalence changes, depending on geographic region and the age of the animals (swine younger than two months are usually seronegative or slightly positive while seropositivity values in pigs over that age often exceed 80%). The approximate estimated prevalence rates (2, 34) are as follows:

- USA and New Zealand: 91-100%
- Australia and Canada: 60%
- China: 30%
- The Netherlands: 25%
- Spain: 75%
- United Kingdom: 65-85%
- Germany: 75-80%.

If the possibility of zoonotic transmission is accepted, it appears clear that the higher the prevalence in

animals, the greater the risk of transmission to humans. In this perspective, the above prevalence data can give rise for concern. In Italy, unlike other countries, information on the presence and prevalence of the infection in pigs is not currently available.

Pathogenesis

The faecal-oral route is thought to be the principal mode of transmission. There is no evidence of vertical virus transmission (35, 36). Experimentally, transmission of the infection from infected to in-contact uninfected animals has been demonstrated, thereby confirming the contagiousness of the virus (31, 35, 36). Experimentally, faecal-oral transmission occurs but is difficult to reproduce and may require repeated virus exposure.

For this reason, the intravenous route is usually preferred (31, 35). As is the case for humans, is still not clear how the virus, once it has penetrated inside the host, reaches the liver and which are the primary replication sites. Using the polymerase chain reaction (PCR) or *in situ* hybridisation in animals experimentally infected by the intravenous route, it is possible to detect viral RNA in several extra-hepatic tissues, also in the absence of viraemia, up to 20-27 days *pi*.

However, the negative strand form of the virus, which is the replicative form, has not only been detected in the liver but principally in the intestine and in the lymph nodes (7, 59). Viraemia can last for about two weeks, but the virus can be detected in the faeces for longer (about three to four weeks). Seroconversion occurs about two to three weeks *pi* (22, 35, 36, 59). Tissues in which the virus first replicates and persists (from 3 to 27 days *pi*) are the liver, small intestine, colon and lymph nodes (22, 59). These observations, together with the fact that during experimental infections viral RNA can be detected in faeces earlier than in the bile and in quantities that are tenfold higher, have led to

the speculation that, after entering by the oral route and before inducing viraemia, the virus replicates in the gastrointestinal tract (35, 36). Virological studies conducted on serum and faeces samples from swine herds have shown that HEV RNA can be primarily detected in pigs of two to five months of age, while generally, animals younger than two months and older than six to eight months are negative (2, 8, 10, 26, 37, 41, 42, 49, 50, 56, 62). Considering these observations and given that maternal immunity is thought to last about two months, natural infection is thought to occur at approximately two to three months of age (22, 29, 37). Viraemia follows initial infection, lasting for one to two weeks and then the virus is excreted in the faeces for about three to four weeks (at three to five months of age) with subsequent seroconversion and clearing of the infection by the immune system (23, 26, 35, 36). HEV infection would therefore be relatively short and would eliminate itself in just few weeks (37, 61).

From a serological point of view, passive maternal immunity has been shown to decline after three to four weeks of age and to terminate after eight to nine weeks. However, as previously mentioned, it is uncommon to find seropositive animals below the age of one to two months, probably due to the low sensitivity of the serological tests used (37). After infection, pigs develop an immune response against the virus characterised by an early IgM response, followed after about a week by an increase of the IgG.

IgM decreases rapidly (in one to two weeks), while IgG rises constantly for several weeks. Seroconversion occurs after the viraemic phase at approximately three to four months of age (antibodies peak at four months) and animals show high serological titres until five to six months of age when the IgG titre slowly starts to decline (23, 26, 37, 59, 62).

Clinical features

HEV virus appears to be attenuated and not particularly virulent for domestic swine, while there is no information available on the virulence in wild boars. The study of the disease in naturally or experimentally infected pigs has shown that HEV normally leads to subclinical infections with signs of hepatitis detectable only histologically (22, 35, 36, 37, 59); this is characterised by mild multifocal sinusoidal and periportal lymphoplasmocitary infiltrations and small areas of vacuolar degeneration and necrosis of hepatocells (22, 32, 35, 36, 37, 59). In some cases, lymphoplasmocitary enteritis and a multifocal interstitial lymphoplasmocitary nephritis have been detected (32). In experimentally infected animals, macroscopic lesions, such as mild to moderate enlargement of the mesenteric and hepatic lymph nodes, have sometimes been detected (22). It is of interest to note that human strains experimentally inoculated in pigs usually lead to more severe histopathological lesions than swine strains (22, 35).

Some authors have suggested that the virus in pigs could behave in the same way as the human hepatitis A virus that is virulent only in adults and not in juveniles (36, 37). This means that the disease would not develop clinically because the majority of the adult pigs would be protected from infection, having encountered the virus at a juvenile stage, thus having developed protective active immunity (37).

In a recent study, twelve seronegative gilts were inoculated intravenously with a swine HEV strain and no clinical signs or fever were observed in the inoculated gilts or their foetuses during the experimental period. Mild multifocal lymphohistiocytic hepatitis was observed in four of twelve inoculated gilts while there was no significant effect of swine HEV on foetal size,

viability, offspring birth weight or weight gain (29).

It is thought that HEV, although initially appearing as a mild pathogen, may be able to act in synergy with other viral agents, such as porcine circovirus type 2 (PCV2), leading to a severe disease (13).

Information on the possible control strategies of HEV infection on swine farms is not presently available. However, given the analogy with human beings where HEV antibodies seem to be protective (58), the possible use of indirect control strategies to prevent the virus from spreading within herds and to minimise risks for human health, should not be overlooked in the future.

Evidence of zoonosis transmission

Commencing in the early 1990s, HEV antibodies were detected in the sera of many animals, such as monkeys, pigs, rodents, cattle, sheep, poultry, dogs and cats in both developed and developing countries (1, 2, 14, 25, 26). Therefore, already at that time the possibility that that human HEV or HEV-like viruses might infect other animal species was seriously questioned. In 1990, using a HEV strain isolated in central Asia from affected patients as inoculum, Usamanov *et al.* (54), were able to experimentally transmit the virus in Large White piglets by intravenous injection. Later, the same research group (55) demonstrated the ability of the virus to spread between piglets although they failed to sequence and characterise the inoculated strain. Since 1995, in endemic areas such as Nepal, human HEV strains have been isolated from the faeces of domestic swine living in close contact with people infected by HEV (9). Following the discovery of HEV-like viruses in pigs in 1997 and a few years later in rodents and chickens (24, 25, 37), the existence of endemic animal reservoirs was questioned and the query raised that some of the sporadic cases in humans reported in industrialised countries may have been of zoonotic

origin. The ability of HEV to cross the species barrier has now been confirmed by experimental infections that have demonstrated that human strains are able to infect non-human primates and that swine strains can infect humans (22, 35, 36, 59). Data available today indicate that all swine strains capable of infecting monkeys belong to genotype 3. These viruses cause subclinical infection, but considering their high genetic variability, some authors do not exclude the possibility that certain strains are more virulent than others. It is also thought that in certain host-related conditions, strains that are normally of lower virulence could lead to a more severe course of disease (22, 35, 36, 41). Other important evidence that supports the possibility of zoonotic transmission of the disease has come from the phylogenetic analysis of human and swine strains isolated in different regions of the world. Many studies have reported strong nucleotide and amino acid similarities between human and swine strains from the same geographic region (2, 8, 16, 23, 26, 37, 38, 41, 49, 57, 59, 62). Recently, a HEV strain isolated from a patient in the UK presented a 100% amino acid identity with two swine strains present in that country (3). Further confirmation of the hypothesis of swine being a probable reservoir of the infection has come from seroepidemiological studies (12, 26, 34, 45, 60) conducted in Greece, Moldova, Taiwan and the USA. In these studies, higher HEV seroprevalence than that found in control populations was detected in people who worked in contact with pigs (swine farmers, swine veterinarians, slaughterhouse workers, people attending animals, swine dealers). In the USA, Meng *et al.* (34) found that 26% of veterinarians are seropositive to HEV compared to 18% of regular blood donors. Again in the USA, Withers *et al.* (60) reported HEV antibody prevalence to be 4.5 times higher in people exposed to contact with pigs than in people who are not. In Taiwan

(26) and Moldova (12), 26.7% and 51.1% of people working in contact with pigs have HEV antibodies, in contrast with 8% and 24.5% of the respective control populations. In Greece, Siochu *et al.* (45) reported a 40% HEV seropositivity rate in pig farmers and 22.2% in slaughterhouse workers, compared to 15.7% among other blood donors. Finally, the direct evidence that swine and probably other animal species can infect humans has been recently demonstrated in Japan where some cases of hepatitis E have been associated with the ingestion of uncooked meat or organs from pigs, wild boar or deer (33, 51, 52, 61). In all the suspected cases, affected people reported to have ingested uncooked animal meat or organs a few days before the onset of clinical signs. In one of these episodes, a HEV strain genetically identical to the strain isolated from the affected patients was found in the meat of the Sika deer considered to be involved in the outbreak and which was still stored in the freezer of the patient (52). A subsequent epidemiological survey confirmed that the ingestion of raw deer meat should definitely be considered a risk factor for HEV infection (53).

Laboratory diagnosis

In humans

It has not yet been possible to grow HEV in cell culture. Partial results have been obtained using human lung cells and monkey cells, in particular hepatocytes, but these have never yielded acceptable quantities of virus (15). Laboratory diagnostic methods include molecular techniques and electron microscopy for direct diagnosis and serological techniques for indirect diagnosis aimed at detecting HEV antibodies. Diagnosis by electron microscope, commencing with faeces samples, is scarcely used today due to the lack of sensitivity of this method. Direct diagnosis is therefore generally performed by seeking to identify the viral genome in faeces

or serum samples by reverse transcription-polymerase chain reaction (RT-PCR); a nested RT-PCR is often used to enhance the sensitivity of the test. Amplification of the PCR product for identification and characterisation of the virus genotype is usually performed by sequencing, cloning or by restriction with endonuclease enzymes. A real-time PCR has recently been developed; this technique is not only able to quantify the virus load, but greatly enhances the rapidity, sensitivity and specificity of the test (39). Serological diagnosis is generally performed using ELISA, or less commonly the Western blot. Target antigens in those assays are either recombinant HEV proteins or synthetic HEV peptides that correspond to immunodominant epitopes of structural HEV proteins, such as ORF2 and ORF3 (14, 34). In general, ORF2-derived recombinant antigens have proved to be more sensitive and more specific. ORF2 also express epitopes able to induce the production of neutralizing antibodies and that are more conserved (90.5%) in the different strains than epitopes contained in ORF3 (73.5%) (58).

Today, ORF2 or parts thereof, have been expressed successfully in different recombinant systems, such as prokaryote cells, yeast, animal cells (particularly insect cells) and plant cells (58). Serological tests are able to discriminate between IgM and IgG; determination of IgM anti-HEV is useful for the diagnosis of acute infection, whereas the presence of anti-HEV IgG indicates an infection that is not necessarily recent. Several studies performed in industrialised countries have reported antibody prevalences ranging between 1% and 20%. Some of these values appear to be relatively high compared with the low prevalence of clinical disease in these areas. It still remains unclear whether the high anti-HEV seroreactivity in non-endemic areas reflects subclinical or anicteric HEV infection, serological cross-reactivity with other agents, false

positivity of serological tests, subclinical infection with swine HEV or other HEV-like viruses, or a combination of all these factors (1, 14). Unfortunately, serological tests currently used differ considerably and also have varying degrees of sensitivity and specificity, thus often complicating the interpretation and comparison of results reported in some studies.

In swine

Diagnosis in swine is performed with the same techniques as those used for humans, not only testing serum and faeces, but also tissues and organs collected from slaughtered animals or from animals subjected to necropsy. For a direct search of the virus using RT-PCR, samples from swine in which the infection is still active need to be examined, which means from pigs of about three to five months of age, while serological tests give positive results especially when performed in adult animals. Nucleic acid extraction is performed, first with samples of faeces and then with serum or tissue. Specific primers designed only for swine viruses are now available (28, 30). Virus detection in tissues is generally performed first on the liver, as it is used for food, and then on the intestine and lymph nodes (61). In addition to RT-PCR, *in situ* hybridisation and immunohistochemistry can also be used to test organs. These techniques enable localisation of the virus in the tissues and infected cells and offer a reliable instrument with which to correlate the virus with lesions and to identify virus replication sites (7, 21). Detecting the virus in organs, liver *in primis*, is therefore not only important to evaluate the potential zoonotic risk associated with the consumption of infected food, but has opened new possibilities in the understanding of the pathogenesis of the infection (7). Swine strains are genetically and antigenically correlated to human and avian strains. Well-documented literature explains how several epitopes, especially those of ORF2, cross-react between

different species and strains (8, 16, 24, 28, 34, 58). For serological diagnosis, although tests using specific recombinant antigens for swine do exist, antigens derived from human strains are also commonly used, only changing the secondary antibody for revelation (34).

Conclusions

Epidemiological and virological studies conducted in the last few years have now clearly demonstrated that HEV can be considered an emerging zoonosis. Swine seems to represent the major animal reservoir for the virus and this creates public health concerns. The infection can be transmitted through food by the ingestion of infected meat products (3, 33, 51, 52, 61). This mode of transmission, at least in Italy, is unlikely because the virus is inactivated by the process of cooking and because it is normally present only in swine below the age of five months (well below the slaughter age). However, the possibility of cross-contamination between raw meat products and the risk of virus spread in the environment through manure from pig farms, with the consequent possible contamination of vegetables and drinking or bathing water, should also be taken into consideration. Contamination of water, as is the case with hepatitis A virus, could also lead to the contamination of filtering shellfish, thereby further compounding public health risks. Another possible mode of contamination for humans is by direct contact with infected animals. In this case, people such as farmers, workers attending the animals and veterinarians who work in contact with pigs during the viraemic period or when virus is excreted in the faeces, would be at greater risk of infection (12, 34, 45, 60, 61). Furthermore, for such categories, the possibility of infection by indirect contact with instruments and tools contaminated by infected faeces cannot be excluded. The knowledge of these risks should thus encourage

recourse to those hygiene and biosecurity procedures used to avoid or minimise infection. Finally, the discovery of HEV poses a new problem for the practice of xenotransplantation. Recently, xenotransplantation has become the focus of intensive research to solve the shortage of organ donors for transplantations. However, the possibility of transmission of pathogens from pigs to human recipients is of major concern in xenotransplantation. Viruses that are pathogenic or moderately pathogenic for pigs might pose a severe risk to humans. However, non-pathogenic viruses of pigs may also become pathogenic for humans after xenotransplantation as a result of crossing the species barrier, recombination or adaptation in immunocompromised xenotransplantation recipients (37, 41). Furthermore, pigs that recover from swine HEV infection might have a damaged liver (or other organ), which would limit its use for xenotransplantation (37).

The enzootic nature of swine HEV infection in pigs in many countries and its ability to cross the species barrier raise concerns in regard to the possibility of zoonotic transmission and food and environmental safety. Nevertheless, many of the veterinary aspects of infection are not yet known. In regard to virology, the animal strains studied are few and the information available scant in regard to the genetic and evolutive correlation between the different animal and human strains. The natural history of the infection in pigs also requires further study, as does the economic impact of the disease on pig production. The host range of the infection is not fully known and the cases associated with the ingestion of uncooked meat from wild boars and deer in Japan indicate that the role of wild animals especially, and swine and ruminants, should be further considered in the epidemiology of the disease. Despite the obvious health implications of this

emerging zoonosis, information in Europe on the presence and circulation of HEV in swine herds or other animals is rather scarce (2, 10, 56). In countries such as Spain, the Netherlands and the UK, where epidemiological studies have been performed, it has been demonstrated that the virus is circulating actively in the pig herds of these countries (2, 10, 56). If the possibility of zoonotic transmission of the infection is accepted, it is clear that the higher the prevalence in animals, the greater the risk of transmission will be to humans. An evaluation of the presence of HEV in pigs or other domestic and wild species is necessary in Italy and genetic and epidemiological surveys of these viruses need to be conducted to determine whether there is indeed a risk of zoonotic transmission of HEV in Italy.

References

1. Aggarwall R. & Krawczynski K. 2000. Hepatitis E: an overview and recent advances in clinical and laboratory research. *J Gastroenterol Hepatol*, **15**, 9-20.
2. Banks M., Bendall R., Grierson S., Heath G., Mitchell J. & Dalton H. 2004. Human and porcine hepatitis E virus strains, United Kingdom. *Emerg Infect Dis*, **10**, 953-955.
3. Banks M., Heath G.S., Grierson S.S., King D.P., Gresham A., Girones R., Widen F. & Harrison T.J. 2004. Evidence for the presence of hepatitis E virus in pigs in the United Kingdom. *Vet Rec*, **154**, 223-227.
4. Cacopardo B., Russo R., Preiser W., Benanti F., Brancati G. & Nunnari A. 1997. Acute hepatitis E in Catania (eastern Sicily) 1980-1994. The role of hepatitis E virus. *Infection*, **25**, 313-316.
5. Chandler J.D., Riddell M.A., Li F., Love R.J. & Anderson D.A. 1999. Serological evidence for swine hepatitis E virus infection in Australian pig herds. *Vet Microbiol*, **68**, 95-105.
6. Chironna M., Germinario C., Lopalco P.L., Carrozzini F. & Quarto M. 2001. Prevalence of hepatitis virus infections in Kosovar refugees. *Int J Infect Dis*, **5**, 209-213.
7. Choi C. & Chae C. 2003. Localization of swine hepatitis E virus in liver and extrahepatic tissues from naturally infected pigs by in situ hybridization. *J Hepatol*, **38**, 827-832.
8. Choi I.S., Kwon H.J., Shin N.R. & Yoo H.S. 2003. Identification of swine hepatitis E virus (HEV) and prevalence of anti-HEV antibodies in swine and human populations in Korea. *J Clin Microbiol*, **41**, 3602-3608.
9. Clayson E.T., Innis B.L., Myint K.S., Narupiti S., Vaughn D.W., Giri S., Ranabhat P. & Shrestha M.P. 1995. Detection of hepatitis E virus infections among domestic swine in the Kathmandu Valley of Nepal. *Am J Trop Med Hyg*, **53**, 228-232.
10. Clemente-Casares P., Pina S., Buti M., Jordi R., Martin M., Bofill-Mas S. & Girones R. 2003. Hepatitis E virus epidemiology in industrialized countries. *Emerg Infect Dis*, **9**, 448-454.
11. De Donno A., Chironna M., Craca R., Paiano A., Zizza A., Guido M., Carrozzini F., Germinario C. & Gabutti G. 2003. Anti-HEV seroprevalence in the area of Lecce. *Ann Ig*, **15**, 199-205.
12. Drobeniuc J., Favorov M.O., Shapiro C.N., Bell B.P., Mast E.E., Dadu A., Culver D., Iarovoi P., Robertson B.H. & Margolis H.S. 2001. Hepatitis E virus antibody prevalence among persons who work with swine. *J Infect Dis*, **184**, 1594-1597.
13. Ellis J., Clark E., Haines D., West K., Krakowka S., Kennedy S. & Allan G.M. 2004. Porcine circovirus-2 and concurrent infections in the field. *Vet Microbiol*, **98**, 159-163.
14. Emerson S.U. & Purcell R.H. 2003. Hepatitis E virus. *Rev Med Virol*, **13**, 145-154.
15. Emerson S.U., Nguyen H., Graff J., Stephany D.A., Brockington A. & Purcell R.H. 2004. *In vitro* replication of hepatitis E virus (HEV) genomes and of an HEV replicon expressing green fluorescent protein. *J Virol*,

- 78, 4838-4846.
16. Engle R.E., Yu C., Emerson S.U., Meng X.J. & Purcell R.H. 2002. Hepatitis E virus (HEV) capsid antigens derived from viruses of human and swine origin are equally efficient for detecting anti-HEV by enzyme immunoassay. *J Clin Microbiol*, **40**, 4576-4580.
 17. Fabrizi F., Lunghi G., Bacchini G., Corti M., Pagano A. & Locatelli F. 1997. Hepatitis E virus infection in haemodialysis patients, a seroepidemiological survey. *Nephrol Dial Transplant*, **12**, 133-136.
 18. Garkavenko O., Obriadina A., Meng J., Anderson D.A., Benard H.J., Schroeder B.A., Khudyakov Y.E., Fields H.A. & Croxson M.C. 2001. Detection and characterisation of swine hepatitis E virus in New Zealand. *J Med Virol*, **65**, 525-529.
 19. Gessoni G. & Manoni F. 1996. Hepatitis E virus infection in north-east Italy, serological study in the open population and groups at risk. *J Viral Hepatitis*, **3**, 197-202.
 20. Grieco A., Miele L., Gasbarrini G. & Grillo R. 2001. Sporadic HEV hepatitis in Italy. *Gut*, **48**, 580.
 21. Ha S.K. & Chae C. 2004. Immunohistochemistry for the detection of swine hepatitis E virus in the liver. *J Viral Hepat*, **11**, 263-267.
 22. Halbur P.G., Kasorndorkbua C., Gilbert C., Guenette D., Potters M.B., Purcell R.H., Emerson S.U., Toth T.E. & Meng X.J. 2001. Comparative pathogenesis of infection of pigs with hepatitis E viruses recovered from a pig and a human. *J Clin Microbiol*, **39**, 918-923.
 23. Haqshenas G., Shivaprasad H.L., Woolcock P.R., Read D.H. & Meng X.J. 2001. Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. *J Gen Virol*, **82**, 2449-2462.
 24. Haqshenas G., Huang F.F., Fenaux M., Guenette D.K., Pierson F.W., Larsen C.T., Shivaprasad H.L., Toth T.E. & Meng X.J. 2002. The putative capsid protein of the newly identified avian hepatitis E virus shares antigenic epitopes with that of swine and human hepatitis E viruses and chicken big liver and spleen disease virus. *J Gen Virol*, **83**, 2201-2209.
 25. He J., Innis B.L., Shrestha M.P., Clayson E.T., Scott R.M., Linthicum K.J., Musser G.G., Gigliotti S.C., Binn L.N., Kuschner R.A. & Vaughn D.W. 2002. Evidence that rodents are a reservoir of hepatitis E virus for humans in Nepal. *J Clin Microbiol*, **40**, 4493-4498.
 26. Hsieh S.Y., Meng X.J., Wu Y.H., Liu S.T., Tam A.W., Lin D.Y. & Liaw Y.F. 1999. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *J Clin Microbiol*, **37**, 3828-3834.
 27. Huang F.F., Haqshenas G., Guenette D.K., Halbur P.G., Schommer S.K., Pierson F.W., Toth T.E. & Meng X.J. 2002. Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. *J Clin Microbiol*, **40**, 1326-1332.
 28. Huang F.F., Sun Z.F., Emerson S.U., Purcell R.H., Shivaprasad H.L., Pierson F.W., Toth T.E. & Meng X.J. 2004. Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. *J Gen Virol*, **85**, 1609-1618.
 29. Kasorndorkbua C., Halbur P.G., Thomas P.J., Guenette D.K., Toth T.E. & Meng X.J. 2002. Use of a swine bioassay and a RT-PCR assay to assess the risk of transmission of swine hepatitis E virus in pigs. *J Virol Methods*, **101**, 71-78.
 30. Kasorndorkbua C., Thacker B.J., Halbur P.G., Guenette D.K., Buitenwerf R.M., Royer R.L. & Meng X.J. 2003. Experimental infection of pregnant gilts with swine hepatitis E virus. *Can J Vet Res*, **67**, 303-306.
 31. Kasorndorkbua C., Guenette D.K., Huang F.F., Thomas P.J., Meng X.J. & Halbur P.G. 2004. Mode of transmission of swine hepatitis E virus (HEV) in pig. *In: Proc. 18th International Pig Veterinary Society (IPVS) Congress*,

- 27 June-1 July, Hamburg. IPVS, Perry, Iowa, Vol. 2, 600.
32. Marcato P.S. & A. Perillo 2000. Le epatiti virali del suino e una nuova zoonosi, l'epatite E suina. *In: Proc. XXVI Meeting Annuale Società Italiana di Patologia ed Allevamento dei Suini (SIPAS)*, 24-25 March, Piacenza, Italy, 163-171.
 33. Matsuda H., Okada K., Takahashi K. & Mishiro S. 2003. Severe hepatitis E virus infection after ingestion of uncooked liver from a wild boar. *J Infect Dis*, **188**, 944.
 34. Meng X.J., Purcell R.H., Halbur P.G., Lehman J.R., Webb D.M., Tsareva T.S., Haynes J.S., Thacker B.J. & Emerson S.U. 1997. A novel virus in swine is closely related to the human hepatitis E virus. *Proc Natl Acad Sci USA*, **94**, 9860-9865.
 35. Meng X.J., Halbur P.G., Haynes J.S., Tsareva T.S., Bruna J.D., Royer R.L., Purcell R.H. & Emerson S.U. 1998. Experimental infection of pigs with the newly identified swine hepatitis E virus (swine HEV), but not with human strains of HEV. *Arch Virol*, **143**, 1405-1415.
 36. Meng X.J., Halbur P.G., Shapiro M.S., Govindarajan S., Bruna J.D., Mushahwar I.K., Purcell R.H. & Emerson S.U. 1998. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J Virol*, **72**, 9714-9721.
 37. Meng X.J., Wiseman B., Elvinger F., Guenette D.K., Toth T.E., Engle R.E., Emerson S.U. & Purcell R.H. 2002. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol*, **40**, 117-122.
 38. Nishizawa T., Takahashi M., Mizuo H., Miyajima H., Gotanda Y. & Okamoto H. 2003. Characterization of Japanese swine and human hepatitis E virus isolates of genotype IV with 99% identity over the entire genome. *J Gen Virol*, **84**, 1245-1251.
 39. Orru G., Masia G., Orru G., Romanò L., Piras V. & Coppola R.C. 2004. Detection and quantitation of hepatitis E virus in human faeces by real-time quantitative PCR. *J Virol Methods*, **118**, 77-82.
 40. Pavia M., Iiritano E., Veratti M.A. & Angelillo I.F. 1998. Prevalence of hepatitis E antibodies in healthy persons in southern Italy. *Infection*, **26**, 32-35.
 41. Pei Y. & Yoo D. 2002. Genetic characterization and sequence heterogeneity of a Canadian isolate of swine hepatitis E virus. *J Clin Microbiol*, **40**, 4021-4029.
 42. Pina S., Buti M., Cotrina M., Piella J. & Girones R. 2000. HEV identified in serum from humans with acute hepatitis and in sewage of animal origin in Spain. *J Hepatol*, **33**, 826-833.
 43. Romanò L., Tanzi E. & Zanetti A. 1999. The role of HEV in acute non-A, non-C hepatitis and the identification of a new variant HEV in Italy. *Ann Ig*, **11**, 451-459.
 44. Schlauder G.G., Desai S.M., Zanetti A.R., Tassopoulos N.C. & Mushahwar I.K. 1999. Novel hepatitis E virus (HEV) isolates from Europe: evidence for additional genotypes of HEV. *J Med Virol*, **57**, 243-251.
 45. Siochu A., Froesner G., Tassis P.D., Kyriakis C.S., Alexopoulos C. & Kyriakis S.C. 2004. First report of the prevalence of anti-hepatitis E virus (anti-HEV) IgG in blood serum of blood donors, slaughtermen and swine farmers in Greece. *In: Proc. 18th International Pig Veterinary Society (IPVS) Congress*, 27 June-1 July, Hamburg. IPVS, Perry, Iowa, Vol. 1, 367.
 46. Stroffolini T., Menchinelli M., Dambruoso V., Menniti Ippolito F., Costantino A., Rapicetta M., Lecce R. & Taliani G. 1996. Prevalence of hepatitis E in a central Italian town at high endemicity for hepatitis C virus. *Ital J Gastroenterol*, **28**, 523-525.
 47. Sun Z.F., Larsen C.T., Huang F.F., Billam P., Pierson F.W., Toth T.E. & Meng X.J. 2004. Generation and infectivity titration of an infectious stock of avian hepatitis E virus (HEV) in chickens and cross-species infection of turkeys with avian HEV. *J Clin Microbiol*, **42**, 2658-2662.
 48. Takahashi K., Kang J.H., Ohnishi S., Hino K., Miyakawa

- H., Miyakawa Y., Maekubo H. & Mishiro S. 2003. Full-length sequences of six hepatitis E virus isolates of genotypes III and IV from patients with sporadic acute or fulminant hepatitis in Japan. *Interirology*, **46**, 308-318.
49. Takahashi M., Nishizawa T., Miyajima H., Gotanda Y., Iita T., Tsuda F. & Okamoto H. 2003. Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. *J Gen Virol*, **84**, 851-862.
 50. Takahashi M., Nishizawa T. & Okamoto H. 2003. Identification of a genotype III swine hepatitis E virus that was isolated from a Japanese pig born in 1990 and that is most closely related to Japanese isolates of human hepatitis E virus. *J Clin Microbiol*, **40**, 869-870.
 51. Tamada Y., Yano K., Yatsushashi H., Inoue O., Mawatari F. & Ishibashi H. 2003. Consumption of wild boar linked to cases of hepatitis E. *J Hepatol*, **38**, 827-832.
 52. Tei S., Kitajima N., Takahashi K. & Mishiro S. 2003. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet*, **362**, 371-373.
 53. Tei S., Kitajima N., Ohara S., Inoue Y., Miki M., Yamatani T., Yamabe H., Mishiro S. & Kinoshita Y. 2004. Consumption of uncooked deer meat as a risk factor for hepatitis E virus infection: an age-and sex-matched case-control study. *J Med Virol*, **74**, 67-70.
 54. Usmanov R.K., Balaian M.S., Dzhumaliev D.I., Alymbaeva D.B., Korolev M.B., Karas F.R., Zamiatina N.A., Berdikozhova S.K., Karymshakova C.H.T. & Kozhomkulov E.T. 1991. Experimental hepatitis E infection in piglets. *Vopr Virusol*, **36**, 212-216.
 55. Usmanov R.K., Balaian M.S., Kazachkov Iu A., Alymbaeva D.B., Zamiatina N.A., Dzhumaliev D.I. & Voronina O.V. 1994. Further study of experimental hepatitis E in piglets. *Vopr Virusol*, **39**, 208-212.
 56. Van der Poel W.H., Verschoor F., Van der Heide R., Herrera M.I., Vivo A., Kooreman M. & De Roda Husman A.M. 2001. Hepatitis E virus sequences in swine related to sequences in humans, The Netherlands. *Emerg Infect Dis*, **7**, 970-976.
 57. Wang L. & Zhuang H. 2004. Hepatitis E: an overview and recent advances in vaccine research. *World J Gastroenterol*, **10**, 2157-2162.
 58. Wibawa I.D., Muljono D.H., Mulyanto, Suryadarma I.G., Tsuda F, Takahashi M., Nishizawa T. & Okamoto H. 2004. Prevalence of antibodies to hepatitis E virus among apparently healthy humans and pigs in Bali, Indonesia: identification of a pig infected with a genotype 4 hepatitis E virus. *J Med Virol*, **73**, 38-44.
 59. Williams T.P., Kasorndorkbua C., Halbur P.G., Haqshenas G., Guenette D.K., Toth T.E. & Meng X.J. 2001. Evidence of extrahepatic sites of replication of the hepatitis E virus in a swine model. *J Clin Microbiol*, **39**, 3040-3046.
 60. Withers M.R., Correa M.T., Morrow M., Stebbins M.E., Seriwatana J., Webster W.D., Boak M.B. & Vaughn D.W. 2002. Antibody levels to hepatitis E virus in North Carolina swine workers, non-swine workers, swine, and murids. *Am J Trop Med Hyg*, **66**, 384-388.
 61. Yazaki Y., Mizuo H., Takahashi M., Nishizawa T., Sasaki N., Gotanda Y. & Okamoto H. 2003. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J Gen Virol*, **84**, 2351-2357.
 62. Yoo D., Willson P., Pei Y., Hayes M.A., Deckert A., Dewey C.E., Friendship R.M., Yoon Y., Gottschalk M., Yason C. & Giulivi A. 2001. Prevalence of hepatitis E virus antibodies in Canadian swine herds and identification of a novel variant of swine hepatitis E virus. *Clin Diagn Lab Immunol*, **8**, 1213-1219.
 63. Zanetti A.R. & Dawson G.J. 1994. Hepatitis type E in Italy: a seroepidemiological survey. Study Group of Hepatitis E. *J Med Virol*, **42**, 318-320.
 64. Zanetti A.R., Schlauder G.G., Romano L., Tanzi E., Fabris P., Dawson G.J. & Mushahwar I.K. 1999. Identification of a novel variant of hepatitis E virus in Italy. *J Med Virol*, **57**, 356-360.