Maisons-Alfort, 17 February 2013

The Director General

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

concerning the
"Request to assess the risks related to contamination of delicatessen meats products derived from raw pork liver with hepatitis E virus (HEV)"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES primarily ensures environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are made public.

The French Agency for Food, Environmental and Occupational Health and Safety (ANSES) received a formal request on 16 January 2012 from the Directorate General for Food (DGAL) to assess the risks related to contamination of delicatessen meats products derived from raw pork liver with the hepatitis E virus (HEV).

1. BACKGROUND AND PURPOSE OF THE REQUEST

A. Background information and questions posed in the DGAL request

The DGAL requested that ANSES examine the following questions concerning the selection of raw materials for the manufacture of raw pork liver-derived products, diagnostic tests for hepatitis E in liver homogenates, and the kinetics of the virus in finished products.

1. Is there a maximum slaughter age above which the probability of liver contamination, and therefore the risk for the consumer, can be considered negligible to low, particularly for products that are consumed raw?
2. What would be the minimum slaughter age enabling the possible presence of HEV in pig livers to be reduced to an acceptable level?
3. What criteria could be used to certify farms in terms of HEV?
4. Concerning diagnosis and further to the analyses carried out as part of the 2011 monitoring programme, what is the timeframe for the development of diagnostic tests for routine use as self-monitoring, with an affordable cost, to determine the HEV status of liver homogenates?
5. What additional work would be required to make this type of test available?

6. Concerning the presence of HEV in finished products, is it possible that an interval between actual manufacture and marketing could lead to a satisfactory reduction in the HEV risk to acceptable levels, given the use-by date?

For several years, ANSES has been highly active on this emerging topic regarding assessment of the transmission risk of hepatitis E to humans via food. The Agency has published a number of risk assessment opinions and has participated in several national research and investigation projects in order to develop knowledge that is essential in assessing the risks related to this virus (see Annex 1).

B. Scope of the expert appraisal

Some of the questions identified needed to be reformulated, and since certain issues do not fall within the Agency's areas of expertise, they were excluded from the scope of the ANSES expert appraisal (acceptable level for the reduction of the possible presence of HEV in pig livers, acceptable cost of tests to determine the HEV status of liver homogenates, acceptable HEV risk level).

Following reformulation, the following questions were addressed by the Agency:

- What data are available on the link between the slaughter age of animals and the presence of the HEV virus in the liver?
- Concerning analytical methods:
    - status of available methods and on-going development activities,
    - update on required conditions and possible difficulties regarding routine use as part of self-monitoring,
    - expected timeframes for the development of tests for routine use (identification of the possible additional work required).
- Regarding the impact on consumer health, what is the relevance of certification of farms as HEV-free? If necessary, identification of corresponding certification criteria (identification of various possibilities and their limits).
- What are the effects of processing methods for pork products on the impact on survival of VHE?

2. ORGANISATION OF THE EXPERT APPRAISAL[1]

The appraisal was carried out in accordance with the French NF X 50-110 Standard "Quality in Expertise – General Requirements of Competence for Expert Appraisals (May 2003)".

The collective appraisal was carried out between 29 November 2012 and 12 February 2013 by the Expert Committee (CES) on Assessment of the biological risks in foods, leader on this project and the Expert Committee on Animal health. This appraisal was based upon an initial report issued by a working group of rapporteurs from both committees and experts from ANSES laboratories.

Hearings of professional organisations, i.e. the French federation of the delicatessen meat industry (FICT) and the French Pig and Pork Producers' Association (INAPORC), provided additional information on zootechnical and economic aspects in the swine farming industry and on delicatessen meat products derived from raw pork liver.

[1] The English version of this section contains more details than the French

3. ANALYSIS AND CONCLUSIONS OF THE EXPERT COMMITTEES

A. Human epidemiology of hepatitis E in France: current data

In France, indigenous cases of hepatitis E have been described since 1996 in several regions (Bohme, Hadjadj *et al.* 1998; Corne, Yeche *et al.* 1997; Coton, Delpy *et al.* 2005; De Ledinghen, Mannant *et al.* 1996; Dupuy, Mayaudon *et al.* 1998). Since the start of the 2000's, several factors have contributed to understanding the epidemiology of this disease in France in terms of incidence, prevalence, risk factors and circulating genotypes. These include the creation of a National Reference Centre (NRC) in 2002, and the investigation of several episodes of grouped human cases.

1. Data concerning indigenous hepatitis E cases reported in France

a) Reported cases

For the first time in France, an article published in 2006 described the 13-month follow-up of a cohort of 23 patients with indigenous acute hepatitis E in south-western France (Peron, Mansuy *et al.* 2006). Most of the patients were male and the mean age was 54 years. The strains identified were all of genotype 3.

In 2007, the National observatory of cases for acute hepatitis E, set up by the National association of hepato-gastroenterologists of general hospitals (ANGH), recorded a total of 53 cases (10 before 2005, 14 in 2005, 24 in 2006, and 5 in 2007). Of these 53 cases, 90% were indigenous. 68% of affected patients were male, their mean age was 56 years, and 85% lived in Southern France. The strains isolated in the 14 viraemic patients were of genotype 3f. The main suspected sources of contamination were water (watering of vegetable gardens by river water or from private wells, consumption of water from a private well or spring), and consumption of shellfish (Renou, Moreau *et al.* 2008). The article does not indicate whether products derived from raw pork liver were taken into account in the study.

The National Reference Centre (NRC) on enterically transmitted hepatitis (hepatitis A and E), created in April 2002, collects samples to confirm diagnosis or for typing. Between 2002 and 2011, there was an increase in the number of patients for whom blood or stool samples were sent to the NRC HEV for diagnosis of hepatitis E, and the number increased five-fold between 2006 and 2011 (Table 1)[2]. The number of diagnosed indigenous cases increased from 9 in 2002 to 249 in 2011 (Table 1). Cases were reported from all regions of mainland France, but most were from the South. More than half of the indigenous cases were from Midi-Pyrénées, Languedoc-Roussillon or Provence-Alpes-Côte d'Azur (Nicand, Bigaillon *et al.* 2009; Nicand, Enouf *et al.* 2005). This trend could reflect a real increase in the number of cases, but could also be explained by a higher number of diagnosed cases. The reason is that clinicians increasingly suspect hepatitis E, as shown by the rise in the number of samples received at the NRC, without any increase in the proportion of certain or probable cases diagnosed among the tested cases (Table 1).

[2] www.cnr.vha-vhe.aphp.fr

Table 1: Number of cases of hepatitis E diagnosed by the NRC HEV, France, 2002-2011. *Source: NRC reports on enterically transmitted hepatitis viruses (A and E)*

Years	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
Number of patients tested										
	209	155	233	327	583	1 012	1 700	2 150	2 549	3 429
Number of certain or probable cases										
Total	13	14	20	39	38	107	180	206	232	266
Imported*	4	11	4	19	14	10	21	23	16	17
Indigenous	9	3	16	20	24	97	159	183	216	249
% of positive results among tested cases										
	6.2	*9.0*	*8.5*	*11.9*	*6.5*	*10.5*	*10.5*	*9.6*	*9.1*	*7.6*

** travel to an endemic area in the 3 months preceding onset of the disease*

In 2008 and 2009, among the cases of indigenous hepatitis E documented by the NRC, there were 10 reports of consumption of Corsican delicatessen meats, liver sausages or figatelli, two to 10 weeks before the onset of clinical signs. In four cases, patients had consumed delicatessen meats in Corsica: pork liver sausages (two cases), figatelli (one case) and local delicatessen meats (one case). Among the remaining six cases, none of the patients had travelled to Corsica but five had consumed figatelli and one Corsican delicatessen meats.

In 2010, the French Institute for Public Health Surveillance (InVS) and the NRC carried out a prospective descriptive study of the indigenous cases of acute hepatitis E diagnosed by the NRC between 1 January and 31 December 2010. A total of 139 indigenous cases were included. The study confirmed the regional differences with 65% of cases from the south-east and south-west regions of France[3]. Eight patients indicated that they developed Parsonage-Turner syndrome (PTS) (neuralgic amyotrophy) or intense disabling pain in one or both shoulders at the start of the disorder that could be suggestive of this syndrome. In all cases, consumption of pork products was reported. In more than a third of cases (39%), patients had consumed products containing raw pork liver (figatelli, Toulouse liver sausages). The only significant regional differences identified (south-east, south-west, north) were that a higher proportion of patients living in the south-east consumed products derived from raw pork liver (52%), while a higher proportion of those in the south-west lived in a rural environment (76%) and had a garden (67%). No episode of grouped cases was detected during the year under study (E. Couturier, personal communication).

A prospective case-control study conducted between 2004 and 2009 concerning 37 cases of hepatitis E, and 148 organ transplant control cases from the south-east of France, demonstrated that the only risk factor independently associated with indigenous HEV infection was the consumption of game meat (68% *versus* 47%; OR[4] = 2.32; CI = 95% 1.04-5.15) (Legrand-Abravanel, Kamar *et al.* 2010).

b) Recent data

Like in other industrialised countries, indigenous cases in France were found to be of genotype 3, with predominance of a cluster of sub-genotype 3f (Peron, Mansuy *et al.* 2006; Renou, Moreau *et al.* 2008). A recent French study comparing viral sequences of HEV in cases of indigenous hepatitis E and in a representative sample of pig livers collected from slaughterhouses showed the same proportion of sub-types in the human and swine populations (3f, 74%; 3c, 13%; 3e, 5%), and

[3] Most cases involved men (74%); mean age was 54 years. The reported genotype in 122 cases was: 3f in 91 cases (74%), 3c in 17 cases (14%), 3e in 7 cases (6%), 3a in 2 cases (2%), 3b in 1 case (1%), not determined in 4 cases. More than half of the cases (59%) had pre-existing comorbidities, including 12% kidney transplants and 19% requiring treatment with an immunosuppressant.

[4] OR: Odds Ratio

more than 99% homology between the viral sequences of human and animal origin (Bouquet, Tesse *et al.* 2011). Two articles published in 2012 describe for the first time the presence of genotype 4 in three indigenous cases (Bouquet, Tesse *et al.* 2011; Colson, Borentain *et al.* 2007; Tesse, Lioure *et al.* 2012).

Clinically, severe indigenous cases of fulminant hepatitis E have been reported in several studies (Dupuy, Mayaudon *et al.* 1998; Mennecier, Nicand *et al.* 2000; Peron, Bureau *et al.* 2007). Since 2009, a few publications have documented neurological manifestations in association with HEV, particularly Parsonage-Turner syndrome or neuralgic amyotrophy. In 2011, a study conducted in France and the United Kingdom showed that of 126 patients with acute or chronic hepatitis E of genotype 3, seven presented neurological disorders and one bilateral neuralgic amyotrophy (brachial neuritis) (Kamar, Bendall *et al.* 2011). Cases of transfusion-associated hepatitis E have also been reported in France (Colson, Coze *et al.* 2007).

2. Seroprevalence studies

Several recent seroprevalence studies carried out in various populations, using diagnostic tests with higher sensitivity than those in earlier studies, have all demonstrated a high seroprevalence that varies depending on geographical area and population group and that increases with age.

- A study in 1998 blood donors from Ile-de-France and Pays de la Loire found mean seroprevalence of anti-HEV antibodies of 3.20%, increasing with age (Boutrouille, Bakkali-Kassimi *et al.* 2007).

- In a study carried out in 2003-2004, the overall prevalence in 512 blood donors from the Midi-Pyrénées region was found to be 16.6%, with highest values among hunters (Mansuy, Legrand-Abravanel *et al.* 2008). A subsequent analysis on the sera of these blood donors with a more sensitive validated test (Bendall, Ellis *et al.* 2010) revealed a prevalence of 52.5% [95% CI 48.2-56.8], three times higher than that observed with the test used in the first study. These results suggest that hepatitis E is hyperendemic in the Midi-Pyrénées region (Mansuy, Bendall *et al.* 2011).

- A 2009-2010 study in mainland France among the general population involving a representative sample of the French population of 5300 people aged 6 to 49 years showed an overall seroprevalence of anti-HEV antibodies of 4.9% [95% CI 4.1-5.8]. The prevalence was found to be higher in the south-west and south-east regions, 9.0% [95% CI 5.9-13.4] and 7.1% [95% CI 5.5-9.0] respectively, than in the northern regions, 3.4% [2.6-4.4]. Seroprevalence increases with age, regardless of the region where the study subjects live (Lepoutre, Antona *et al.* 2011).

- A study involving 593 forestry workers in Champagne-Ardenne, Burgundy, Franche-Comté, Alsace and Lorraine, from whom samples were taken in 2002 and 2003, and 135 professionals not exposed occupationally to wild animals from whom samples were taken in 2002, 2003 and 2011 (control population) revealed a significantly higher risk of HEV infection (OR = 2.2; p = 0.003) in woodcutters and forestry workers in close contact with wild animals (prevalence = 37%), than in non-exposed professionals (prevalence = 19%), game wardens (prevalence = 20%) and silviculturists (prevalence = 25%) (Carpentier, Chaussade *et al.* 2012). Prevalence was also significantly higher in Alsace (prevalence = 44%) and in Lorraine (33%) than in three other regions (Franche-Comté 23%, Burgundy 17%, Champagne-Ardenne 12%). Prevalence increased significantly with age.

- A national study undertaken between September 2011 and March 2012 in 304 swine farmers, 231 forestry workers and 322 tertiary sector workers from the north-west, north, north-east, south-west, south-east and Corsica also found that HEV seroprevalence was significantly higher in swine farmers (prevalence = 44%) *versus* workers in the tertiary sector (prevalence = 26%) (OR = 2.5; p = 0.0001), in workers living in the south (prevalence = 41%) *versus* those in the north

(prevalence = 31%) (OR = 1.47; p = 0.02) and among consumers of figatelli (prevalence = 29% in non-consumers *versus* 38% in occasional consumers, and 66% in frequent consumers), with a risk multiplied by 1.7 (OR = 1.7; p = 0.003) among occasional consumers *versus* non-consumers, and multiplied by 4.5 (OR = 4.48; p < 0.0001) in frequent consumers (Carpentier, Chaussade *et al.* 2012; Chaussade 2012).

3. Description of episodes of grouped cases of hepatitis E in France

Since 2005, five episodes of grouped cases of hepatitis E, including three foodborne illness outbreaks have been investigated. Consumption of figatelli was the most plausible source in two of these outbreaks.

- Between April 2005 and June 2006, six indigenous cases of hepatitis E were reported from a restricted geographical area near the Gapeau plain (Var *département*). The exposure risks were found to be consumption of water from a private well in four cases, and the presence of a domestic pig in one case. Water samples from the Gapeau river and samples taken from several animal species (goats, sheep) were screened for HEV by PCR to evaluate the possible role of water and the animal reservoirs in occurrence of these cases. HEV (genotype 3) was found in one water sample. The exact source of contamination in these cases has not been determined (unpublished data, Local health and social affairs authority (DDASS du Var), Dr A. Deccopet).
- In 2006, two cases of indigenous familial hepatitis E were attributed to consumption of dried pork about 4 weeks before onset of jaundice (Deest, Zehner *et al.* 2007).
- During summer 2007, in the Vaucluse *département*, three indigenous familial cases of hepatitis E (genotype 3f) were related to a meal shared by four people one month before onset, during which figatelli were served. The three consumers of raw figatelli presented hepatitis E. The fourth person who did not eat figatelli did not develop the disorder (InVS and CIRE 2007).
- In September 2008, the relationship between consumption of raw figatelli and hepatitis E was suggested in a case-control study carried out in the families of three symptomatic patients (genotype 3f). Acute or recent hepatitis E was diagnosed among seven of 13 family members who consumed raw figatelli *versus* zero cases among the five who did not consume the dish (p = 0.04). Of 12 batches of figatelli purchased in various supermarkets in the south-east, but from batches distributed after those consumed by the study patients, seven were found by PCR to be positive. Sequencing found two viral strains in these figatelli (genotypes 3f and 3e) close to those in the patients who consumed this product (Colson, Borentain *et al.* 2010).
- In 2011, investigation of 11 grouped cases of hepatitis E (eight symptomatic and three asymptomatic kidney transplant patients) occurring between January and March 2011 among patients in the Bouches-du-Rhône (10 cases) and Var *départements* (one case) found a high frequency of consumption of figatelli (6/11, 54.5%). Epidemiological, veterinary, and microbiological investigations did not support a common source of contamination. The viral genotypes identified among the 10 patients were different (four 3f, four 3c, and two 4a). The virus of genotype 4a was found in two patients who had eaten raw figatelli. The sequences of the virus were genetically similar (96.5%) to those recently described in swine in Belgium and the Netherlands (Colson, Romanet *et al.* 2012).

Key points concerning human epidemiology of hepatitis E in France

- ✓ The number of cases of indigenous hepatitis E diagnosed in France has increased since the implementation of a monitoring system by the NRC in 2002. This increase is associated with an increase in the number of diagnostic tests performed. The observed incidence is higher in the southern regions of the country.
- ✓ Several recent seroprevalence studies among different populations demonstrate that seroprevalence may be high (up to 50%). It increases with age, is higher in the south of France, and in populations who are exposed occupationally, such as swine famers and woodcutters in contact with wild animals. The contrast between high seroprevalence and the low number of diagnosed cases suggests that the majority of infections are asymptomatic or cause few symptoms. HEV infections can however be severe (fulminant hepatitis) and may become chronic in immunodepressed patients, particularly transplant subjects.
- ✓ In France, like in other industrialised countries, the viruses found in indigenous cases are primarily of genotype 3, with a predominance of a cluster of sub-genotype 3f.
- ✓ Various risk factors and sources of contamination have been identified or are suspected based on different studies and the investigation of sporadic or grouped cases. These include transfusion, contact with swine, rural environments, game meat consumption, consumption of water from boreholes or wells, shellfish, or products containing raw pork liver, and at-risk groups such as hunters or woodcutters. Consumption of products derived from raw pork liver, and in particular figatelli, appears to be one of the most significant risk factors, specifically for populations in the south-east of France.

B. Qualitative and quantitative aspects of HEV transmission on swine farms

Several species are hosts to the virus, but the main animal reservoir for HEV is swine and more generally Suidae. Although infection in the domestic pig or breeding pigs (*Sus scrofa domesticus*) is asymptomatic, the virus multiplies readily and is shed in high quantities.

Most studies in breeding conditions show that the main source of HEV shedding is growers, primarily from three months of age, and above all in the first month of fattening (60%), followed by weaners (41.7%) (Fernandez-Barredo, Galiana *et al.* 2006).

However, it has also been demonstrated among herds in Spain that nearly 16% of sows shed the virus postpartum and 17% prepartum, indicating a possibility of transmission from sow to sow, and from sow to piglet (Casas, Cortés *et al.* 2011; De Deus, Casas *et al.* 2008; Fernandez-Barredo, Galiana *et al.* 2006). In another study carried out in Italy, a high level of shedding was found in multiparous sows (more than two litters) and in gilts and young sows, but to a lesser extent (Di Bartolo, Martelli *et al.* 2008). Therefore, horizontal transmission from the sow to the piglet by contact infection, and vertical transmission *in utero* cannot be ruled out (De Deus, Casas *et al.* 2008). The sow can shed the virus but can also potentially transmit the virus to its foetus via the transplacental route in the event of viraemia during gravidity. Viral RNA has also been detected in foetal livers following abortion (Hosmillo, Jeong *et al.* 2010). These results are however controversial. Indeed, an experimental study (Kasorndorkbua, Thacker *et al.* 2003) did not show vertical transmission following intravenous inoculation of gestating gilts. However, despite the practical difficulties of observing these shedding processes in the sow, it is not possible to rule out this category of animal as a reservoir for HEV in infected swine farms. Sows may maintain spread of the virus on swine farms.

The virus is shed primarily via the faecal route in swine, leading to accumulation of HEV in the living environment of animals on infected farms. Studies have demonstrated a relationship between HEV shedding by pigs and its presence in manure pits on these farms (Fernandez-Barredo, Galiana *et al.* 2006). Depending on the type of floor surface (bedding or grating with pre-pits for manure storage),

the animals remain in constant contact with manure, resulting in varying degrees of direct exposure to this environmental source of HEV. When pens are fitted with pre-pits, transmission via manure suspensions may be possible, particularly during mixing and emptying.

Several studies have detected the virus in the urine of swine that were in contact with fellow pigs inoculated intravenously or infected naturally (Banks, Bendall *et al.* 2004; Bouwknegt, Rutjes *et al.* 2009; Bouwknegt, Teunis *et al.* 2011). It appears that urine is also a major route of HEV transmission in swine, given the volumes excreted daily and the longer shedding times of the virus via urine (Bouwknegt, Rutjes *et al.* 2009).

On breeding farms, in view of the faecal and urinary shedding routes, drinking water or feed may also be indirect propagation vectors for a group of livestock, particularly if the feed and water supplies can easily be soiled by excrements (Fernandez-Barredo, Galiana *et al.* 2006). Therefore, repeated daily contact between pigs in the same pens in a confined space, as well as transfers at different stages of production (changes in facilities or environments) appear to accelerate spread of HEV within farms (Bouwknegt, Frankena *et al.* 2008; De Deus, Casas *et al.* 2008; Kasorndorkbua, Guenette *et al.* 2004).

These findings confirm that the faecal-oral pathway is the main route of HEV transmission in swine (Bouwknegt, Rutjes *et al.* 2009; Casas, Pina *et al.* 2009; Kasorndorkbua, Guenette *et al.* 2004), although several studies highlight the difficulty of inoculation via the oral route (Bouwknegt, Lodder-Verschoor *et al.* 2007; Kasorndorkbua, Halbur *et al.* 2002). It is estimated that infection by oral inoculation requires a dose of viral particles 4 times higher (about 15 g of faeces per day at 10^8 genome equivalents (gEq) per g for 3 consecutive days) than the dose required for intravenous inoculation (Bouwknegt, Teunis *et al.* 2011). Experimentally, a minimum load of 10^6 gEq/g appears to be needed to infect swine orally, and so that they are able to shed the virus and transmit it to other animals (N. Rose, personal unpublished communication, HEVECODYN project). Propagation on farms via the faecal-oral transmission route suggests that high viral loads probably accumulate in the environment in order for the transmission process to be maintained.

Persistence of the virus on breeding farms depends on (i) the intrinsic capacity of the virus to persist in the animals' environment, (ii) the likelihood of regular reintroduction into the farms, and (iii) the ability of the virus to be maintained and to spread in the population. The third criterion can be measured by the basic reproduction number (R_0) of the virus, which indicates the number of secondary infections generated by an infected pig over the entire period of shedding, in a fully susceptible population. The higher this rate, the more readily the virus can spread between animals, and the greater its ability to persist in the population. In theory, below an R_0 threshold of 1, the virus will die out in the population (in the absence of re-introduction). For HEV, research conducted by a Dutch team (Bouwknegt, Frankena *et al.* 2008), demonstrated that this ratio can be estimated to be 8.8, implying that an infectious animal can theoretically contaminate more than eight others during the infectious period. This experimental estimation is however based on repeated one-to-one contacts, and on the production of naturally infected animals (to ensure contacts with susceptible animals) after initial exposure of these index animals to pigs inoculated intravenously. Likewise, experimental models on groups of swine inoculated orally and in direct or indirect contact with susceptible pigs (separated pens), provide preliminary estimates confirming that the virus is able to spread in a susceptible population and to be maintained, but that its spread appears to be strongly restricted if the animals are not in direct contact (on-going HEVECODYN project funded by the French National Research Agency (ANR)).

The presence of the virus in the wild animal reservoir, and particularly in the wild boar population, has been reported in many articles in the literature in most countries where indigenous hepatitis E cases have been described: Japan (Sakano, Morita *et al.* 2009; Sonoda, Abe *et al.* 2004); Germany (Adlhoch, Wolf *et al.* 2009; Schielke, Sachs *et al.* 2009); Netherlands (Rutjes, Lodder-Verschoor *et al.* 2010); Italy (Martelli, Caprioli *et al.* 2008); Spain (De Deus, Peralta *et al.* 2008) and France (Kaba,

Davoust *et al.* 2009; Payne, Rossi *et al.* 2011). Reported prevalence is however highly variable between studies and countries. In France, the results show an apparent seroprevalence between 7.2 and 22.7% depending on the *département,* with higher seroprevalence in Southern France (Payne, Rossi *et al.* 2011). Wild boar populations are thus a substantial reservoir likely to represent a risk to swine populations farmed in open-air conditions. There are however no data on HEV prevalence on this type of farm. The estimated prevalence in domestic swine is nonetheless significantly higher than the estimated prevalence in wild animals (Sakano, Morita *et al.* 2009), suggesting factors favouring persistence on swine farms independent of regular introduction from wild animals.

Key points concerning HEV transmission on swine farms

- ✓ Growers (weaners and fatteners) constitute the main source of HEV shedding. The role of sows as a reservoir on infected farms cannot be ruled out.
- ✓ The faecal-oral route is the main HEV transmission pathway in swine. Persistence of the virus on breeding farms depends on (i) the intrinsic capacity of the virus to persist in the animals' environment, (ii) the possibility of regular reintroduction into the farms, and (iii) the ability of the virus to be maintained and to spread in the population (R_0>1).
- ✓ Given the faecal-oral route of propagation, pig-to-pig transmission is dependent on accumulation of high viral loads in the environment. The propagation process within farms is therefore closely related to animals' levels of contact with their excrements. Hygiene factors such as cleaning, disinfection, emptying of pre-pits, and sub-floor areas, as well as husbandry practices including mixing animals with different infectious statuses during the farming period, are likely to have a significant impact on spread of the virus within the population.

C. Relationship between slaughter age and HEV contamination of pig livers

1. Slaughter age of swine in France

There are two main categories of animals that are likely to be used as sources of raw liver-containing products: fattening pigs and cull animals (mainly sows and boars).

For delicatessen meat, payment for carcasses is calculated using:

(i) a base price determined by the Breton Pork Market with a 56% LMP (lean meat percentage),

(ii) a range of 80 to 102 kg with a premium of 2 eurocents in the range 85 – 97 kg (hot carcass weight without kidneys or skirt meat),

(iii) with a premium or discount depending on the LMP of the carcass (+2 to +4 eurocents per additional LMP percentage and -2 cents to -4 cents for fatty carcasses).

It is therefore not the age of the animals that determines the time of slaughter, but the live weight estimated or measured by the farmer. Breeders are penalised if they send animals for slaughter that are too heavy or too light (out of range).

Breeding farm performance is partially measured based on the age of animals at 115 kg of live weight. The lower the age the better the growth performance of the livestock since minimising this criterion is one factor that guarantees revenue for the breeder. In 2011, the standard age[5] at 115 kg was 183 days (SD = 10) for Brittany, 39% of swine farms were at less than 180 days, and 3% were at more than 200 days (Ifip 2012). Based on 2011 technical economic management data published by IFIP concerning breeder-fattener stock farmers, the mean age at a mean weight of 116.2 kg at the end of

[5] Standard age is measured based on mean weight on finishing and using a growth curve taking into account average daily gain (ADG) for the period.

the fattening period is 185 days (SD = 18, CI = 95%) (Source: IFIP: mean result calculated based on mean values per swine farm for 1747 farms).

In certain livestock sectors, specific quality charters and labels require that animals be slaughtered at a minimum age, for instance 182 days for free-range “Label Rouge” quality-certified swine. In others, breeders aim to produce heavy livestock, for example free roaming Gascony Noir de Bigorre pigs, which are bred for longer periods of time, and swine that are slaughtered at 12 months. The volumes are however quite low. In other swine farming sectors for heavier pigs, standard slaughter ages are about 182 days.

Breeding animals, primarily sows, are slaughtered at an adult age when they are culled to renew breeding stock. In 2010, the mean age of cull sows was 35 months (SD = 5.3) for Brittany, and 32.9 months (SD = 5.8) nationally, with a mean of 5.1 to 5.4 litters per sow. In Brittany, 66.3% of swine farms cull sows before 36 months, and 9% at more than 42 months.

2. Relationship between age and HEV shedding in swine

Since the end of the 2000s, a number of studies have sought to describe the infection dynamics of the virus by showing that the prevalence of HEV RNA in faeces and in serum in swine depends on the production stage, and therefore on the age of the animal (Annex 2). The results obtained in the various studies are fairly similar. In most cases, **within the swine population of a farm**, the animals collectively shed the virus over a long period of time, approximately from 1.5 to 5 months of age, with manifest viraemia between 2 and 4 months of age. Durations and times to onset of viraemia and shedding appear to be similar (Seminati, Mateu *et al.* 2008). In another study, shedding was observed over a very long period in the 10 studied animals: from 40 to 100 days of age, i.e. for about 9 weeks (Kanai, Tsujikawa *et al.* 2011). In most available studies, the number of animals observed was generally very low, resulting in poor accuracy of the estimated number of shedding animals, thus explaining the differences found between investigations.

However, in the vast majority of studies, peak faecal shedding is found at around 3 to 4 months of age (De Deus, Casas *et al.* 2008; Fernandez-Barredo, Galiana *et al.* 2007; Takahashi, Nishizawa *et al.* 2005) and very few animals are found by PCR to be positive after 6 months of age, depending on the study: less than 10% (McCreary, Martelli *et al.* 2008; Nakai, Kato *et al.* 2006), or even no animals (De Deus, Seminati *et al.* 2007).

The faecal shedding data as a function of age presented in Annex 2 are summarised below in the form of a meta-regression second order polynomial model for these parameters (Table 2), taking into account the respective weights of publications calculated using the inverse of the sum of inter- and intra-study variance for a given age. The mean predicted response of the model and its confidence interval are presented in Figure 1.

Table 2: Second order polynomial model for the relationship between prevalence of faecal HEV shedding and animal age, 13 publications.

	Coefficient	Standard deviation	t value	Pr (>|t|)
Constant	-0.0815	0.074	-1.11	0.27
age	0.01	0.002	4.64	<0.0001
age^2	$-5.08.10^{-5}$	$1.2.10^{-5}$	-4.27	0.0001

R^2 (adjusted): 0.33
F-statistic: 11.2 with 2 and 39 ddl, p-value: <0.0001

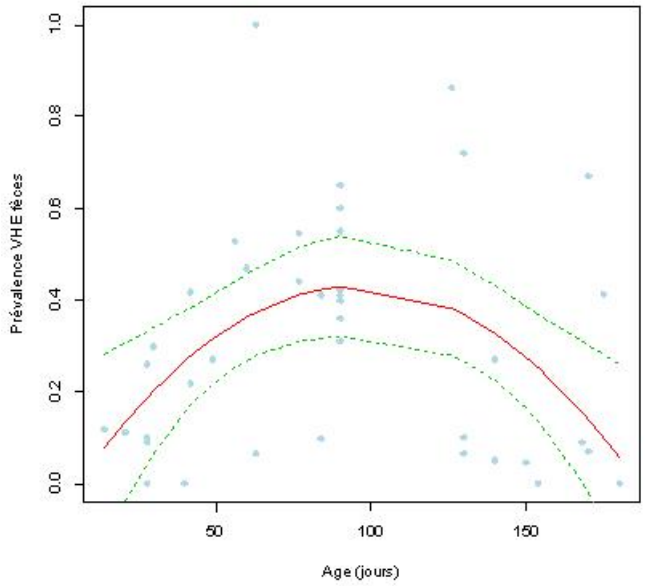

Figure 1 : Predicted response of the second order polynomial model for prevalence of faecal shedding as a function of age, 13 publications.

Despite considerable variability between the studies, the model indicates probable maximum faecal shedding for the population between 90 and 120 days of age. It also shows that the prevalence of shedding can no longer be extrapolated beyond 188.5 days of age, on average. There is no study enabling analysis beyond 175 days. The response values from the model for prevalence of faecal shedding at the age of 175 and 185 days are 11% [95% CI 0-16%] and 3% [95% CI 0-17%], respectively. These predictions are consistent with the estimated prevalence of contamination in livers from fattening pigs sent for slaughter in France (4% [95% CI 0.02-0.06] (Rose, Lunazzi *et al.* 2011).

Since the end of 2010, certain observational studies have provided more precise data on infection dynamics, taking into account individual variability by implementing regular longitudinal follow-up of swine from birth to slaughter. In the study conducted by Casas *et al.* (Casas, Cortés *et al.* 2011), IgM antibodies which are produced very soon after infection but decline rapidly, were detected for the first time in 20 study pigs aged 7 to 13 weeks on the six stock farms. On slaughter, at around 25 weeks, IgG antibodies, which appear much later but persist for longer periods than IgM, were present in 50 to 100% of test animals on five of six swine farms. Likewise, 11.5% of the 96 animals slaughtered were found positive by PCR (liver and bile). In some studies, the various immunoglobulin isotypes were assessed and demonstrated later appearance of IgG (from 15 weeks of age) compared to IgA and IgM which appeared at 12 weeks of age in naturally infected swine (45 piglets in one farm) (De Deus, Casas *et al.* 2008). In this study, viral RNA was found in sera of swine at all ages, with the highest prevalence observed at 15 weeks of age, and in the faeces and lymph nodes from 9 weeks of age, with a peak between 12 and 15 weeks (faeces and lymph nodes, bile and liver in necropsied animals). At 18 weeks of age, HEV could still be detected in liver (2/5) and in faeces (2/5) in necropsied swine. A correlation was also found between viraemia and IgG and IgM seroconversion at 15 weeks of age. These field studies carried out in actual conditions demonstrate collective infection dynamics and show several differences that could be explained by highly variable individual dynamics.

Intra-farm seroprevalence distributions observed in a national survey on HEV prevalence at slaughterhouses (Rose, Lunazzi *et al.* 2011) suggest highly variable individual infection dynamics. Close individual follow-up of successive cohorts on infected swine farms provides insight into individual variability (study on-going, ANR/HEVECODYN project). Initial results obtained (three

cohorts in one infected swine farm) indicate that the probability of contamination of livers at slaughter is closely related to the interval between infection and slaughter of the animals. Preliminary estimates based on this first farm show that the risk period is **an infection/slaughter interval of less than 20 days**. This is consistent with experimental data on sequential slaughter after infection with HEV (ANR/HEVZOONEPI report). During these studies, animals were infected intravenously and slaughtered at 4, 8, 14 and 21 days post-inoculation (n=3 pigs at each time point). Quantification data concerning the genomic load in pig livers after slaughter show a maximum load 8 days post-inoculation, followed by a gradual decline, and the absence of viral genome at 21 days post-inoculation (Figure 2). These results suggest that the animals eliminate the virus from the liver by 21 days post-inoculation via the intravenous route.

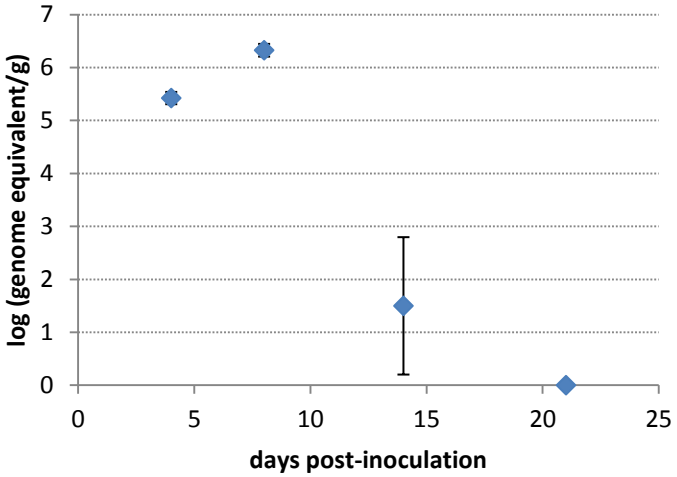

Figure 2: Genomic HEV load in the liver at different post-inoculation times (n=12 pigs) following intravenous inoculation

In real conditions, the probability of the presence of virus in the liver cannot be directly inferred from the slaughter age since the exact date of infection is not known. Within infected swine farms, most infections take place in the first and second thirds of the fattening stage, as described in the literature. However, considerable individual variability is found as shown by the survival curve representing the probability of non-infection before a given age (Figure 3).

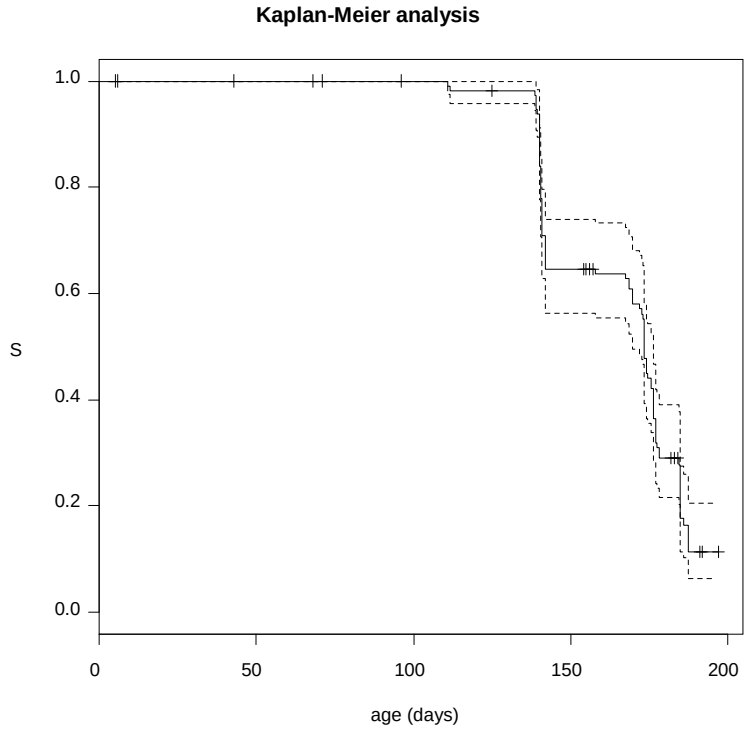

Figure 3: Survival analysis of HEV infection age data (n=120 individually tested pigs, one HEV infected farm) (On-going ANR/HEVECODYN project).

Although there are few data on sows, a study conducted in Italy (Di Bartolo, Martelli *et al.* 2008) showed that the prevalence of shedding was higher in the oldest sows (> 2 litters) *versus* gilts and young sows (53.4% *versus* 38.6% and 43.1%, respectively). These results suggest that adult pigs are likely to be re-infected during their productive lifetime (transient immunity, re-infection by different strains) or, if potential chronicity is considered, that they are likely to shed the virus again during decreased immunity.

3. Factors influencing the age of infection

The data presented above show that infection dynamics vary, with shedding and seroconversion at different times following infection. These variations suggest that there are factors that affect virus propagation on swine farms.

The presence of maternal antibodies in the piglet does not stop infection but delays onset of viraemia and seroconversion (Dos Santos, Vitral *et al.* 2009; Kanai, Tsujikawa *et al.* 2011). The duration of presence of antibodies is also dependent on antibody titre in mothers (Casas, Cortés *et al.* 2011), which is strongly correlated with the age of the sow (Klobasa, Butler *et al.* 1987). IgG immunoglobulin remains until 9 weeks of age in piglets born to highly seropositive sows, compared to 1 to 3 weeks in those born to sows with low seropositivity (De Deus, Casas *et al.* 2008). The same patterns were found in earlier studies (Meng, Purcell *et al.* 1997). Passive immunity lasting for longer periods of time is likely to delay the infectious process in the piglet. It is important to note that some piglets remain seronegative despite being born to seropositive sows. This may be explained by insufficient or inadequate consumption of colostrum, or by adoption practices during lactation (Casas, Cortés *et al.* 2011).

The layout of the breeding facility and the nature of contacts between animals within a farm (multiple pigs in a pen, multiple pens in a unit, and multiple units in a barn, etc.) can affect HEV transmission (Bouwknegt, Frankena *et al.* 2008). In order to stop propagation of infection, HEV-free swine should

be separated from infected stock immediately after weaning, and the two groups should not be mixed subsequently in order to minimise the number of HEV positive pigs at finishing.

In a modelling study, the effect of fictive late or early vaccination on infection dynamics of the virus was assessed (Backer, Berto *et al.* 2012). Later vaccination at 10 weeks of age would be a better strategy since it would reduce the proportion of infectious pigs at slaughter age. By vaccinating at 10 weeks of age, the infectious period is shortened and the number of infectious animals at slaughter age is reduced, while vaccinating at 3 weeks of age reduces the transmission rate early on, but the proportion of infected animals at slaughter is higher.

Key points concerning slaughter age of swine and HEV infection

- Slaughter of swine concerns fattening pigs and cull animals (mainly sows and boars).
- In pigs, the factor affecting slaughter is the live weight of the animal as measured or estimated by the farmer and not age.
- On the basis of a model generated from published data, the prevalence of faecal shedding of HEV is highest at the age of 90 to 120 days, and stops after a mean of 6 months.
- Preliminary results of on-going studies appear to show that an interval between infection and slaughter of less than 20 days increases the risk of contamination of the liver.
- On infected swine farms, most infections occur in the first and second thirds of the fattening stage, but there is considerable individual variability.
- Adult pigs are likely to shed the virus following re-infection (transient immunity, re-infection by different strains).
- In real conditions, the probability of presence of the virus in the liver cannot be directly inferred from the slaughter age, since the exact infection date is not known.

D. HEV analytical methods

1. Diagnostic methods in animals

Identification of HEV infection involves detection of anti-HEV antibodies or detection of the HEV genome by molecular amplification. Like most hepatitis viruses, HEV is difficult to culture *in vitro*. Detection of the viral genome does not necessarily imply the presence of infectious virus. The only model enabling confirmation of infectious virus is the experimental swine model, but it cannot be used routinely. Another factor that may limit analysis of samples infected with HEV is its classification as a biosafety level 3 infectious agent, requiring handling in a laboratory or animal housing facility with a high containment level (L3, A3). No vaccine or treatment is currently available for this zoonotic virus.
For a long time, HEV detection was performed by a few research laboratories using their own “in-house” methods. Over the past few years, with identification of the zoonotic origin of hepatitis E, several commercial serological or molecular tests have been developed for use in humans or animals. The ANSES Maisons-Alfort Animal Health Laboratory has approved in-house two commercially available tests for serological diagnosis in swine (Barnaud, Rogee *et al.* 2012; Rose, Boutrouille *et al.* 2010). However, the commercial release of at least four new test kits would require a more thorough comparative analysis before recommending routine use of one of these tests. Although there is only one serotype of HEV, the performances of these tests vary depending on the genotype of the antigens used (genotype 1: Se 0.47 [0.39–0.55], Sp 0.98 [0.95–0.99]; genotype 3: Se 0.92 [0.81–0.99], Sp 0.98 [0.93–0.99]) (Rose, Boutrouille *et al.* 2010). Moreover, some of these tests only detect IgG while others detect all the classes of immunoglobulin (IgA, IgM, and IgG). Multiple immunoglobulin tests are also able to identify recent infections which involve a higher risk of liver contamination in animals.
Methods for molecular detection of HEV in faecal matter, serum, muscle, and the liver in swine have also been developed and validated in-house by the Maisons-Alfort Animal Health Laboratory, on the

basis of a non-commercial method (Rose, Lunazzi *et al.* 2011). This detection technique involves conventional RT-PCR for sequence typing, coupled with real-time RT-PCR for quantification of HEV in genome equivalents. Since then, at least two real-time RT-PCR methods have been placed on the market. To simplify routine use in laboratories, validation of these commercial kits would be required, given that HEV is a virus with considerable genetic variability. A recent study conducted by the NRC HEV demonstrated the differences in test sensitivity for the detection of various sub-types of HEV depending on the genomic region amplified (Abravanel, Sandres-Saune *et al.* 2012). These genomic regions are not specified in the various molecular diagnostic kits in order to maintain confidentiality. The performance of these kits must be evaluated with the major subtypes circulating in the swine population in France (3e, 3c and 3f), but also with other genotypes such as genotype 4. Human cases associated with genotype 4 have recently been reported (see Epidemiology of HEV in France), suggesting that this genotype has been introduced into French herds. The first European swine farm (Belgium) infected with genotype 4 was described in 2011 (Hakze-van der Honing, van Coillie *et al.* 2011).

2. Analytical methods for HEV in meat-based food matrices

Assessing the probable transmission of HEV via consumption of food containing contaminated pork liver requires methods that can detect HEV in meat-based food matrices. There are several types of products suspected as sources of contamination: dry sausage derived from raw pork liver (dry liver sausage, figatelli, fitone), dry salted liver, fresh liver sausages, and liver dumpling (quenelle) dough. These complex matrices are composed primarily of liver, fats, salt and spices.

There is currently no standardised method in food safety for viral diagnosis. The European Committee for Standardisation (CEN) is working on a draft standard for the detection of norovirus and the hepatitis A virus in food (plant matrices, fruits, and seafood). The recommendations of this group on the various molecular detection methods, and the controls used to validate diagnostic results have been described in two publications (Giuffrida, Troia *et al.* 2011; Lees and Tag 2010). When the project for the development of a standard was initiated by the CEN, HEV had not been identified as an infectious agent with a risk of transmission by food. However, it has now become essential to develop a sensitive and specific technique for the detection of HEV in food, in line with general CEN recommendations. The two main obstacles to viral diagnosis in food safety are the concentration of viruses in food, which can be low, and the presence of RT and/or PCR reaction inhibitors in the food matrices.

It is important to note that unlike norovirus or HAV contaminations which occur mainly on the surface of food, e.g. vegetables and fruits, or through concentration in shellfish, HEV contamination is intrinsic in the raw material due to infection of liver cells, which leads to differences in the analytical methods.

3. Available methods and on-going development

a. Available methods

In Europe and more widely at the international level, there are few studies that focus on the role of pork-derived food products in the transmission of HEV. Only a few research laboratories, such as the AHVLA in the United Kingdom or the ISS in Italy, have worked on HEV detection throughout the swine production chain and in food (fresh sausages) (Berto, Martelli *et al.* 2012; Di Bartolo, Diez-Valcarce *et al.* 2012). The detection method for HEV described in these two studies is identical. It involves grinding of the samples in a lysis buffer solution, followed by extraction of RNA. In both studies, a process control consisting of addition of murine norovirus (MNV) was used from the extraction phase. Molecular detection of HEV by real-time RT-PCR from RNA follows the protocol described by Jothikumar in 2006 (Jothikumar, Cromeans *et al.* 2006). The extraction yield is estimated by MNV quantification at the end of the analysis.

As part of a study on the resistance of HEV to heat treatments used in the agro-food industry, the Animal Health Laboratory in Maisons-Alfort developed a quantitative detection method for HEV in a liver pâté-type food matrix. This study made it possible to validate in-house an extraction method for nucleic acids in complex foods consisting of meat (see section on composition below) (Barnaud, Rogee *et al.* 2012). This method is also based on direct grinding of the matrix but in normal saline solution, followed by extraction of total RNA. The presence of reaction inhibitors was evaluated by overloading the samples with synthetic HEV RNA. Molecular detection of HEV by real-time RT-PCR has also been carried out according to the protocol described by Jothikumar in 2006.

In 2011, a monitoring plan for HEV was set up to determine the prevalence of HEV in 400 products containing raw pork liver. In the framework of this plan, ANSES's Maisons-Alfort Animal Health Laboratory developed and validated in-house extraction methods for matrices consisting of dry sausage, dry salted liver, and dumpling dough. These methods also involve grinding of samples in a saline solution, followed by RNA extraction. Before the analysis, fat is removed manually from the sausages using a scalpel. HEV genome detection was carried out applying the same real-time RT-PCR method. A control of the presence of reaction inhibitors was also applied by adding synthetic HEV RNA (Report submitted to the Directorate General for Food (DGAL)).

Also as part of the monitoring plan, the ANSES Food Safety Laboratory (LSA) in Maisons-Alfort developed another method including an elution-concentration step using polyethylene glycol (PEG) and an MNV process control (Personal communication by S. Perelle, Unit of virology in food and water, LSA). Molecular detection of HEV is carried out by duplex RT-qPCR with an MNV process control (Martin-Latil, Hennechart-Collette *et al.* 2012). Likewise, as part of an earlier survey of the prevalence of HEV in pig livers at the slaughter stage, an RNA extraction technique was developed using only the liver matrix (Rose, Lunazzi *et al.* 2011). The extraction technique also involved grinding of livers in normal saline solution but did not include pre-treatment, i.e. fat removal. This simple matrix has few if any RT-PCR inhibitors.

Table 3: Summary of analytical methods for HEV in meat matrices

Year	Type of sample	Method of homogenisation	Process control	RNA extraction method	Genetic amplification method	Control of RT-PCR inhibition	Bibliographic reference
2011	Liver	Mechanical (Fast-Prep) in lysis buffer	None	Silica column	RT-qPCR Jothikumar N, *et al.* 2006	Viral RNA overload	(Rose, Lunazzi *et al.* 2011)
2012	Fresh sausage	Mechanical (mortar) in lysis buffer	MNV	Silica column	RT-qPCR Jothikumar N, *et al.* 2006	Included in process control (MNV)	(Berto, Martelli *et al.* 2012)
2012	Fresh sausage	Mechanical (mortar) in lysis buffer	MNV	Silica column	RT-qPCR Jothikumar N, *et al.* 2006	Included in process control (MNV)	(Di Bartolo, Diez-Valcarce *et al.* 2012)
2012	Liver pâté	Mechanical (Ultra Turrax) in normal saline	None	Silica column	RT-qPCR Jothikumar N, *et al.* 2006*	Synthetic RNA overload	(Barnaud, Rogee *et al.* 2012)
2012	Figatelli Fitone Dry salted liver Dumpling Dried liver sausage	Pre-test fat removal (figatelli, fitone, dried liver sausage) Mechanical (Ultra Turrax) in normal saline	None	Silica column	RT-qPCR Jothikumar N, *et al.* 2006*	Viral RNA overload	Personal communication N. Pavio
2012	Figatelli Fitone Dry salted liver Dumpling Dried liver sausage	Pre-test fat removal (figatelli, fitone, dried liver sausage) Mechanical (Stomacher) and elution-precipitation PEG centrifugation	MNV	Silica beads	RT-qPCR Martin-Latil, Hennechart-Collette *et al.* 2012	Included in process control (MNV)	Personal communication S. Perelle

Detection limit: 5 copies of genome equivalent per reaction

b. On-going developments

The ANSES Food Safety Laboratory is currently working on the optimisation of the HEV extraction method developed (elution-precipitation), in order to obtain higher yields for dry salted livers and dumpling dough.

4. Prerequisites and possible difficulties for routine use as part of self-monitoring

The methods described above were validated by several laboratories but none are recognised as reference methods. It is however possible to transfer methods to laboratories that have the necessary equipment. Analysis of meat-based food matrices requires rotating grinding devices which are not commonly found in non-specialised diagnostic laboratories. Pre-test fat removal from the matrices is essential but time-consuming and cannot be done automatically, which limits the number of analyses that can be performed simultaneously.

The primary limiting factor remains HEV's classification as a class 3 infectious agent, requiring handling in laboratories with a high containment level (L3). HEV doses, in genome equivalents, that may be present in faeces, liver or even figatellu slices (10^6 to 10^9 gEq/g) (Rose et al 2011, personal communication N. Pavio), are higher than the estimated ID_{50} infectious dose via the oral route in humans (10^5 gEq). An exemption on the basis of risk assessment as per the Order dated 16 July 2007 "establishing technical prevention measures… where workers are likely to be exposed to pathogenic biological agents"[6], may be granted for handling in laboratories with a containment level of 2+ or even 2. In addition, concerning serological analyses, it may be possible to waive this regulatory requirement of containment level 3 (with a containment level 2) for handling of sera, for instance if it can be demonstrated that prior decomplementation enables inactivation of HEV and maintains immunoglobulin integrity. Likewise, a containment level below 3 could be proposed for PCR and serological analyses following a risk assessment specifically taking into account the primarily oral and non-airborne route of viral transmission, the high frequency on swine farms and in the environment, and the biosafety measures implemented in the respective laboratories.

There are several types of laboratories that carry out self-monitoring:

- company laboratories, but most of them are small or medium-sized facilities that are not fully equipped laboratory structures. This applies particularly in the case of companies manufacturing products that contain raw pork liver, since a large proportion of producers are artisans. Concerning larger companies that do have a laboratory, these facilities are not classified as containment level 3 (L3);
- service-provider laboratories, some of which currently carry out virological analyses (detection of norovirus or HAV), but are not necessarily L3;
- technical centre laboratories which, similarly, are not all L3 facilities;
- departmental laboratories, but not all are equipped as L3 facilities.

[6] Order dated 16 July 2007 "establishing technical prevention measures… where workers are likely to be exposed to pathogenic biological agents" stipulates (Article 3 §II): "concerning agents classified as group 3, indicated with an asterisk (such as HEV) in the list annexed to the aforementioned Order dated 18 July 1994, as amended, that are not usually transmissible in the air, risk assessment should make it possible to determine whether the concentration or quantity of the pathogen of interest and the type of activities enable an exemption to be granted concerning certain specific level 3 containment measures".

In all these cases, method transfer and training are needed before these laboratories can become operational in carrying out routine analyses.

5. Expected timeframe required for the development of tests for routine use

a. Identification of possible additional activities

Since analysis of finished products is more complex than analysis of pork liver, another solution could be to test raw materials, i.e. pork liver itself, **after mixing and before other ingredients are added.** Given that the manufacturing process does not appear to have any inactivation effect on HEV, contamination of raw materials may be a good indicator of the risk of exposure to HEV.
The ANSES Animal Health Laboratory proposes to pursue work on detection of HEV in liver, paying specific attention to determining the effect of dilution of contaminated liver on the detection threshold of the method developed. In this study, livers contaminated with variable levels of HEV would be mixed with variable quantities of healthy liver. The objectives would be to specify the operating procedure for analyses of homogenates of livers, and to define a methodology applicable to self-monitoring. Another objective in the case of preliminary slaughterhouse testing would be to evaluate the possibility of pooling livers (5, 10 or more) before conducting analyses in order to reduce costs. The effectiveness of MNV-type or mengovirus-type process controls also need to be evaluated.
In addition, the newly available HEV molecular detection methods need to be evaluated. There are differences in sensitivity for the detection of the various HEV sub-types depending on the genomic region amplified (Abravanel, Sandres-Saune *et al.* 2012). The performances of these kits must be evaluated with the major sub-types circulating in the swine population in France (3e, 3c and 3f) but also with other emerging genotypes such as genotype 4.
Moreover, evaluation of the performances of these new serological and virological tests, and coordination of the transfer procedure to laboratories, require that a National Reference Laboratory be designated for monitoring of HEV in swine farms and in products containing raw pork liver intended to be eaten raw.

b. Expected timeframes

Conducting this type of study would require technical and financial support for a period of 12 months, taking into account:

- method validation,
- identification of laboratories able to carry out these analyses,
- transfer of the method or methods and training for laboratories.

As a result, designation of a reference laboratory appears to be necessary.

Key points concerning analytical methods

- HEV must be handled in a level 3 (L3) containment laboratory. It may be possible to propose a containment level lower than 3 following an assessment of the risks in the corresponding laboratories.
- Serological and virological detection methods for HEV in animals are available but none are recognised as a reference method. Moreover, there is no reference laboratory.
- HEV detection methods for various meat-based matrices (liver pâté, dry sausages, dry salted liver and dumpling dough) are available but are not recognised as reference methods.
- Analyses of food matrices require a rotating grinding machine.

- Transfer of these validated methods by ANSES to laboratories with the necessary equipment is however possible.
- Since analysis of finished products is more complex than analysis of pig liver, analysis of the raw material could be considered. This could be carried out after mixing livers and before adding other ingredients.
- It would be necessary to pursue work on the detection of HEV in pork livers in order to specify the operating procedure for analyses of liver homogenates, and to define a methodology applicable to self-monitoring.

E. Implementing certification of farms with regard to HEV

With the aim of providing raw materials that are HEV-free to be processed by agro-food companies into products containing raw liver, certification of swine farms could be considered. This would require powerful diagnostic tools that could be used routinely by analytical laboratories, along with a sampling plan to qualify swine farms.

1. Benefits and limitations of serological certification of swine farms

Large-scale serological certification of swine farms could be considered. Data are available concerning the intrinsic characteristics of the serological tests that could be used (Casas, Pina *et al.* 2011; Rose, Boutrouille *et al.* 2010; Zhang, Mohn *et al.* 2011). However, as mentioned above, the commercial kits recently placed on the market would need to be analysed in-depth. The benefit of carrying out serological tests on muscle fluid at the slaughterhouse has recently been highlighted (Casas, Pina *et al.* 2011). Data also show that there is a close relationship between the level of seroprevalence within the herd and the probability of detecting positive livers in animals from the same farm (Rose, Lunazzi *et al.* 2011). In this study, the probability of finding the virus in the liver was significantly higher in animals from farms where the seroprevalence at the end of fattening was higher than 25%: OR=6.7 [2.1–21.6]. These data suggest that farms that are at risk of providing positive livers are those in which the herds have high levels of circulating virus, and where the virus is transmitted at levels higher than 25% of the fattening pig population.

Nonetheless, concerning individual animals, an appreciable proportion of fattening pigs that have positive results in liver virology are seronegative due to extremely late infection having occurred a short time before slaughter (the detection time for seroconversion appears to be 25 to 30 days depending on the sensitivity of the serological test used). In breeder-fattener farms, it therefore appears necessary not only to carry out serological tests on pigs at the end of fattening or at the slaughterhouse, but to supplement this testing with samples from sows in order to establish with certainty the HEV status of the farm. The number of animals to test can be determined based on the theoretical prevalence limit to detect, the level of confidence required, and the performances of the tests used (see Table 4 at the end of this section).

2. Benefits and limitations of virological certification of swine farms

The presence of the virus in faecal matter could be detected since there appears to be high correlation between the presence of the virus in the liver and faecal shedding (HEVZOONEPI report).

Detection could be implemented:

On swine farms, as part of controls before slaughter, by sampling of animal faeces before transfer to the slaughterhouse, or from manure collected from pre-pits for the animals to be tested.

At the slaughterhouse, where faecal matter could be collected *pre mortem* in holding pens.

On the livers directly, after slaughter. This would provide direct results on the risk posed by offal from the tested farms intended for use in products containing raw liver. The test could be performed by PCR on liver specimens sampled from the slaughter chain. This approach may however prove to be extremely costly and difficult to implement, given the slaughter rates and the variability of carcass numbers in each batch (the number of animals per line is highly variable, ranging from a few individuals to more than 200). The sampling plan remains to be defined based on the theoretical prevalence limit to detect, the level of confidence required, and the performances of the tests used. Viral detection on livers could be used as a secondary control, restricted to pre-selected swine farms according to another evaluation method.

3. Certification procedures for farms regarding HEV

Prevalence data for the national swine population appears to indicate that the virus circulates in 65% of farms, and that 24% of farms supply some fattening pigs to the slaughterhouse that have infectious virus contamination of the liver.

It is the farms that supply animals with contaminated livers that are potentially at risk concerning use of pork products, and particularly liver for preparations derived from raw liver. In order to reduce this risk, several certification options can be considered.

The first certification option would be to identify farms that are HEV-free and to authorise pork livers only from these farms for the preparation of products containing raw liver. On these farms, a sampling system could be implemented to guarantee that, in the absence of positive results, intra-farm seroprevalence is below a certain threshold (for example <1%). To give a theoretical example, in a breeder-fattener farm with 200 sows (national mean – Technical management of sow herds (GTTT, IFIP), and a population of about 2200 pigs after weaning at a given time point, it would be necessary to sample at least 305 animals to confirm on the basis of negative results that the intra-farm seroprevalence is not higher than 1%, with a confidence interval of 95% (and assuming availability of a serological test with 92% sensitivity) (Rose, Boutrouille *et al.* 2010). From a practical point of view, and considering the persistence of antibodies in adult animals, it would probably be more useful to test a sample of sows in first-line testing, and if all the results are negative, to regularly confirm the status on groups of fattening pigs at the end of fattening. This is because it is unlikely that a farm could maintain an HEV-free status over time (Table 4). This possibility of changes in status, the high cost of acquiring and maintaining this certification, and the lack of available routine analytical methods, all constitute major obstacles to the implementation of this type of certification.

A different certification procedure would involve identifying farms at low risk of producing animals carrying the virus in their livers. However, data on the dynamics of HEV in farms are currently insufficient, and given the current status of knowledge, it appears difficult to define a threshold at which the risk is low or very low. This option is therefore not workable.

A second option would involve real-time *pre-* or *post-mortem* certification of a batch based on faeces sampling from the farm, targeting the batch that is to be slaughtered or at the slaughterhouse. This option is very costly and requires very complex logistics within the slaughterhouse (Table 4).

A third option not focused on certification of farms would consist in qualifying homogenates of test livers using real-time PCR, with the risk of rejection of most homogenates (Table 4). Pre-selecting a group of farms with an established status could reduce the number of rejected homogenates.

Key points concerning procedures for implementing HEV-free farm certifications to reduce HEV risks

- Certification relies on identification of HEV-free farms to restrict use of pork liver for preparation of raw liver products to these producers. There are two possible strategies:
  1) serological certification based on sampling of sows and/or on fattening pigs in the fattening stage, on the farm or at the slaughterhouse,
  2) analysis of faecal matter on the farm or at the slaughterhouse, indicating the possible presence of the virus in the liver.
  These two strategies could possibly be combined.

- The objectives, obstacles, and means required for the various certification strategies are summarised in Table 4, which also presents a certification option involving liver homogenates. The main limitations identified are as follows:
  i) variability of the "HEV-free" status of farms over time,
  ii) the lack of available analytical methods for routine use. Moreover, the performances of the methods available on the market need to be evaluated since there are differences in method sensitivity,
  iii) the cost and complexity of implementing certification.

- Given these limits, it does not currently appear possible to implement a certification system for HEV-free farms.

Table 4: Summary of certification options regarding HEV: advantages and obstacles

	Objective	Means required	Sampling method	Advantages	Obstacles
Certification of HEV-free farms	Identify HEV-free farms	Identification of a sub-group of eligible farms HEV-free certification: sampling to verify seroprevalence < threshold, confidence level Characteristics of the serological test used (sensitivity, specificity)	Serum	Identification of farms eligible for production of raw pork liver	Variability over time of the "HEV-free" status Theoretically covers a relatively small proportion of farms (<35%) Complex logistics within slaughterhouses, additional segmentation Not applicable to pure fattener farms Cost
Pre- or *post-mortem* certification of batches in real time	Establish real-time virology status based on samples taken from animals within a batch	Sampling of batches in the holding pen (pre-mortem) or on the line (post-mortem) Virological analyses (PCR) with just-in-time results for batch release	Faeces	Specific knowledge on the status of batches used in the raw pork liver sector.	Very complex logistics, difficult to manage in the slaughterhouse Extensive sampling to ensure that a positive animal is not likely to contaminate the whole batch Cost of analyses
	Objective	Means required	Sampling method	Advantages	Obstacles
Certification of homogenates of pork livers	Determine the status of homogenates	Sampling and analysis of homogenates (PCR) in real-time for release to the raw pork liver sector Upstream traceability of liver batches	Pork liver homogenates	Moderate cost Immediate risk management depending on the result	Risk of rejection of most homogenates as a result of homogenisation and the individual prevalence of contaminated livers[7]

[7] If possible, with pre-selection of a group of farms with a known status to avoid rejection of a too large number of homogenates.

F. Effects of food processing on the fate of HEV in delicatessen products derived from raw pork liver

The effects of processing procedures such as cooking and drying on the hepatitis E virus can only be evaluated using data on the infectious nature of the virus. The hepatitis E virus is practically impossible to culture and the viral genome can only be quantified using molecular biology techniques, which do not make it possible to determine the infectious status of the virus. As a result, technological food processing can only be assessed at this time on the basis of studies carried out on animals.

1. Effects of thermal treatment

To our knowledge, three studies have been conducted to assess the effect of thermal treatment on the survival of the hepatitis E virus.

- A study by Tanaka (Tanaka, Takahashi *et al.* 2007) using a cell culture system based on the PLC/PRF/5 hepatocarcinoma cell line showed that heating to 70°C for 10 minutes is required to inactivate faecal suspensions of HEV at 6 x 10^4 gEq. However, incubation at 56°C for 30 minutes did not inactivate HEV.
- A study by Feagins (Feagins, Opriessnig *et al.* 2008) using a bioassay showed that virus present in the liver could be infectious in pigs. However, stir-frying pork liver cut into cubes of 0.5 to 1 cm² at 191°C or cooking the cubes in boiling water resulted in a core temperature of 71°C for 5 minutes leading to inactivation of the virus present in the liver by natural contamination. By contrast, incubation at 56°C for 1 hour was insufficient to obtain complete inactivation of the hepatitis E virus.
- A study published by Barnaud (Barnaud, Rogee *et al.* 2012) using a bioassay aimed to determine the effect of various heat treatments on the infectivity of the hepatitis E virus. Complex food matrices were used in this study: homogenates containing 30% infected liver (10^8 gEq of HEV/g) and 48% fat (liver pâté comparable to figatellu). The thermal treatments applied were as follows: 71°C for 5, 10 and 20 minutes, 68°C for 5, 10 and 20 minutes, 62°C for 5, 20 and 120 minutes. These findings indicate that only heat treatment at 71°C for 20 minutes inactivates HEV completely. Treatment at 68°C for 20 minutes does not inactivate HEV, and treatment at 62°C for 120 minutes has no effect on survival of HEV.

A comparison of these three studies shows that the presence of fat in the homogenates (48%) may have a protective effect for the virus against heat treatment. Accordingly, the faecal suspension and plain liver cubes had higher sensitivity to heat treatment. In the absence of other data, the recommendation of treatment at 71°C for 20 minutes can be made to ensure inactivation of HEV, even though this approach is very strict in terms of safety since study results were based on contamination of homogenates before thermal treatment with nearly 10^7 gEq of HEV/g and intravenous administration, as opposed to the oral route. In oral administration, the virus must cross the intestinal barrier to reach the general circulation.

2. Effects of drying

There are no current data on the effects of drying on the hepatitis E virus, nor on its survival depending on salt content or on activity in water (a_w). HEV is a small non-enveloped virus able to survive in the environment and to cross the gastric barrier. It is excreted in bile containing biliary salts (detergent effect). HEV is therefore resistant in adverse conditions.
In the absence of data and given a_w values and/or the salt concentration in delicatessen meats, drying as used by producers of these products must be considered ineffective.

3. Conclusions concerning food processing

Delicatessen products made from raw pork liver can be divided into three categories:

i. Products cooked by the producer (Alsatian liver sausages or dried Alsatian liver sausages, liver mousse (pâté) and Alsatian liver dumplings). The manufacturing processes for these products involve at least one cooking step performed by the producer. Cooking can be considered effective, provided that it includes treatment at a minimum of 71°C for 20 minutes.

ii. Products cooked by consumers (fresh liver sausages from the south west). These products do not undergo thermal treatment by the producer but only by the consumer. Recommendations should be provided to the consumer to ensure that cooking is sufficient to inactivate HEV.

iii. Products consumed raw (figatelli, dry liver sausages, dry salted liver). The manufacturing processes for these products must be considered ineffective to inactivate HEV, and it is important to note that the virus remains infectious for the full shelf life of the product. As a result, extending the shelf life (use-by date in most cases) or implementing an interval between manufacture and marketing must be considered ineffective in inactivating the HEV virus.

If HEV-free pork livers are not pre-selected, the only way to reduce the risk related to these products is to use livers that are heat-treated before they are processed (see IFIP study). This recommendation can also be applied to products intended to be cooked by the consumer.
Use of high-pressure processing (HPP) on finished products could be an alternative to inactivate HEV. There are currently no data on the effectiveness of high pressure processing on the hepatitis E virus, the effectiveness of HPP on hepatitis A virus (HAV) has been demonstrated (Kingsley and Chen 2009).

Key points concerning the effects of food processing on HEV

- Thermal treatment to achieve an internal temperature of 71°C for 5 minutes is recommended for decontamination of pork liver homogenates, and for 20 minutes for complex food matrices such as those of figatelli.
- Drying as performed by producers of products derived from raw pork liver cannot be considered effective in inactivating HEV (Afssa 2009). As a result, extending the interval between manufacture and marketing would be not useful.
- The effects of high pressures on HEV should be evaluated (known efficacy on HAV).

G. Summary of risk management options for HEV in products derived from raw pork liver: from farm to fork

The diagram below presents the risk management options for public health regarding the risk of HEV in products derived from raw pork liver.

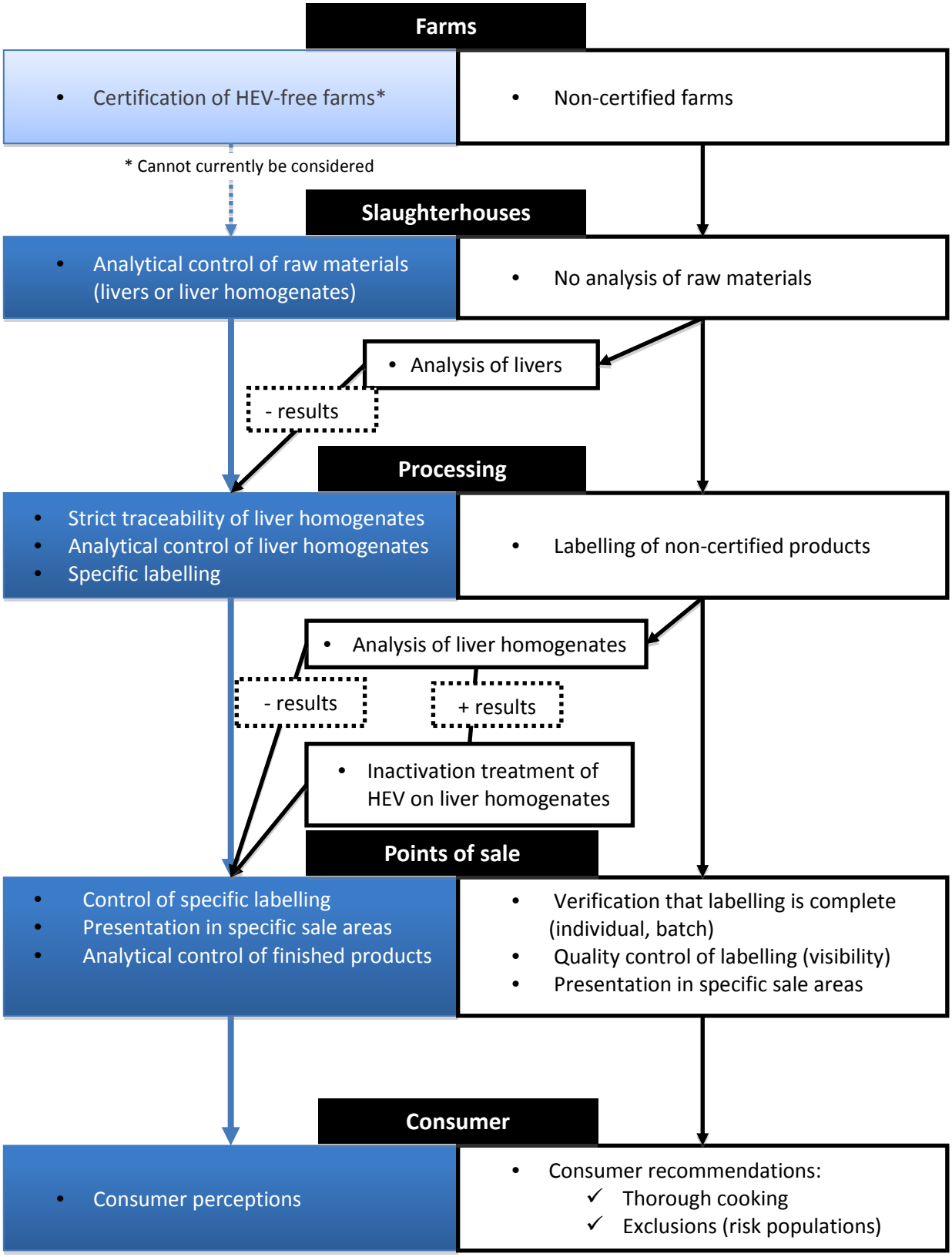

Figure 4: Public health management options regarding the risk of HEV in products derived from raw pork livers

The left of the diagram (blue background) presents the conditions for implementing a certification process to guarantee to the consumer the absence of detectable HEV in products derived from raw pork liver, bearing in mind that certification of HEV-free farms is currently not possible. The right of the

diagram (white background) shows the raw liver-based product life cycle with no specific guarantee regarding the HEV risk.
Selection after analysis of non-contaminated livers or liver homogenates during the manufacturing process, and/or application of adequate thermal treatment to the livers or homogenates are two measures that could, depending on their effectiveness, enable potentially contaminated products to be sold as items with low HEV risk under a different form of certification.
A certification procedure requires, among other things, regular control capabilities relying on effective analytical tools for routine use, particularly on farms, at processing facilities and at points of sale. The effectiveness of the certification system also relies on traceability of pork livers, and requires a reference on the product labelling for all items containing pork liver. This labelling should provide the consumer with information on the possible dangers related to consumption of these products. The coexistence of two sectors for raw pork liver-containing products, with different food safety statuses for the same products, may pose a problem: complexity in terms of customer perception given the two types of information provided for the same product, and the risk of confusion between products with different food safety statuses. Likewise, a certification system for all products derived from raw pork liver would require the same level of guarantee in terms of traceability and clear, understandable information.
A cost-benefit analysis with a quantitative approach is theoretically possible for a single measure or a combination of measures, provided that the necessary data are available. As a minimum, the following data are required to carry out an assessment of the risk for the consumer: contamination data for the incriminated products, dose-response data, product consumption and preparation data for products derived from raw pork liver, effectiveness of cooking, survival of the virus in dried products. A benefit-risk analysis requires evaluation of the quantified effectiveness of the potential measures and of their associated costs.
Along with the measures that could be taken upstream to reduce the risk of contamination of livers, certain measures can already be implemented with the aim of protecting consumers, such as better information on risks. The effectiveness of these measures could be assessed on the basis of a reduction in the proportion of cases related to consumption of products derived from raw pork liver among the cases of hepatitis E recorded by the monitoring system (see epidemiological data).
Regardless of the measure adopted, a combination of several measures would most probably be more effective than a single measure.

Conclusions of the Expert Committees (CES) on Biorisk and Animal health

The number of indigenous cases of hepatitis E in France is on the rise, and seroprevalence is high but variable between regions. Consumption of products derived from raw pork liver, particularly figatellu, appears to be one of the most important risk factors, particularly in patients living in the south-east of the country. Analysis of the HEV sequences isolated in humans and in animals suggest a zoonotic source of human contamination, irrespective of the route of contamination.

In conclusion, the following responses are proposed:

Concerning the relationship between the slaughter age and the probability of contamination of livers by HEV: the probability of the presence of the virus in pork livers decreases with age and after 20 days post infection. The age of animals at the time of infection is highly variable and is difficult to determine. In practice, the probability of the presence of the virus in the liver cannot be directly inferred from the slaughter age of the animals.

Concerning certification of HEV-free farms: certification relies on identification of HEV-free farms so that only pork livers sourced from these farms can be used to prepare products derived from raw liver. This type of certification requires serological analyses of samples taken from sows and/or from

fattening pigs on farms or at the slaughterhouse, and/or virological analysis of faecal matter on farms or at the slaughterhouse, as an indicator of the possible presence of the virus in the liver. In view of the constraints presented in this opinion, certification of HEV-free farms cannot be considered at this time.

Concerning the timeframe required to develop diagnostic tests for routine use as part of self-monitoring to determine the HEV status of liver homogenates: there is no standardised method for the detection of the HEV genome in food matrices. Validated methods for the detection of HEV in various meat-based food matrices (liver pâté, dry sausage, dry salted liver and dumpling dough) are available and could be transferred to laboratories for routine use.
Additional studies on the operating procedures for these analyses and the methodology applicable to self-monitoring for HEV detection in pork liver are required before these tests can be made available.

Concerning the effects of food processing on the survival of HEV in products containing pork: thermal treatment at a minimum of 71°C for 20 minutes is required to inactivate HEV in a complex matrix (composition similar to that of figatellu). If swine with HEV-free livers are not pre-selected, the only measure to reduce the risk would be to use livers treated thermally (71°C for 5 minutes minimum) for the manufacture of processed products. Drying, as carried out by producers of products derived from raw liver, cannot be considered effective in inactivating HEV. As a result, extending the interval between manufacture and marketing of these products would not be beneficial. Alternative methods of inactivation, such as high-pressure processing, could be examined.

Along with the measures that could be taken upstream to reduce the risk of contamination of livers, certain measures can already be implemented with the aim of protecting consumers:

- thermal treatment of livers,
- clear, understandable information on all products derived from raw pork liver placed on the market, highlighting to consumers the need to cook these products thoroughly,
- information to physicians and to people who may develop serious forms of the disease (immunodepressed patients, individuals with chronic liver disease, pregnant women) concerning the risk of hepatitis E and preventive measures. Hepatitis E screening could potentially be considered for these subjects who, in the case of negative results, would be provided with information by their doctor on the risks related to consumption of raw products of this type.

4. CONCLUSIONS AND RECOMMENDATIONS OF THE AGENCY

The French Agency for Food, Environmental and Occupational Health & Safety endorses the conclusions of the Expert Committees (CES) on Biorisk and Animal health.

The Director General

Marc Mortureux

KEY WORDS

HEV; Shedding; Liver contamination; Swine farming; Slaughter; Certification; Processing.

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ANNEXES

Annex I: Background information on ANSES's work in this area

Participation in research and investigations

Concerning the prevalence of the virus and assessment of the risk of zoonotic transmission

ANSES participates in national projects aimed at evaluating the prevalence of the hepatitis E virus in the swine and wildlife reservoirs and assessing the risk of zoonotic transmission of the hepatitis E virus via food (ANR/HEVZOONEPI programme).
In addition, an ongoing study is seeking to determine the apparent prevalence and viral load of hepatitis E virus in porcine muscle at the slaughter stage. This study is being carried out in collaboration with the French Pork and Pig Institute (IFIP).
Similarly, a study has been implemented to determine the apparent prevalence and the viral load of hepatitis E virus in products derived from raw pork liver intended to be consumed raw or cooked.

Concerning virus dynamics

ANSES is coordinating a project aimed at assessing the dynamics of the hepatitis E virus in associated ecosystems: from pig farms to the environment and shellfish (ANR/HEVECODYN programme).

Concerning the impact of cooking processes on the fate of the virus

A study on the effects of cooking processes on the fate of the hepatitis E virus was carried out in 2010 in collaboration with the IFIP.

Concerning the identification of infectious virus in positive HEV samples
ANSES is developing *in vitro* HEV culture models in order to correlate positive molecular detection of HEV with the presence of infectious particles. A study on the presence of infectious virus in products derived from raw pork liver was carried out in collaboration with two European laboratories (United Kingdom and Netherlands) (Berto, Martelli *et al.* 2012). Another study was carried out on the development of two human and porcine hepatocyte models on the basis of animal samples (liver, faeces, bile) (Rogee, Talbot *et al.* 2012).

Assessment documents published by the Agency
ANSES has published several opinions and expert appraisals concerning assessment of the risks related to hepatitis E virus.

AFSSA Opinion of 30 April 2009 concerning the risk of human contamination with the hepatitis E virus (HEV) after ingestion of figatelli (raw sausages derived from pork liver) http://www.anses.fr/Documents/MIC2009sa0101.pdf. This Opinion was issued in an emergency context, in response to Request 2009-SA-0101 from the Directorate General for Food, after consultation of an *ad hoc* Emergency collective expert assessment group (GECU).

This Opinion was issued further to the reports by Prof. René Gerolami, Head of the Hepato-gastroenterology Department of the Marseille Hôpital de la Conception, and by Dr Philippe Colson, Virology laboratory of Hôpital Timone in Marseille, of about 20 human cases of hepatitis E each year in the Marseille Public University Hospital System. They identified consumption of raw figatelli as the common factor in these patients, and presented a communication on this topic on 10 April 2009. The communication was widely publicised by the local and national press. In this context, AFSSA received an urgent formal request from the DGAL. The Opinion issued in response to the request provided information on:

- the background and epidemiological data related to this virus;

- information on this health risk;
- information on raw sausages derived from pork liver;
- replies to the questions posed by the DGAL concerning risks to the consumer, the effects of drying and of cooking.

AFSSA Opinion of 23 September 2009 concerning the hepatitis E virus: detection methods, risks for the consumer and risks for the environment (in response to Request No 2009-SA-0146 of the DGAL/DGS) http://www.anses.fr/Documents/MIC2009sa0146.pdf. The Opinion was issued after consultation of an *ad hoc* Emergency collective expert assessment group, and of the ANSES Expert Committees (CES) on Microbiology and Animal Health.

This opinion provided answers to the issues identified in Requests No. 2009-SA-0101 and No 2009-SA-0146. It is therefore a comprehensive document on the topic of the hepatitis E virus, including:

- information provided in the AFSSA Opinion of 30 April 2009 (in response to Request No 2009-SA-0101), on the following questions:
    - does consumption of raw sausages derived from pork liver, such as figatelli and Toulouse liver sausages, contaminated with the hepatitis E virus, pose a health risk for the consumer?
    - does drying these products help to reduce the risks for consumer health, and if so, what is the recommended drying protocol?
    - does cooking these products help to reduce the risks for consumer health, and if so, what is the recommended cooking protocol?
- information on the risks of contamination with the hepatitis E virus via consumption of pork, wild boar and deer meat;
- information in response to the three questions posed in Request No. 2009-SA-0146:
    - an opinion on the HEV detection methods available depending on the type of matrix (liver, dried, raw or cooked finished products) and the conditions of use (routine, other);
    - an opinion and if necessary a study protocol aimed at collecting specific data on the behaviour of HEV in products during cooking and in dried, salted, or smoked products, depending on the initial viral load, in order to evaluate the effects of these various treatments on inactivation of the HEV virus and to propose practical procedures for effective treatment;
    - an opinion on the conditions of persistence of the virus in manure on swine farms and on the possible risk related to spreading of swine manure and on inactivation procedures, if necessary.

ANSES Opinion of 4 October 2010 concerning a proposed sampling plan for a 2011 monitoring programme for the hepatitis E virus (HEV) contamination in products derived from raw pork liver (in response to Request No 2010-SA-0170 from the DGAL).

This programme, which covered the year 2011, included 400 samples in 40 establishments producing products containing pork liver (liver sausages, liver dumplings, figatelli and dry salted liver). Its aim was to determine the apparent prevalence of HEV contamination in products derived from raw pork liver.

ANSES Opinion of 13 October 2010 regarding analytical capabilities for the detection of hepatitis E virus in products derived from raw pork liver (in response to Request No 2010-SA-0171 from the DGAL).

Hepatitis E virus is difficult to culture *in vitro* and detection is primarily molecular. In the absence of standards and standardised detection methods, qualitative and quantitative analytical methods have

been developed and validated in-house in ANSES laboratories. These techniques enable detection of the HEV genome in animal samples and in meat-based food matrices.

Data sheet on foodborne microbial hazards concerning the hepatitis E virus dated November 2010 (in response to ANSES Internal Request No 2010-SA-0145)
http://www.anses.fr/Documents/MIC-Fi-HepatiteE.pdf. This information sheet was validated by the ANSES Expert Committees (CES) on Microbiology and Animal health.

This descriptive document, intended for agro-food sector professionals, presents information concerning the characteristics of the virus (biological properties, sources, transmission routes), foodborne disease in humans (type of disease, dose/effect and dose/response relationships, epidemiological data), the role of food (main foodstuffs to consider, industry-level inactivation processes, monitoring in food), and hygiene considerations.
This information could be useful to professionals to take into account this biological hazard when writing their guides to good hygiene practice and application of principles of hazard analysis and critical control points (HACCP).

Annex II: Virological prevalence of HEV in swine depending on age according to the literature

Country	Number of animals sampled	Age	Prevalence HEV RNA (%)			Source
			Faeces	Serum	Bile	
France	100 pigs 107 pigs	3 months 6 months	65 0	22 0	- 0	(Kaba, Davoust *et al.* 2009)
Spain	19 sera, 8 faeces 6 sera, 5 faeces 15 sera, 10 faeces 10 sera, 5 faeces	3-6 weeks 8-10 weeks 12-13 weeks 22 weeks	0 100 40 0	0 66.7 86.7 0	- - - -	(Seminati, Mateu *et al.* 2008)
Spain	ND 23 17 20 ND	<1 month 1 month 2 months 3 months >3 months		0 30 47 55 0		(De Deus, Seminati *et al.* 2007)
Spain	20 pigs 22 pigs 20 pigs 27 pigs	0-4 weeks 5-12 weeks 13-20 weeks 21-24 weeks	10 41 5 7	20 32 10 11	- - - -	(Fernandez-Barredo, Galiana *et al.* 2007)
Spain	18 24 20 20 28	maternity post-weaning 1st month fattening 2nd month fattening 3rd month fattening	11.1 41.7 60 5 7.1	- - - - -	- - - - -	(Fernandez-Barredo, Galiana *et al.* 2006)
Taiwan	11 sera 67 sera 255 sera 112 sera 76 sera	<2 months 2 months 3-4 months 5-6 months >7 months	0/20 9/22 8/12	0 4.5 1.2 1.8 0	- - - - -	(Wu, Chen *et al.* 2002)
Italy	64 pigs 37 pigs	<120 days >120 days	42.2 27	- -	- -	(Di Bartolo, Martelli *et al.* 2008)
Canada	51 pigs 51 pigs 51 pigs 51 pigs	2 weeks 8 weeks 18 weeks 22-29 weeks	11.8 52.9 86.2 41.2	0 2 47.1 11.8	- - -	(Leblanc, Ward *et al.* 2007)
United Kingdom	50 pigs 50 pigs 45 pigs	3-5 weeks 10-12 weeks 22-24 weeks	26 44 8.9	- - -	- - -	(McCreary, Martelli *et al.* 2008)
Hungary	204 pigs total	1-4 weeks 5-10 weeks 11-16 weeks >17 weeks	9 27 36 10	- - - -	- - - -	(Forgach, Nowotny *et al.* 2010)
Denmark	32 pigs 33 pigs 32 pigs	4-8 weeks 9-12 weeks 13-22 weeks	21.9 54.5 71.9	- -	- -	(Breum, Hjulsager *et al.* 2010)
Thailand	26 pigs 50 pigs	2-4 months other	- -	39 0	- -	(Cooper, Huang *et al.* 2005)
Mexico	92 faeces, 125 sera	2-4 months	31	6.4	-	
Italy	85 pigs 49 pigs	80-120 days <80 days	- -	- -	46.9 20	(Martelli, Toma *et al.* 2010)
China	167 pigs 143 pigs 135 pigs 109 pigs	<10 weeks 10-15 weeks 16-20 weeks >20 weeks	6.6 9.8 6.7 4.6	- - - -	- - - -	(Zhang, Yang *et al.* 2009)
China, Mongolia	101 pigs 132 pigs 123 pigs	2 months 3 months 4 months	- - -	7 9 9	- - -	(Jinshan, Jirintai *et al.* 2010)
Belgium	420 pigs	< 6 months		4		(Thiry D, Mauroy A *et al.* 2012)