EuroReference
journal of Reference
anses
French agency for food, environmental and occupational health & safety

Journal No. 13
Winter 2015

Research

Autochthonous cases of hepatitis E: where does the virus come from? Impact of pig slurry treatment on reduction of the viral load and prevalence of the virus in food substrates

Fabienne Loisy-Hamon[1,2] (fabienne.loisy@ceeram.com), Géraldine Leturnier[1]
1. Ceeram, La Chapelle sur Erdre, France
2. bioMérieux Industry, Marcy L'Étoile, France.

Abstract
In recent years, several autochthonous hepatitis E cases and a high seroprevalence have been reported. A potential source of contamination is the consumption of pork products or food contaminated by an environmental source. The objective of this study was to evaluate the prevalence of hepatitis E virus (HEV) in food samples, not only pork products, and to evaluate pig slurry as a potential source of environmental contamination.
A large prevalence study was conducted on 440 food samples collected in international food companies in 2011 as part of assessment of viral risks in their Hazard Analysis & Critical Control Points (HACCP) plan, including pork liver sausages, shellfish, fruits, vegetables, herbs and spices, process water, and ready-to-eat foods. The kit hepatitisE@ceeramTools™ was used for real time RT-PCR detection. Samples were also tested for norovirus GI, norovirus GII, and hepatitis A virus (HAV). A study was also conducted on pig slurry collected from 3 pig breeding farms positive for HEV to evaluate the persistence of HEV after various slurry treatments.
The results obtained for HEV demonstrate a prevalence of 0.9% with positive samples including pork liver sausage, pepper and bay leaf powder. On the 440 samples tested, the prevalence levels for norovirus GI, GII and HAV were 2.95%, 8.6% and 0.45%, respectively.
Concerning untreated pig slurry, 67% was positive for HEV. After treatment, 27% of pig slurry was still positive for HEV. Among this, 30% of treated pig slurry was positive for HEV after composting, 50% after dehydration, and only 5.6% of the pig slurry treated by anaerobic digestion was positive for HEV.
To our knowledge, this is the first large-scale study conducted on HEV prevalence in food samples to try to understand the origin of autochthonous hepatitis E cases and the potential origin of contamination in food samples. Our results demonstrate that HEV prevalence in food samples is in the same range as HAV. Spreading of pig slurry does not appear to be an agricultural practice at risk for HEV. These results demonstrate that types of foods other than pork liver products do not seem to be a potential source of contamination. This study could be helpful to evaluate the origin of human hepatitis E cases and to better prevent autochthonous HEV cases.

Introduction
The hepatitis E virus (HEV) causes acute hepatitis outbreaks with enteric transmission in humans that are fairly similar to hepatitis A epidemics though generally more severe (Emerson and Purcell, 2003). Although most hepatitis cases resolve spontaneously, fatal outcomes are reported (1-2% of cases). The risk of fulminant hepatitis in pregnant women can reach 25%, even though these cases have to date been reported only in emerging countries (Smith, 2001). Chronic hepatitis is also more and more frequently reported, especially in immunodepressed patients (Bihl and Negro, 2009; Gerolami *et al.,* 2008; Kamar *et al.,* 2008). Recently, many sporadic cases of hepatitis E unrelated to travel to endemic areas have been reported in developed countries. In France, the National Reference Centre (NRC) for entero-transmissible hepatitis has described a significant increase in the number of human cases of hepatitis E between 2002 (9 cases, creation of the NRC) and 2011 (249 cases), partly related to better diagnosis of this pathogen (Nicand *et al.,* 2011; Roque-Afonso, 2011).
In 1997, Meng *et al.* demonstrated genetic similarities between a new porcine virus (porcine HEV) and a strain of human HEV (Meng *et al.,* 1997). This discovery pointed to the potential role of porcine HEV strains in autochthonous human cases. As a result, many studies were carried out in various animal populations and showed that HEV is able to infect many animal species, including pigs, its primary reservoir (Cooper *et al.,* 2005; de Deus *et al.,* 2008; Meng *et al.,* 1997). A direct link between consumption of infected products and cases of autochthonous human hepatitis was reported following ingestion of raw deer meat (Tei *et al.,* 2003), raw wild boar meat (Tamada *et al.,* 2004), and raw liver sausages called figatelli (Colson *et al.,* 2010).
Bivalve molluscs can concentrate viral particles during the filtration process involved in their method of nutrition. The hepatitis E virus has been detected in shellfish collected in various regions of Europe and Asia (Crossan *et al.,* 2012; Donia *et al.,* 2012; Li *et al.,* 2007). Maunula *et al.* (2013), described the presence of HEV in raspberries.
Pigs infected by HEV shed the virus for 3 to 4 weeks in large amounts. Pig farming practices therefore result in high-dose exposure of animals to HEV (Kasorndorkbua *et al.,* 2005). The HEV status in pig slurry stored in installations such as concrete and earth basins remains to be studied, along with the impact of slurry treatment on elimination of the hepatitis virus.
The study objectives were as follows:
- to evaluate the prevalence of HEV compared to prevalence rates observed for noroviruses and hepatitis A in various food substrates sampled at manufacturers as part of HACCP plans, including a limited number of products containing pig liver;
- to determine whether spreading of products following treatment of pig slurry constitutes an at-risk practice that could lead to contamination of crops.
-

Materials and methods
Hepatitis E virus, mengovirus and samples
Development and validation of the detection method for hepatitis E virus were carried out using the available international WHO standard for this virus. This standard corresponds to HEV-positive plasma measured in international units and containing 250,000 IU/mL. HEV-positive pig faeces provided by ANSES (Dr Nicolas Rose, Swine epidemiology and welfare unit, Ploufragan, France) were used to supplement this validation.
Mengovirus vMC0, used as a process control, was obtained from the CeeramTools® Mengo Extraction Control kit (Ceeram,

Research

La Chapelle-sur-Erdre, France). In compliance with ISO/TS 15216, a yield of 1% mengovirus validates the process.
Detection of the foodborne viruses norovirus (NV), hepatitis A (HAV), and hepatitis E (HEV) by real-time RT-PCR was performed on 441 samples of various food substrates (**Table 1**) undergoing viral analysis as part of HACCP plans in agro-food businesses operating in Europe. This enabled determination of prevalence data.
The following food substrates were evaluated in this study: 230 samples of various herbs and spices, 77 fruits, 62 process waters, 36 shellfish (oysters and mussels), 20 pork-free ready-to-eat meals, 12 vegetables, and 4 figatelli.
The analysed substrates were mainly from Europe but some were from Asia or Africa, for example the spices. Specific information on the origin of samples was however difficult to obtain and these particulars are not detailed in this article.
122 faeces and pig slurry samples and 44 samples of compost from a field study were collected to evaluate the impact of various treatment methods for pig slurry (spreading, composting, anaerobic digestion, and dehydration). Two collection networks were used: a private network of veterinary laboratories in the Morbihan department and ANSES (Dr Nicolas Rose, Swine epidemiology and welfare unit, Ploufragan, France). Samples were taken at various stages of slurry treatment in swine farms located in Brittany. The treatment process was evaluated through sampling at different stages depending on the type of treatment: faecal matter, compost, raw and treated water, and semi-liquid muddy water from lagooning.

Elution / concentration of viral particles in food substrates and extraction of viral RNA

For the samples of shellfish, fruits and vegetables, and herbs and spices, the method outlined in Standard ISO/TS 15216 was applied. In short, after adding mengovirus vMC0, 2 g of digestive tissue of shellfish were treated with proteinase K by incubation for 1 h at 37°C, then 15 min at 60°C. After centrifugation at 3000 g at room temperature for 5 min, the supernatant was collected. Viral capsid lysis was performed on 500 µL of supernatant using NucliSens® lysis buffer (bioMérieux) by incubation at 56°C for 30 min. RNA was then extracted and purified using NucliSens® reagents (bioMérieux) as per the supplier's recommendations.
To 25 g of fruit or vegetable samples, and to 5 g of dried herbs and spices, 40 mL of TGBE buffer (tris-glycine-beef extract buffer, pH 9.5) were added, together with the mengovirus vMC0 control extract. Bags were then agitated constantly for 20 min at room temperature. The supernatant obtained was centrifuged for 20 min at 10,000 g at 4°C. pH was adjusted to 7.2 +/- 0.2. Viral particles were precipitated with polyethylene glycol (PEG) (1/4 Vol) under agitation for 1 h at 4°C, then centrifuged for 30 min at 10,000 g at 4°C. The pellet was then re-suspended in 500 µL of phosphate buffered saline (PBS1X) then clarified using chloroform/butanol. After 15 min of centrifugation at 13,500 g at 4°C, the upper aqueous phase was retained for lysis. Nucleic acids were extracted as described previously.
For the samples of process waters, mainly loaded with particles, an alternative method that was more suitable than Standard ISO/TS 15216 was applied. One litre was concentrated by cross-flow filtration using a filter cartridge (Sartorius) after adding mengovirus vMC0. After rinsing the cartridge with 20 mL of glycine buffer, a 40 mL concentrate was obtained. Secondary concentration was then performed by incubation in 50% PEG for 1 h at 4°C, followed by centrifugation for 20 min at 11,000 g at 4°C. The pellet was then suspended in 1 mL of PBS1X and clarified using chloroform/butanol. After 15 min of centrifugation at 13,500 g at 4°C, the upper aqueous phase was collected and the viruses lysed, and nucleic acids extracted as described previously.
Concerning figatelli, the process developed by the Maisons-Alfort Laboratory for Food Safety, Enteric viruses unit (Martin-Latil *et al.* 2014 *EuroReference*) was applied. In short, 30 mL of distilled water were added to 3 g of the substrates ground in a Stomacher bag (2 min, 260 rpm). The elution was performed at room temperature under agitation for 10 min after addition of mengovirus vMC0. The homogenate was clarified by centrifugation for 15 min at 8000 g at 4°C, and viral particles were then precipitated with PEG (1/4 Vol) for 2 h at 4°C and concentrated by centrifugation for 30 min at 8000 g. The eluate was recovered for lysis.

Elution / concentration of viral particles in pig faeces/slurry and composts and extraction of viral RNA

A 10% to 50% suspension of faeces or slurry was prepared in PBS. The suspension was then clarified by centrifugation for 30 min at 3000 g at 4°C. The supernatant was collected then clarified for a second centrifugation for 15 min at 10,000 g at 4°C. If the resulting supernatant was not clear, the second centrifugation step was repeated. Lysis and extraction of RNA was performed using 500 µL of suspension with NucliSens® reagents (bioMérieux) as described previously.
For "solid" samples (for example sawdust compost), 5 g of sample were taken and transferred to a filter bag containing 40 mL of TGBE buffer (tris-glycine-beef extract, pH 9.5). The bags were agitated constantly for 20 min at room temperature. Through the filter, the supernatant was recovered then centrifuged for 20 min at 10,000 g at 4°C. The pH of the supernatant obtained was adjusted to 7.2 +/- 0.2. Ten milliliters of PEG-NaCl 5X were then added to 40 mL of supernatant and agitated for 1 h at 4°C then centrifuged for 30 min at 11,000 g at 4°C. The pellet was then suspended in 1 mL of PBS1X and clarified using chloroform/butanol. After 15 min of centrifugation at 13,500 g at 4°C, the upper aqueous phase was collected and the viruses lysed and nucleic acids extracted.
For "semi-liquid" samples, for example samples from settling basins or lagooning basins, a protocol similar to the one used for the extraction of HEV in faeces or slurry was applied. Three millilitres of sample were taken to apply the protocol described previously.

Quantitative RT-PCR

The nucleic acid extracts obtained were tested using the real-time RT-PCR kit hepatitisE@ceeramTools™, following the supplier's recommendations and with SDS7300 or SDS7500 systems (Applied Biosystems). RNA extracted from the food substrates was also tested for NoVGI, NoVGII, and HAV using the real-time RT-PCR kits norovirusGI@ceeramTools™, norovirusGII@ceeramTools™, and hepatitisA@ceeramTools™ (Ceeram, La Chapelle-sur-Erdre, France). Positive controls containing RNA extracted from virus suspensions and a negative control containing all the reagents except the RNA extract were included in each set of experiments. The internal amplification control (IAC) contained in the hepatitisE@ceeramTools™ kit made it possible to validate each reaction. In addition, each RNA extract was tested undiluted and diluted

Research

to 1/10th in duplicate. All the samples were characterised by a cycle threshold (Ct). A standard curve for each viral target was produced using serial dilutions of viral suspensions. Mengovirus extraction yields were calculated for each sample based on the corresponding standard curve.

Results

Evaluation of HEV prevalence in food

The sampling plan for the prevalence study included all the at-risk substrates described in ISO/TS 15216 and in the Directive on the application of general food hygiene practices to control of viruses in food. The samples were analysed as part of self-monitoring to address the viral risk in an HACCP plan. The number of samples per type of food substrate was however dependent on the production activities of the food processor businesses involved in this study.

For the 441 samples analysed, 7 types of substrate were represented. Analyses were carried out in 2011. For all these samples, a minimum yield of 1% mengovirus vMCO was obtained, thus validating the test results.

Among the 441 samples analysed, the presence of HEV genomes was found in 2 figatelli out of 4 and in 2 herbs and spices out of 230, indicating prevalence of about 0.9% of HEV contaminated food. The prevalence for all analysed samples not containing pork was 0.46% and 0.9% only for the samples of herbs and spices, versus 50% for samples of figatelli containing pig liver. The obtained Ct values for figatelli samples were 31.23, corresponding to an amount of 4775 genome copies/gram and 30.18, corresponding to 9603 genome copies/gram. The Ct values for samples of pepper and bay leaf powder were 36.4 and 37.2, respectively. The viral load in these samples was not quantifiable; it was below 500 genome copies/test sample, corresponding to the limit of quantification of the method.

Genotyping of the identified positive samples was not carried out since the very low viral load did not enable recovery of sufficient material to obtain a workable result.

On the same samples, the prevalence rates for norovirus GI, norovirus GII, and HAV were 2.95%, 8.6% and 0.45%, respectively. The number of positive samples and the prevalence by substrate analysed is given in **Table 1**.

For the food substrate most represented in this study, herbs and spices, the prevalence rate for HEV, HAV, and NoVGII was about the same at less than 1%. For NoVGI, 8 samples were found to be positive of out 230 analysed, corresponding to a higher prevalence than for the other viruses at 3.5%.

Evaluation of pig slurry treatment on reduction of HEV viral load

Of the 20 initially selected farms, three (A, B and C) were found to be positive with HEV levels sufficiently high to carry out the study. A total of 123 raw slurry samples taken from basins or directly from animals in different housing areas were analysed. The presence of HEV nucleic acids was found in 82 samples, i.e. 67% positive samples. The results obtained for slurry on the various farms are presented in **Table 2**.

Table 2. Samples of slurry and treated slurry analysed by farm

	Number of samples	Number of slurry samples	Number of positives	Number of treated samples	Number of positive treated samples
Farm A	58	48	26	10	3
Farm B	70	54	43	16	11
Farm C	38	20	13	18	1
Total	**166**	**122**	**82**	**44**	**12**

Among the positive samples, the viral concentrations were variable. For *farm A*, the observed contamination levels in untreated slurry ranged from absence of detection to 1.46×10^6 genome copies/g, with a lower mean for the whole farm at 2.26×10^4 genome copies/g. Concerning *farm B*, contamination levels in untreated slurry ranged from absence of detection to 3.97×10^5 genome copies/g, with a mean of 2.53×10^4 genome copies/g for the whole farm. On *farm C*, contamination levels in untreated slurry ranged from absence of detection to 7.74×10^3 genome copies/g, with a mean of 1.5×10^3 genome copies/g for positive samples for the whole farm.

Since each farm has its own treatment system, three types of treatments were evaluated.

Farm A used sawdust composting to treat slurry.

Farm B used a slurry dehydration treatment plant leading to three types of products that can be exploited: fermented compost, settling basin supernatant, and lagooning water.

Farm C also used an anaerobic digestion treatment plant for slurry, leading to three types of products that can be exploited: raw slurry, treated slurry, and lagooning water. The results obtained for the different farms are shown in **Table 3**.

Of the 166 samples analysed, 122 were slurry samples and 44 were samples from slurry treatment. Of these 122 samples, 82 (67%) were identified as HEV-positive, with contamination levels ranging from 118 genome copies/g to 1.46×10^6 genome copies/g.

Table 1. Prevalence data for analysed food substrates

Type of substrate	Number of samples analysed	Number of HEV positives	Prevalence of HEV (%)	Other food viruses Number of positive samples (prevalence in %)		
				NoVGI	NoVGII	VHA
Herbs and spices	230	2	0.9	8(3.50)	1 (0.45)	1 (0.45)
Fruits	77	0	0	0 (0)	2 (2.60)	0 (0)
Process waters	62	0	0	0 (0)	3 (4.85)	0 (0)
Shellfish (oysters, mussels)	36	0	0	5 (13.9)	32 (88.9)	0 (0)
Prepared meals	20	0	0	0 (0)	0 (0)	0 (0)
Vegetables	12	0	0	0 (0)	0 (0)	1 (8,3)
Figatelli	4	2	50	0 (0)	0 (0)	0 (0)
Total	**441**	**4**	**0.9**	**13 (2.95)**	**38 (8.6)**	**2 (0.45)**

Research

For the treated samples, the presence of HEV was identified in 12 samples, i.e. 27% of treated samples, with concentration levels ranging from 85 genome copies/g to 3.34×10^4 genome copies/g.
On *farm A*, which treats slurry by sawdust composting, of the 10 analysed composts, 3 were found to be HEV-positive, with low contamination levels of 17 to 740 genome copies/g.
On *farm B*, which uses a slurry treatment plant, of the 6 samples of compost tested, 3 were found to be negative. For the 3 positive samples, contamination levels ranging from 100 to 6680 genome copies/g were detected. Four samples from settling basins were tested: all were HEV-positive with levels ranging from 80 to 600,000 genome copies/mL. For the lagooning basin test sample, 1030 genome copies/mL were detected.
On *farm C*, of the 6 samples of raw slurry sampled at the plant, a single sample was found to be positive with 416 genome copies/g. For the 12 other samples taken at the plant, corresponding to treated slurry or lagooning basin samples, none were identified as positive.
On average, the quantity of HEV detected in treated slurry samples was lower than in untreated slurry samples.
More detailed data on HEV reduction levels are given in **Table 3**.
On *farm A*, treating slurry by sawdust composting, a logarithmic reduction in viral load of 1.88 was observed.
On *farm B*, using a treatment plant, the mean reduction for all treated samples was 0.76. Reduction was 0.79 in fermented compost, 0.1 in supernatant from a settling basin, and 1.39 in the lagooning basin.
On *farm C*, a mean reduction of 3.29 was calculated. The reduction was 0.56 in raw slurry and 3.17 in both treated slurry and in the lagooning basin.

Discussion

The first part of this study aimed to evaluate the prevalence of HEV in various food substrates and not only in pork products identified as presenting a risk. This large study on 441 samples showed HEV prevalence of 0.9% in all the food substrates, a prevalence rate lower than that observed for noroviruses in the same samples. However, it is similar to rates found for hepatitis A virus. In the study performed by Maunula *et al.* (2013), an HEV prevalence of 0.98% was found in raspberries. These data for raspberries are similar to the overall prevalence rate found in this study.
The prevalence of HEV in the most represented substrates in this study, i.e. 230 samples of herbs and spices, was identical to the prevalence rate found for the samples overall. Moreover, the prevalence rate for HAV and NoVGII of 0.45% is comparable to data found for HEV, *versus* 3.5% for NoVGI. The herbs and spices are mainly produced in tropical areas of Africa, South America, and Asia, mostly using traditional methods. They are exposed to many sources of contamination, particularly microbiological: irrigation with water of insufficient sanitary quality, contact with soil and with untreated biological soil improvers, as well as handling by farmers or harvesters who are potentially vectors of contamination. The zoonotic properties of HEV also appear to suggest animal contamination, unlike HAV and noroviruses which do not have animal reservoirs. Assessment of bibliographic data on the microbial quality of these raw materials shows that the samples present highly diversified contamination, with the presence of enteric bacteria and yeasts and moulds in high quantities, particularly in untreated products (McKee *et al.,* 1995; Garcia *et al.,* 2001; Omafuvbe *et al.,* 2004; Hara-Kudo *et al.,* 2006; Choo *et al.,* 2007). The data obtained in this study confirm the potential risk related to these substrates through the presence of enteric viruses. The data must however be interpreted with caution since the viral load in these samples was very low and must be considered in relation to the infectious dose in humans.
Concerning the other substrates, Serracca *et al.* (2012) did not demonstrate the presence of HEV in ready-to-eat meals (110 samples). These results confirm those in our study on the same type of food substrate. None of the mollusc samples tested were found to be HEV positive. However, of 153 samples of molluscs tested, Diez-Valcarce *et al.* (2012) found an HEV-positive rate of 3%. These data suggest that bivalve molluscs may be a substrate with greater risk for HEV and for noroviruses GI and GII, as well as hepatitis A virus, due to their filtering activity that could concentrate the viruses present in a contaminated environment. In our study, 2 of the samples of raw pig liver products (figatelli) out of 4 presented contamination with hepatitis E virus. These findings are consistent with those reported by Martin-Latil *et al.* (*EuroReference*, 2014) indicating that 1 in 3 pig liver-containing products was contaminated by HEV. The data in this study demonstrated an HEV prevalence rate equivalent to that of HAV, and variability of the presence of the virus depending on the analysed substrates.
The second part of this study involved evaluation of pig slurry as a potential source of contamination of the environment and of food. Three swine farms were identified as positive for the virus and were interesting in that they used three different systems of

Table 3. Impact of treatment

	Raw slurry initial viral load		Treatment			Logarithmic reduction in viral load (initial load-load after treatment)
			Type of treatment	Viral load after treatment		
	Copies/g	Log10		Copies/g or mL	Log10	
Farm A	2.26×10^4	4.35	composting	294	2.47	1.88
Farm B	2.53×10^4	4.4	fermented composting	4117	3.61	0.79
			settling basin supernatant	2×10^4	4.3	0.1
			lagooning basin	1030	3.01	1.39
Farm C	1.5×10^3	3.17	raw slurry after anaerobic treatment	416	2.62	0.56
			treated slurry	0	0	3.17
			lagooning basin	0	0	3.17

Research

slurry treatment. The mean viral RNA levels for hepatitis E found for the various farms were relatively constant (104 genome copies/gram of slurry), with the exception of farm C where a lower viral load was detected (103 genome copies/gram of slurry). The loads found in final products following treatment were quite low or even very low for farm C, where the viral load was initially lower. These findings are consistent with those in published studies (Kasorndorkbua *et al.,* 2005; McCreary *et al.,* 2008; Garcia *et al.,* 2013).

The treatment by composting used on farm A appears to be quite effective since a very low viral RNA concentration was found in the final product. Garcia *et al.* (2013) demonstrated that following composting, the final product did not present HEV contamination, suggesting it is safe to use as an agricultural fertiliser. The treatment used on farm C appears to be effective since very low contamination was found in a single final product. On farm B, contamination levels in the various treatment products appear to present a greater risk. Results found for settling basin supernatant and the lagooning basin were consistent with the study carried out by Kasorndorkbua *et al.* (2005). In their study, the authors showed that HEV found in basins and lagoons was infectious after inoculation in pigs. Use of supernatant from settling basins in agriculture such as production of fruit and vegetables could possibly lead to contamination of these foods. This contamination could be a potential risk for humans in the event of consumption. The question of the infectious dose in humans remains nonetheless open.

In conclusion, our results show that the prevalence of HEV in food samples is similar to that for HAV in substrates such as herbs and spices. The origin of contamination by HEV could not be determined. Human activities, whether direct or indirect through contaminated water, or animal sources through spreading or wildlife, could be the source of contamination. Evaluation of pig slurry and of products resulting from slurry treatment shows, however, that spreading treated pig slurry does not appear to constitute a practice with an HEV risk.

Acknowledgements

The part of the study concerning the impact of pig slurry treatment on elimination of hepatitis E virus was funded by the French National Research Agency (project HEVECODYN ANR-2010-CESA-010).

References:

Bihl, F., F. Negro. 2009. Chronic Hepatitis E in the Immunosuppressed: A New Source of Trouble? *J Hepatol*, 50: 435-37.

Choo, E., S.S., Jang, K., Kim, K.G., Lee, S., Heu, S, Ryu. 2007. Prevalence and genetic diversity of *Bacillus cereus* in dried red pepper in Korea. J Food Prot, 70 : 917-922.

Colson, P., P. Borentain, B. Queyriaux, M. Kaba, V. Moal, P. Gallian, L. Heyries, D. Raoult, R. Gerolami. 2010. Pig Liver Sausage as a Source of Hepatitis E Virus Transmission to Humans. *J Infect Dis*, 202 : 825-34.

Cooper, K., F. F. Huang, L. Batista, C. D. Rayo, J. C. Bezanilla, T. E. Toth, et X. J. Meng. 2005. Identification of Genotype 3 Hepatitis E Virus (HEV) in Serum and Fecal Samples from Pigs in Thailand and Mexico. Where Genotype 1 and 2 HEV Strains Are Prevalent in the Respective Human Populations. *J Clin Microbiol*, 43, : 1684-88.

Crossan, C., P. J. Baker, J. Craft, Y. Takeuchi, H. R. Dalton, L. Scobie. 2012. Hepatitis E Virus Genotype 3 in Shellfish, United Kingdom. *Emerg Infect Dis*, 18 : 2085-87.

De Deus, N., M. Casas, B. Peralta, M. Nofrarías, S. Pina, M. Martín, J. Segalés. 2008. Hepatitis E Virus Infection Dynamics and Organic Distribution in Naturally Infected Pigs in a Farrow-to-Finish Farm. *Vet Microbiol*, 132 : 19-28.

Diez-Valcarce, M., P. Kokkinos, K. Söderberg, M. Bouwknegt, K. Willems, A.6M. de Roda-Husman, C.-H. von Bonsdorff, *et al.* 2012. Occurrence of Human Enteric Viruses in Commercial Mussels at Retail Level in Three European Countries ». *Food Environm Virol*, 4 : 73-80.

Donia, D., M. C. Dell'Amico, A. R. Petrinca, I.a Martinucci, M. Mazzei, F. Tolari, M. Divizia. 2012. Presence of Hepatitis E RNA in Mussels Used as Bio-Monitors of Viral Marine Pollution. *J Virol Met*, 186 : 198-202.

Emerson, S.U., R. H. Purcell. 2003. Hepatitis E Virus. *Revi Med Virol*, 13 : 145-54.

García, M., S. Fernández-Barredo, M. T. Pérez-Gracia. 2014; Detection of Hepatitis E Virus (HEV) through the Different Stages of Pig Manure Composting Plants. *Microb Biotechn*, 7 : 26-31.

Garcia S., F., Iracheta, N., Heredia. 2001. Microbiological Survey of retail herbs and spices from Mexican markets. *J Food Protect*, 64 : 99-103.

Gérolami, R., V. Moal, C. Picard, P. Colson. 2009. Hepatitis E Virus as an Emerging Cause of Chronic Liver Disease in Organ Transplant Recipients. *J Hepatol,* 50 : 622-24.

Hara-Kudo, Y., L.K., Ohtsuka, Y., Onoue, Y., Otomo, I., Furukawa, A., Yamaji, Y.,Segawa, K., Takatori. 2006. *Salmonella* prevalence and total microbial and spore populations in spices imported to Japan. *J Food Prot*, 69 : 2519-2523.

ISO/TS_15216-2, 2013. Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -- Part 1: Method for quantitative detection. International Organization for Standardization, Geneva, Switzerland

Journal No. 13
Winter 2015

Research

ISO/TS_15216-2, 2013. Microbiology of food and animal feed -- Horizontal method for determination of hepatitis A virus and norovirus in food using real-time RT-PCR -- Part 2: Method for qualitative detection. International Organization for Standardization, Geneva, Switzerland

Kamar, N., J. Selves, J.-M. Mansuy, L. Ouezzani, J.-M. Péron, J. Guitard, O. Cointault, *et al.* 2008. Hepatitis E Virus and Chronic Hepatitis in Organ-Transplant Recipients. *N Engl J Med*, 358 : 811-17.

Kasorndorkbua, C., T. Opriessnig, F. F. Huang, D. K. Guenette, P. J. Thomas, X.-J. Meng, P. G. Halbur. 2005. Infectious Swine Hepatitis E Virus Is Present in Pig Manure Storage Facilities on United States Farms, but Evidence of Water Contamination Is Lacking. *Appl Environm, Microbiol*, 71 : 7831-37.

Martin-Latil, S., C. Hennechart-Colette, S. Perelle. 2014. Method for HEV detection in raw pig liver products and it implementation for naturally contaminated food. *Euroreference*, this issue.

Maunula, L., A. Kaupke, P.Vasickova, K. Söderberg, I. Kozyra, S. Lazic, W. H.M. van der Poel, *et al.* 2013. Tracing enteric viruses in the European berry fruit supply chain. *Intern J Food Microbiol*, 167 : 177-85.

McCreary, C., F. Martelli, S. Grierson, F. Ostanello, A. Nevel, M. Banks. 2008. Excretion of Hepatitis E Virus by pigs of different ages and its presence in slurry stores in the United Kingdom. *Vet Rec*, 163 : 261-65.

McKee L.H. 1995. Microbial contamination of spices and herbs: A review. *Food Sc. Tech*. 28 : 1-11

Meng, X. J., R. H. Purcell, P. G. Halbur, J. R. Lehman, D. M. Webb, T. S. Tsareva, J. S. Haynes, B. J. Thacker, S. U. Emerson. 1997. A novel virus in swine is closely related to the human Hepatitis E Virus. *PNAS*, 94 : 9860-65.

Nicand, E., Enouf, V., Caron, M., 2005. Hépatite E, bilan d'activité du Centre national de référence des hépatites entéro-transmissibles, France, 2002-2004. *BEH*, 33, 167-168.

Omafuvbe, B.O., D.O., Kolawole. 2004. Quality Assurance of Stored Pepper (*Piper guineense*) Using Controlled Processing Methods. *Pakistan J Nutr*, 3 : 244-249.

Roque-Afonso, A.M., 2011. Rapport d'activité du CNR des virus des hépatites à transmission entérique A et E. 36 pp.

Serracca, L., I. Rossini, R. Battistini, M. Goria, S. Sant, G. De Montis, C. Ercolini. 2012; Potential Risk of Norovirus Infection due to the Consumption of "Ready to Eat" Food. *Food Environm Virol*, 4 : 89-92.

Smith, J. L. 2001. A Review of Hepatitis E Virus. *J Food Protect*, 64 : 572-86.

Tamada, Y., K. Yano, H. Yatsuhashi, O. Inoue, F.Mawatari, H. Ishibashi. 2004; Consumption of wild boar linked to cases of hepatitis E. *J Hepatol*, 40 : 869-70.

Tei, S., N. Kitajima, K.Takahashi, S. Mishiro. 2003. Zoonotic Transmission of Hepatitis E Virus from Deer to Human Beings. *Lancet*, 362, 9381: 371-73.

Tian-Cheng, L., T. Miyamura, N. Takeda. 2007. Detection of Hepatitis E Virus RNA from the Bivalve Yamato-Shijimi (*Corbicula Japonica*) in Japan. *Am J Trop Med Hyg*, 76 : 170-72.