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OPINION

of the French Food Safety Agency regarding the hepatitis E virus: methods for detection, risks to the consumer and risks to the environment

THE DIRECTOR-GENERAL

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Review of the request

On 15 May 2009 the Directorate General for Food (DGAL) and the Directorate General for Health (DGS) submitted a request to the French Food Safety Agency (AFSSA) for an opinion on methods for detecting the hepatitis E virus, and on the behaviour of the virus in pig slurry, and during cooking, drying, salting or smoking of products made from pig liver.

Questions asked

AFSSA's opinion on the following issues is requested for 1 September 2009:

- An opinion on the available methods for detecting the hepatitis E virus (HEV) depending upon the nature of the matrix (liver, dry, raw, or cooked final products) and on the conditions of their use (routine, other, etc.);
- An opinion, and if necessary, a study protocol for the purpose of gathering specific data on the behaviour of HEV in products during cooking and in dried, salted or smoked products according to the baseline viral load, in order to assess the impact of these different treatments on the inactivation of HEV and to suggest practical methods of effective treatment;
- An opinion on the conditions for viral persistence in slurry from pig production, on the risk from spreading pig slurry and on the inactivation procedures, if any.

Moreover, this request reiterates that information is also requested on the risk of consuming meat from pork, wild boar and venison.

Background

This request follows an AFSSA Opinion issued on 20 April 2009 concerning the risk of human contamination by the hepatitis E virus (HEV) following consumption of Figatelli sausage (raw pork liver sausage), in response to a request from the DGAL dated 16 April 2009.

AFSSA issued an Opinion at that time in response to three questions:

- Is the consumption of raw pork liver sausage (such as Figatelli, Toulouse liver sausage) infected with the hepatitis E virus likely to pose a consumer health risk?
- Would drying these products be likely to reduce the consumer health risk? If so, what drying protocol should be recommended?
- Would treatment by prior cooking these products be likely to reduce the consumer health risk? If so, what cooking protocol should be recommended?

Moreover, in its conclusion, the Opinion also stated that further analysis of the risk of contamination by the hepatitis E virus resulting from consuming meat from pork, wild boar, and venison would be provided later.

Following the conclusions reached by AFSSA in this Opinion, the DGAL and the DGS now seek additional information on the above-mentioned issues.

Assessment Method

The 'Risk of human contamination by the hepatitis E virus due to consumption of Figatelli' (emergency collective expert assessment group (ECEAG) created on 23 April 2009 in response to Request no. 2009-SA-0101 has been asked to respond to this second request. Its composition is listed in **Annex 1**.

Scientific and technical support on estimates of the consumption of pork, wild boar or venison products was provided by AFSSA's 'Food consumption observatory – nutritional epidemiology' unit.

The conclusions of the ECEAG were presented to AFSSA's Scientific Panels on 'Animal health' and 'Microbiology', on 8 and 9 July 2009 respectively.

This Opinion is a comprehensive document on this topic and includes:

- the data provided in AFSSA's 30 April 2009 Opinion;
- general answers on the risks of contamination by the hepatitis E virus from consuming meat from pork, wild boar and venison;
- general answers to the three questions in the new request.

Discussion

1. Background and epidemiological situation

a. Background

The hepatitis E virus is recognised as the primary agent of acute hepatitis in countries with low standards of hygiene, where it follows an endemo-epidemic pattern of development. In 'industrialised' countries, cases of hepatitis E were reported initially in those having spent time in an endemic area (12), particularly among the military (23). However, since 1997 cases of autochthonous hepatitis E in the United States in patients who had not spent time in an endemic area (55) have revealed a new pattern for HEV infection, which has raised the question of their origin.

The discovery of natural infection by HEV in primates and pigs suggests exposure to the virus and possible interspecies transmission (16). The hypothesis of a zoonotic origin in autochthonous cases has been raised since 1997 in the United States due to the isolation of a variant of HEV in pigs (called Swine HEV) that is genetically similar to human variants found in autochthonous cases discovered at the same time and associated with genotype 3 (74). Since then, many variants of HEV have been isolated in both humans and animals, frequently with close genetic proximity that strengthens the zoonosis hypothesis. Evidence of a zoonotic component in the case of HEV was finally provided by the observation since 2003 of around ten cases of food-borne transmission of the virus in Japan, from contaminated pork, wild boar or venison meat that was raw or undercooked (61, 101).

b. Clinical signs of hepatitis E in humans

The incubation period for hepatitis E ranges from between three and eight weeks, with an average of 40 days (86). Nearly half of cases are asymptomatic or show few symptoms. The disease's prodromal phase (fever, asthenia, digestive disorders) is sometimes absent or brief, and at times can last up to two weeks. Its clinical picture is then similar to hepatitis A (32, 83, 86). This array most often combines asthenia, mucocutaneous jaundice and hepatomegaly. In addition, there are various clinical signs of digestive disorders such nausea, vomiting, and abdominal pain. Some patients also have fever that is generally moderate. The evolution of this disease is usually favourable, typically with spontaneous healing without sequelae, after two to four weeks of progression. In 1 to 2% of cases, however, complications of a fulminant form of hepatitis E develop (52), involving life-threatening conditions for which liver transplant is often the only solution. The risk factors that have been identified for fulminant hepatitis are:

- The existence of an underlying liver pathology in those individuals (84).
- Pregnancy: in fact, it appears that a higher incidence of fulminant hepatitis associated with HEV has been reported in pregnant women in endemic areas, up to 20% during the third trimester. Several prospective studies conducted in India in particular, address the relationship between hepatitis E and pregnancy (42, 48, 51, 54).

Complications such as chronic hepatitis and cirrhosis have also been observed in immunocompromised patients (31, 35, 46, 47). The severity of hepatitis E appears to be well above that of hepatitis A with a respective mortality rate of 0.4-4% versus 0.1-2% (82).

c. Epidemiological situation in France

General data

It should be noted that hepatitis E is not a notifiable disease in France. Surveillance of hepatitis E is carried out by the National Reference Centre (NRC) for enterically-transmitted hepatitis (hepatitis A and E) created in 2002 and located in Paris. For information, **Annex 2** provides the algorithm for interpretation of the biological profiles used by the NRC. The virology laboratories of the Toulouse and Marseille university hospitals perform routine serological and molecular diagnosis of hepatitis E and collaborate with the NRC for synthesising the results.

Table 1 shows the number of cases of hepatitis E diagnosed by the HEV NRC and differentiates between imported cases (subjects spending time in endemic areas in the three months prior to onset of the disease), autochthonous cases or those of unspecified epidemiological context. Note that in the 25% of cases where the epidemiological context is not specified, genotyping of the virus identifies genotype 3f, the predominant genotype found in Europe.

The cases were diagnosed in all the metropolitan areas with a strong concentration in the south. Each year, more than half of the autochthonous cases occurred in individuals living in the Midi-Pyrenees or Provence-Alpes-Côte d'Azur (PACA) regions (15).

Since 2002, an increase in autochthonous cases of hepatitis E has been observed. This may be due to a real increase in the incidence of the pathology or to an effect associated with screening and/or more reliable diagnosis.

In fact, a parallel surge in requests for analyses addressed to public and private laboratories has been noted, along with greater attention from health professionals including gastroenterologists (**Table 1**). In the Midi-Pyrénées region, where the efficacy of the diagnosis has been fairly consistent given the involvement of local clinicians and virologists, the number of cases has remained constant for three years, supporting the hypothesis of a stable epidemiological situation (65).

Table 1: Number of autochthonous hepatitis E cases in France between 2002 and 2008.

Years	2002	2003	2004	2005	2006	2007	2008
Number of patients tested	209	155	233	327	583	3500*	5500*
Confirmed cases							
- imported	4	11	4	19	14	14	23
- autochthonous	9	3	16	20	24	97	146
-unspecified epidemiological context						5	49
Total	13	14	20	39	38	116	218

Description of nine isolated autochthonous cases documented by the Hepatitis E NRC, between 2008 and 2009, reported as resulting from the consumption of Figatelli or liver sausages:

Among the first seven cases, five were living in the PACA region; three cases had visited Corsica in the two to 10 weeks prior to the onset of symptoms and four others had no recollection of visiting Corsica. All had eaten pork liver sausage (two cases), Figatelli (four cases) or local Corsican delicatessen meats (one case). The molecular characterisation of the virus indicates that it is genotype 3f, the predominant genotype found in Europe.

Two other cases are still being investigated:

In March, 2009, a patient with viraemia died from fulminant hepatic failure. The viral genotype was characterised as type 3f. This patient had an underlying liver pathology. Consumption of raw Figatelli was identified in the two to 10 weeks prior to the onset of symptoms (the end of December

2008). Screening for HEV markers in the Figatelli could not be carried out because they had all been consumed. This patient was exposed to other potential hepatitis E risks, including drinking water from a private well (however, the results of a sampling of well water revealed no HEV).

In March 2009, another autochthonous case living in Marseilles had eaten traditionally made Figatelli from Corsica. This Figatelli was consumed during a meal shared by four people. Only the diner who ate a small piece of raw Figatelli served at this meal contracted hepatitis E. No other potential source of contamination has been identified.

Description of two episodes of outbreaks of food-borne hepatitis E that occurred in the South of France between 2007 and 2008:

During the summer of 2007, a familial outbreak of three cases of autochthonous hepatitis E (confirmed by PCR and genotype 3f sequencing) that occurred in the Vaucluse *département* was investigated by CIRE Sud [the Inter-regional Epidemiology Unit for southern France]. What these three cases had in common was a single meal shared one month earlier by four people, during which Figatelli was served. The three consumers of raw Figatelli contracted hepatitis E. The fourth guest, who had not eaten the Figatelli, did not become ill. No other common source of contamination was identified.

In March 2009, the gastroenterology and virology departments of the Marseilles Public Hospitals (AP-HM) reported a case of clinical hepatitis E diagnosed in September 2008 (by positive PCR and genotype 3f sequencing). This case was related to four other oligosymptomatic cases with HEV seroconversion. All these patients had taken part in the same family meal at the beginning of August 2008 in Corsica. Among the 10 guests, all had eaten raw Figatelli, except one guest who tested PCR-negative and seronegative.

2. Information on the hazard¹

a. Identification of the hazard and route of transmission

Characteristics of the hepatitis E virus:

Hepatitis E virus is a non-enveloped RNA virus, with the genome encoding three open reading frames = ORF (ORF1, ORF2 and ORF3). It was recently classified in the *Hepeviridae* family.

Human HEV strains are indistinguishable from animal strains

Four mammalian HEV genotypes have been differentiated (1 to 4), each genotype itself being divided into subtypes (24 subtypes).

Genotypes 1 and 2 are found only in humans, whereas genotypes 3 and 4 are found in both humans and animals. Hepatitis E occurs in certain cases as a zoonosis.

Genotype 1 (5 subtypes, a to e) consists of human HEV strains responsible for epidemics but also for sporadic cases in Africa and Asia. Genotype 2 (2 subtypes, a and b) has a more restricted distribution in Mexico and in some countries in Africa (Chad, Nigeria). Strains of genotype 3 are essentially derived from industrialised countries and are both human and animal. To date, the genotype 3 viruses (10 subtypes, a to j) have been identified only in sporadic cases. Genotype 4 (7 subtypes, a to g), on the other hand, is a genotype that is mainly found in humans and animals in Southeast Asia.

Many swine virus strains (genotypes 3 and 4) have been identified worldwide. Each time, phylogenetic analyses have confirmed a close genetic proximity between human and animal strains suggesting that zoonotic transmission takes place (58).

In practice, on the basis of genetic sequences used for the comparison of isolates, it appears to be impossible to differentiate between human and animal strains, the variability within species being at least as great as the variability between species. (6, 9, 37, 41, 80).

¹ Part of the summary of the literature in this Opinion is based on the thesis of Marulier Fleuriane, Zoonotic hepatitis E, veterinary PhD thesis, National Veterinary School of Alfort, defended in February 2009.

In addition to the four genotypes found in mammals, there is an avian genotype responsible for hepatosplenomegaly in chickens. This avian strain is not transmissible to primates or pigs (39).

Only recently, a new isolate was identified in rabbits but initial sequence data do not make it possible to formally classify it as one of the four known genotypes (112).

The human and animal strains of genotype 3 and 4 are transmissible between species

The first experimental study on a possible crossing of the species barrier was conducted in 1988 (73). In this study, the Swine HEV strain of genotype 3 was transmitted by intravenous route (IV) to two rhesus monkeys and a chimpanzee, which developed viraemia and seroconversion. The human strain of human genotype 3 US-2 was transmitted to SPF [specific-pathogen-free] pigs. The same type of work was performed for genotype 4: Arankalle *et al.* have shown that inoculating rhesus monkeys with an Indian swine strain of genotype 4 resulted in viraemia and seroconversion in these animals (5). In 2008, in the United States, the team of Feagins *et al.* inoculated primates and pigs with the human strain of genotype 4 TW6196E (28) resulting, in both cases, in infection of the animals with seroconversion, viraemia and faecal shedding of HEV.

There are documented cases of zoonotic transmission to humans by consumption of contaminated meat

Currently, there are two cases that have been reported in the literature in which scientific evidence makes it possible to prove the zoonotic origin of contamination and to compare isolates. Both cases of contamination occurred in Japan:

Table 2: Confirmed cases of zoonotic transmission to humans by consumption of contaminated meat

No. of cases and incubation times	Animal species	Preparation method	Genotype	Data indicating zoonotic transmission	Reference country
4 † 40 days	Sika Deer	Slices of raw meat (sushi)	3	100% homology between sequences of patients and that of the rest of the frozen meat. 10 ⁵ GE ² /g of meat	Tei <i>et al.</i> , 2003 Japan: (101)
1 † 60 days	Wild Boar	Stew	3	99.95% homology between sequences of patients and that of the rest of the frozen meat.	Li <i>et al.</i> , 2005 Japan: (61)

The first case was described by Tei *et al.* in 2003 (101) and concerned the consumption of slices of raw Sika deer meat. The meat was kept frozen by the families, which made it possible to screen for HEV. The RNA viral titre was 10⁵ copies/gram. The sequencing showed 100% homology between the isolates from the meat and those from the patients (genotype 3).

The second case was reported by Li *et al.* in 2005 (61) in a 57 year old woman who ate meat stew made from two boars killed in a hunt. Ten people had eaten the meat but only this woman developed clinical hepatitis E. Frozen pieces enabled isolation of HEV in one of the two animals killed. The comparative phylogenetic analyses between ORF2 of the isolate taken from the patient and that taken from the meat led to classification within genotype 3 (nucleotide sequence homology of 99.95%).

In other cases, the origin of the contamination was quite likely food borne, but a comparative analysis could not be made between isolates from patients and those from meat suspected of being the source of the contamination:

² GE: genome equivalent

Table 3: Suspected cases of zoonotic transmission to humans by consumption of contaminated meat

No. of cases and incubation times	Animal species	Preparation method	Genotype	Data indicating zoonotic transmission	Reference country
10 † 14 to 60 days	Pig	Grilled livers, more or less cooked	3, 4	9/10 patients having eaten grilled pig livers.	Yazaki <i>et al.</i> , 2003 Japan: (109)
2 † 30 to 60 days	Wild Boar	Raw liver	4	Sharing and consumption of the same foodstuffs. IgM and IgG positive for both patients. RNA + for one of the two patients.	Matsuda <i>et al.</i> , 2003 Japan: (71)
5 † 39 days	Wild Boar	Grilled (Barbecue)	3	Of 12 people who participated in the same meal: IgM: 8/12, IgG: 11/12, RNA 2/12.	Tamada <i>et al.</i> , 2004 Japan: (98)
1 † 59 days	Wild Boar	Marinated grilled meat (Barbecue)	3	IgM, IgG, RNA positive and strong IgM reactivity in one other person who shared the same dish	Masuda <i>et al.</i> , 2005 Japan: (69)

The first series of cases involved 10 patients who contracted acute or fulminant hepatitis E in Japan between 2001 and 2002 (109). The epidemiological survey revealed that nine of the 10 patients had eaten several servings of pig livers, grilled, but more or less cooked, two to eight weeks before the appearance of symptoms.

In 2003, Matsuda *et al.* reported the case of two brothers who were hospitalised the same day for the same symptoms of acute hepatitis but at two different facilities in Japan (71). The hepatitis E (genotype 4) diagnosis was determined for both patients retrospectively after the death of one of them. Among the risk factors, the regular consumption of raw wild boar livers was noted during the three months prior to the outbreak of the disease. These two people were the only family members to eat the boar liver and were the only ones to develop hepatitis E.

The next cases occurred in 2004 in Japan among 12 members of a local senior citizens association (98). In reference to five clinical cases of hepatitis E among these members, it appeared that the only occasion on which these 12 people were together was at a barbecue during which they ate grilled wild boar. The phylogenetic test performed on isolates of viral RNA found in two of the clinical cases showed a homology of 99.4%.

The last case was reported by Masuda *et al.* in 2005, also in Japan (69). A 71 year old man developed acute hepatitis E. About 60 days earlier, this man had eaten wild boar cheeks with his wife and brother-in-law. Neither of these other two people showed signs of hepatitis. However, serological studies had shown that the brother-in-law was strongly seropositive for anti-HEV IgM and IgG, suggesting a recent subclinical infection.

Moreover, these serological studies have shown the following connections: in the Tei study (100), 89% of individuals seropositive for the anti-HEV antibodies had a history of eating raw venison, as opposed to only 46% of seronegative people, a significant difference (with $p = 0.035$). A German case-control study directed by Wichmann in 2008 also found a significant association between HEV infection and consumption of wild boar meat (OR 4.3; CI 95%) and offal (OR 2.7; CI 95% [1.2-6.2]) (105). Among the patients with autochthonous hepatitis E, 20% reported eating wild boar meat and 41% ate offal in the two months preceding the study as opposed to 6.7% and 18.5% among control individuals.

Thus, a link between the consumption of raw or insufficiently cooked pork, wild boar or venison products and the occurrence of hepatitis E has been reported in various studies.

b. Virus carriage in pigs and prevalence in foodstuffs derived from pigsVirus carriage in pigs - general

Several species are capable of harbouring the virus, but the main HEV animal reservoir is typified by pigs and more generally by the Suidae family. The infection is asymptomatic in domestic or production pigs (*Sus scrofa domesticus*) but they replicate and shed the virus liberally. Numerous articles recount the isolation of RNA in this species on all continents (30, 38, 44, 56, 63, 64, 79, 89, 93, 99, 108, 110, 113). Other swine are targets of HEV. Thus, several studies have made it possible to isolate the virus in wild boar. HEV has been identified in this species in Europe and in Japan, respectively, in the sub-species *Sus scrofa scrofa* (20, 45, 67) and *Sus scrofa leucomystax* (20, 45, 67). Along anecdotal lines, the Tanaka *et al.* study performed in 2004 revealed the presence of the virus in miniature Asian and American domestic pigs used for medical experiments (99). In all cases observed in swine, HEV of either genotype 3 or genotype 4 was involved.

Prevalence in pig farming

Many descriptive studies have been conducted on the hepatitis E virus in pigs in different countries but few are true prevalence studies, i.e., those including a sampling plan to guarantee representative data and the number of observations (farms, pigs) needed to ensure sufficient accuracy of the estimates. The unit of observation is highly variable depending on the studies: estimating the prevalence in animals (average “pig” prevalence, pigs coming from different batches or farms), estimating the intra-farm prevalence, and estimating the “farm” prevalence. The nature of the information collected also varies greatly between studies: seroprevalence (research on IgG antibodies and sometimes IgM and/or IgA by a serological technique), and prevalence of viral RNA (RT-PCR) in the serum, faeces (studies in livestock) or liver (purchase of commercial pig livers).

Serologically, all the studies converge toward a broad dissemination of the virus in pig farms if we consider as a criterion of positivity for a farm the detection of at least one seropositive pig. By using this criterion, 15 out of 15 farms sampled were positive in the U.S. in 1997 (74), 20/22 in New Zealand in 2001 (30), 23/50 in Laos in 2007 (8), 10/10 in Mexico in 2005 (17) and 40/41 in Spain in 2008 (93). A retrospective study concerning 208 farms sampled since 1985 showed that this endemic situation in pig production is not a new phenomenon (204 seropositive farms out of 208 analysed (14)).

At the individual level (pig), at 6 months of age, the average seroprevalence is usually lower, with high variability according to the studies: 56% of seropositive pigs in Japan in 2005 (97), 23% in Argentina in 2006 (77), 81% in Brazil in 2005 (33) and 51% in Laos in 2007 (8). This high variability stems from significant differences between batches on the same farm (4 to 48% for the Argentine study (77), 15 to 100% for the Brazilian study (33)). Breeding animals are also frequently seropositive (over 60% (93)).

In metropolitan France, an ongoing national survey suggests a high seroprevalence with over 90% of farms positive and with rates of serological prevalence of animals in each farm ranging from 2.5 to 80%.

Influence of age in pig farming

The presence of HEV in pigs changes according to their age (10, 72, 107). Animals under one month do not have viral RNA in their serum, likely due to protection against early infection by maternal immunity. Viraemia becomes detectable at two months of age, and then peaks between two and four months (29, 93, 96). The prevalence of RNA from HEV in the serum then decreases gradually until it almost disappears around 5-7 months, the age at which the pigs are slaughtered, depending upon production methods. In terms of virus shedding, the detection of RNA from HEV in the stool begins at around two months of age and the prevalence of animals shedding the virus reaches its maximum between two and 4.5 months. But unlike the serum, the prevalence of positive PCR in animal faeces clearly decreases with age but does not seem to disappear. Thus, according to these three studies, 8% of finishing pigs, at an age eligible for slaughter, i.e., between five and seven months in Taiwan and England, and up to 41% in Canada, show virus shedding.

Dynamics of intra-farm infection

Under actual farming conditions, the dynamics of infection by the hepatitis E virus are very similar to those described for most viral infections in pigs: acquisition of passive immunity transmitted by the sows via colostrum (60% of piglets), gradual decline in these passive antibodies up to 10-12 weeks of age, with seroconversion between 12 and 15 weeks of age corresponding to peak viraemia observed at 15 weeks of age (40% of animals (19)). In this study, the percentage of viraemic pigs increased from nine weeks of age until 15 weeks of age and decreased gradually until slaughter. IgM increased from nine weeks of age and nearly 100% of pigs monitored (n=16) were seropositive (IgG) at 22 weeks of age. These dynamics observed in a Spanish farm are also consistent with observations made in Japan where peak faecal shedding was observed between one and three months of age (75 to 100% of animals) and then decreased at 5-6 months of age (in only 7% of animals) (78). The high seroprevalance at the end of the finishing period revealed effective transmission of the virus between animals of the same group. This was confirmed by the experimental estimation of the basic reproduction number (R0) for the hepatitis E virus estimated at 8.8, revealing the theoretical possibility of one infectious pig coming into contact with a susceptible population and infecting more than eight animals during its infectious period. The duration of this period (ability to infect a susceptible pig after contact) was estimated at 49 days in this same study (10).

Infection in pigs, target organs

The target organ in pigs is primarily the liver and the infection is subclinical (57) although hepatitis lesions have been described in Spain in production pigs with the help of autopsies (identification of histological lesions) in a difficult sanitary context (Porcine post weaning multisystemic wasting syndrome) (68).

Experimentally induced infection studies show an extra-hepatic distribution of the hepatitis E virus. After experimentally-induced infection by intravenous route, the virus is likely to be found in the mesenteric and hepatic lymph nodes, and in the colon and small intestine up to 20-27 days post-inoculation (106). In this study, the virus was also found in the stomach and spleen but was more transient (14 days post-infection) and also occasionally in the kidneys, tonsils, salivary glands and lungs. Only the inoculation of a human strain in the pig enabled detection of viral RNA in the muscle up to 14 days post-infection. One more recent study of pigs inoculated by intravenous route (IV), and pigs coming into contact with them, showed the detection of viral RNA by PCR in the longissimus, biceps femoris and iliopsoas muscles up to 27 days post-inoculation in pigs by IV and up to 27-31 days after the onset of faecal shedding in contact pigs. The results of these studies are based on the amplification of viral RNA by qualitative PCR. There are no data on quantification of the viral load in these various organs.

Presence of HEV in pig liver

The presence of HEV in pork foodstuffs has been demonstrated. The first study of this type was conducted in Japan in 2003, in packaged pig liver sold in grocery stores on the island of Hokkaido (109). The RT-PCR analyses indicated that 2% of the livers tested contained viral RNA. Recently, other studies on pig livers have been conducted in the United States (26), India (53) and the Netherlands (11). The percentage of positive samples was 11.2 and 6.5% respectively.

In France, screening for the virus in slaughterhouse pig livers has shown a prevalence of viral RNA in about 3% of livers entering the food chain (unpublished data communicated by experts).

c. Virus carriage in other species (including wild boar and deer) and contamination of foodstuffs

Virus carriage

The pig is not the only animal to be infected by HEV. Other animals such as wild boar, deer, rats, dogs, cats, mongooses, cattle, sheep, goats and horses may have anti-HEV antibodies and may therefore have been exposed to the virus or to a similar agent. While isolates of genotype 3 and/or 4 have been identified in wild boar and deer, no virus has been connected with other serologically positive animals suspected of being potential viral reservoirs. Pigs, wild boar and deer are therefore true reservoirs but the other animals seem to be only occasional hosts, not responsible for human contamination. On the basis of studies published to date, the avian strain, which is sufficiently distant genetically from other HEV strains, does not represent an identifiable hazard to humans.

Transfer of this strain to non-human primates has not been possible with experimental infection. Experimental infection of chickens by strains of genotype 1 or 3 is not possible either (39) (Prof. XJ Meng, personal communication).

The few available studies on the presence of HEV in wild boar and deer give varying percentages of seroprevalence of 3 to 43% in wild boar and 2 to 35% in deer (**Table 4**). The prevalence of viral RNA in wild boar ranges from 3 to 25%, while one study reported 34% in deer (**Table 4**). In these studies there is no indication as to the age of the animals tested, making it difficult to assess the actual level of contamination from these reservoirs. The HEV strains amplified are of genotype 3 and are genetically very close to the human and pig strains described in the same regions, suggesting inter-species contamination.

Table 4: HEV frequency in wild boar and deer

Country	Animals	Serology	HEV RNA Genotype 3	Type of sample	Reference
Japan	Yezo Deer	34.8% (181/520)	nd	Serum	(102)
Japan	Wild Boar Deer	9% (n=35) 2% (n=117)	nd	Serum	(94)
Japan	Wild Boar wild farmed	25% (100/392) 71% (10/14)	nd 3%	Serum	(75)
Hungary	Wild Boar Deer	n/a	12.2% (9/74) 34.4% (11/32)	Liver	(88)
Germany	Wild Boar	n/a	5.3%	Serum	(45)
Germany	Wild Boar	n/a	15%	Liver	(92)
Italy	Wild Boar	n/a	25% (22/88)	Bile	(67)
Spain	Wild Boar	42.7%	19.6%	Serum	(20)
Netherlands	Wild Boar		4%	Faeces	(90)
France	Wild Boar	3.4% (3/88)	nd	Serums	N. Pavio (unpublished)

nd: not determined

Anecdotally, the presence of HEV (genotype 3) can also be observed in Japanese mongooses (8.3%, 7/84) but no human case has been connected with this reservoir.

Human contamination by derived foodstuffs

Cases of contamination by consuming wild boar or venison products have been described in Japan, but do not specify or give little data on the amount ingested; the cuts of meat or the viral load identified in cases of positive meat are incomplete. In the case described by Tei *et al.*, contamination was due to consuming a 'significant' amount of deer meat as sushi and sashimi (raw). The analysis of a remaining piece made it possible to find 10^5 genome equivalents per gram of meat. There was no indication about the cuts ingested. In the case described by Masuda *et al.*, the wild boar meat was prepared and marinated before being grilled on the barbecue (cooked very rare). The patient had eaten around 80g. The case described by Li *et al.* concerns the consumption of grilled boar meat without specifying either the amount or the cuts.

It should be noted that preparations of wild boar or venison are most often prepared traditionally, and intended for limited distribution. As such, they represent a lower exposure factor for the entire population.

d. Probability of human infection associated with the dose of HEV

In the current state of knowledge, there are no data enabling us to accurately determine the probability of infection for humans according to the dose of HEV ingested. Information on the number of infectious particles by genome of HEV detected by gene amplification is still fragmentary and very likely dependent upon experimental conditions. Nevertheless, the available data allow an approximation of an infectious dose (ID) of 50% by oral route in humans.

In 1994, Tsarev *et al.* titrated a human strain of probable genotype 1 in cynomolgus monkeys, both orally and intravenously. In this experiment, one ID-monkey-oral₅₀ \geq 10⁴ ID-monkey-IV₅₀ (103).

In light of these results, it may be concluded that a minimum of 10⁴ ID-pig-IV₅₀ might be needed to infect primates by oral route. This may be considered as the worst case scenario, since it implies that there is no pig/primate species barrier, which is probably a pessimistic assumption, but one that cannot be excluded given the current state of knowledge.

Moreover, a relationship was established between a number of genome equivalents (GE) defined by RT-PCR (limiting dilution assay) and the infectious dose by intravenous inoculation in pigs for one strain of genotype 3 (73). Thus, a dose of 10⁶ GE corresponds to 10^{4.5} ID-pig-IV₅₀ (49). Therefore, an ID-pig-IV₅₀ would correspond to 10^{1.5} GE. Under these conditions **the fifty percent primate infectious dose by oral route would be \geq 10^{5.5} GE**. Note that a recent study looked at oral transmission in pigs (13). Of the sixteen animals that received 10^{5.3} GE orally, 25% (4/16) showed signs of infection by HEV (faecal shedding and/or viraemia and/or seroconversion), which is highly consistent with the previous estimate calculated from different data.

3. Answers to the questions listed

a. Methods for detecting the hepatitis E virus

There are no permissive cells allowing HEV replication. Currently, there is no standardised method for detecting the HEV genome in food matrices. However, several methods have been used and validated for public health diagnosis (**Table 5**). These methods are routinely used on serum or stool samples from patients suspected of having hepatitis E. These same techniques are used for detecting HEV in pigs (serum and faeces) (**Table 5**). Detection of HEV in pig livers can also be accomplished with a protocol for extracting nucleic acids adapted to this matrix. For finished products, the detection methods stay the same but the nucleic acid extraction process is different from that used for livers, serums and stools. These different methods are reliable and have been validated in several laboratories but none are recognized as reference methods that can be decentralised to routine diagnostic laboratories. The analysis of food matrices requires special pulverising equipment that is not typically found in non-specialised diagnostic laboratories.

Table 1: Summary of different methods in published literature for detecting HEV by real time RT-PCR

Year	Sample	Automation/Probe	Target	Test sample	Detection limit or measurement interval	Comparison with other tests	Comments	Reference
2004	Stool	LightCycler / SYBR Green	ORF2	300 µL of a 10% faecal suspension	10 copies / reaction	Quantitative	One-step RT-PCR	(81)
2004	Serum/Stool	LightCycler / Taqman probes	ORF2	200µL	1000 copies /mL	Qualitative	Two-step RT-PCR	(66)
2005	Serum/Water	RAPID Thermal Cycler/ Taqman Probes	ORF3	250µL	4 to 4.10 ⁹ copies /reaction	Quantitative	One-step RT-PCR	(43)
2006	Serum	ABI PRISM 7000 / Taqman Probes	ORF2	140 µL	1.68 x 10 ¹ copies /reaction	Quantitative	Two-step RT-PCR	(3)
2006	Serum/Stool	LightCycler / SYBR Green	ORF2	100 µL of serum or 10% faecal suspension	4.5 to 4.5. 10 ⁴ copies/mL. Sensitivity of 5 copies/reaction	Quantitative	One-step RT-PCR	(62)
2006	Serum/Stool	LightCycler / Taqman probes	ORF2	200 µL of serum or 10% faecal suspension	10 to 10 ⁹ copies/ reaction	Quantitative	Two-step RT-PCR	(25)
2006	River and filtered waste water	?	?	?	?	Quantitative	?	(4)
2007	Stool/Tissues	Rotor-Gene 3000 / Taqman Probes and PPET (Primer Probe Energy Transfer) Probes	ORF2	140 µL in 5% suspension	1 to 20 copies /reaction	Quantitative	One-step RT-PCR	(34)
2007	Serum	ABI PRISM 7000 / Taqman Probes	ORF3	140 µL	5.6.10 ³ to 5.6.10 ¹⁰ copies/reaction	Quantitative	One-step RT-PCR	(111)
2008	Serum/Stool	Unknown/ Taqman Probes	ORF2	Unknown	20 copies / reaction	Quantitative	Two-step RT-PCR	(87)
2008	Serum/Saliva/Stool	Applied Biosystems 7500 / Taqman Probes	ORF2	200 µL of serum/saliva	25 copies /mL	Quantitative	One-step RT-PCR	(70)

				100 µL of faecal suspension				
2009	Pig Serum and Stool	Stratagene Mx3005P system / Taqman Probes	Several probes	?	?	Quantitative	Various RT-PCR	(104)
2009	Wild Boar Liver	?	ORF2	?	?	Qualitative	One-step nested RT-PCR	(92)

It should be emphasised that:

- a positive PCR result is comparable to the presence of an infectious virus only for products that have not undergone inactivation treatment; as such, the PCR may only relate to raw foodstuffs, and
- only quantitative PCR techniques may provide usable results; thus, the presence of low concentrations of HEV could represent a marginal risk, as for many orally transmitted pathogens. However, the definition of a hygienic standard would require an assessment of the infectious dose by oral route, which is still merely estimated (see **Section 2.d.**). It is advisable to start this kind of assessment without delay on a pig model, considered as a maximalist model of the susceptibility of humans to swine strains. This model would help confirm that the dose of $10^{5.5}$ GE is a good approximation of the oral infectious dose. Ideally, this type of study should also be done in primates. In the current state of knowledge, a standard could range from "no detectable genome" up to a threshold value, which is currently not precisely definable, but should be much less than 10^5 GE by amount consumed.

There are no permissive cells allowing HEV replication. There are now methods of viral genome detection that can be integrated into a risk management approach.

b. Risk to the consumer

Three population groups are particularly likely to develop severe forms of hepatitis E:

- subjects having an underlying liver pathology with a risk of fulminant hepatitis (84)
- immunocompromised subjects with a risk of chronic infection and cirrhosis (47).
- pregnant women (given the current state of knowledge, and although there are incomplete data on this topic regarding strains of genotype 3 or 4), who should be considered potentially at risk from the severe form.

In this section, the risks are analysed according to the type of product concerned.

Categories of products

The following table (**Table 6**) shows categories of pork products and highlights in grey those that are usually or occasionally eaten raw.

Table 6: Families of pork products

Category of products	Examples (non exhaustive list)	Organs	Consumption method	Cooking	
				Core temperature	Time
Dried ham	Raw ham (Bayonne, Vendée, etc.)	Muscle	Dried raw	/	/
Dry sausage	Sausage, French rosette salami, Chorizo	Lean pork, fat	Dried raw	/	/
	Figatelli, Toulouse liver sausage	Lean pork, fat, liver			
Sausages, sausage meat	Sausage spreads, Longaniza, Sobrasada	Lean pork, fat	Raw, may be eaten cooked	/	/
	Chipolata, Morteau sausage	Lean pork, fat	Raw, cook before eating	/	/

Bacon chunks	Chopped, smoked bacon chunks	Belly	Raw, cook before eating	/	/
Offal	Heart, liver		Raw, cook before eating	/	/
Cuts of meat	Chops, roasts	Muscle	Raw (may be marinated), cook before eating	/	/
Andouille [chitterling], andouillette sausage	Andouille de Vire	Stomach, sausage-stuffed pig stomach	Cooked	Precooking 85°C 2 nd cooking 92°C	6 to 7 hours 4 to 5 hours
Black pudding	Blood sausage with onions, Caribbean blood sausage	Blood, fat, rind	Cooked	80°C	20 to 35 min
Cooked ham	Ham, cooked shoulder roast	Muscle	Cooked	> 65°C	> 1 hour
Pâtés, Mousse	Country Pâté, liver mousse, galantine	Lean, fat, offal, rind, liver (depending on the recipe)	Cooked	> 72°C	30-40 min
Jellied products	Trotter, tongue, head cheese	Head, tongue or trotter, fat	Cooked	90-95°C	> 1 hour
Potted meat	Potted meat [<i>rillettes</i>]		Cooked	95°C	> 6 hours
Sausages, Cooked sausages	Sausage cooked with garlic	Lean, fat, belly	Cooked	> 70°C	15 to 20 min
	Liver sausage (Alsace)	Lean, fat, belly, liver			
Tripe	Tripe, Stuffed tripe [<i>Tripoux</i>]	Stomach, trotter	Cooked	90°C-95°C	7 to 8 hours

Regarding deer or wild boar, pâtés, hams or sausages found in stores are often (but not exclusively) traditionally-made products. It may be assumed that the technologies used to prepare these products are similar to those devised for pork.

Food consumption data were taken from the INCA 2 [Individual and National Study on Food Consumption] study which was conducted in 2006-07 on 4079 individuals, from 3 to 79 years of age. Only 2624 adults were asked to provide information as to how the pork meat was consumed. The number of individuals who consumed pork was 2332 (89%). A document attached as **Annex 3** identifies the frequency of pork product consumption patterns and quantifies the very low proportion of venison and wild boar product consumption.

The risks can be graded according to the preparation method (cooked or to be cooked/raw) and, for raw products, the originating organ, with the liver representing, at the current state of knowledge, the organ with the highest viral load (varying between 10^2 and 10^8 GE/gram).

Raw products: Liver preparations (liver sausage, Figatelli)

Figatelli is made of lean pork, pork fat, pig liver (30% minimum imposed by the Code of Practice for delicatessen, cured, and canned meats), wine and various additives, including salt (about 2.5%). The various ingredients are chopped and then forced into a pork gut (casing). The products are then heated at 25°C for 12 hours. After resting for 48 hours the Figatelli can be smoked (cold smoking temperature < 30°C). They are dried for four to six days (at approximately 14-16°C). The weight loss at the end of the process is around 12 to 15%. The pH is around 5 (4.8 to 5.2).

The main ingredient in Figatelli and related preparations is pig liver, which is a potentially virus-laden organ. It should be pointed out that, like all viruses, the hepatitis E virus cannot multiply in food matrices. The manufacturing process does not include any step that could inactivate or eliminate HEV by partitioning. Based on a prevalence of 3% of livers containing viruses, the mixing of pieces needed for manufacturing a batch significantly increases the risk of contamination of the final product, even if it can reduce the average viral load³. Viral safety experiments show that the 'mixing' factor plays a major role in cases of transmission from biological products, uncompensated by the dilution.

Drying takes place at a cold temperature (below 30°C). The literature does not contain any specific data on the fate of HEV in a dried product. This treatment method must be considered ineffective for this type of virus.

Two food-poisoning outbreaks occurring in 2007 and 2008, the first investigated by CIRE Sud and the HEV NRC, and the second by La Timone University Hospital in Marseille, are very likely related to the consumption of Figatelli (see above).

In March, 2009, the AP-HM⁴ tested a batch of seven Figatelli purchased in a Marseille supermarket, using PCR, among which five tested positive by PCR, with whole virus particles identified by electronic microscopy. The sequencing identified two viral strains in these Figatelli (genotypes 3c and 3f). These results (submitted for publication) confirm the presence of infectious virus in this product: in the absence of a proven or probable inactivation process, the presence of viral RNA must be likened to the presence of infectious virus. The viral load in the samples tested, a significant factor in quantifying risk, is not known at this time however, and the data on the frequency of contaminated batches must be completed.

³ For example, the manufacture of a batch of around 2100 Figatelli requires 75 livers. Based on 3% of contaminated livers, the probability of this batch being contaminated (containing at least one contaminated liver) is $1-(0.97)^{75}$, which is around 90%. The mean viral load for this batch will be 1.8 log lower than that of the original liver.

⁴ Assistance Publique – Hôpitaux de Marseille [Marseille Public Hospitals]

The epidemiology of the infection in pigs, the method of preparing liver sausages, the results from sampling conducted on commercial samples, the existence of clustered or sporadic human cases having their probable or possible origin in the consumption of Figatelli, indicate that consuming this type of specialty food raw poses a consumer health risk. Consumption of this kind of product constitutes a significant opportunity for exposure to the virus, even if the number of clinical cases remains low; the relative importance of the factors involved in clinical expression is not known (dose, specific mutations linked to tropism, individual susceptibility factors).

Given the potential severity of the symptoms, the Group believes that this information should be conveyed to the consumers of these products. Moreover, subjects particularly likely to develop very serious forms of hepatitis E are those who have an underlying liver pathology with a risk of fulminant hepatitis, immunocompromised subjects with a risk of chronic infection and cirrhosis, and lastly, pregnant women. These people should be given information that is specifically tailored to the risk incurred.

Raw products: Cured and dried ham (Bayonne, Vendée, etc.), sausage spreads, Longaniza, Sobrasada, dry sausage, French rosette salami, chorizo

To date, no case of human HEV has been reported after consuming this type of product. However, this concept must be considered from the perspective that, factually, almost all human HEV cases are still of unknown origin. An epidemiological investigation is currently underway among blood donors in France in order to identify whether avoiding the consumption of pork products is correlated with a lower serological prevalence in humans. This research should provide data of major importance in the assessment of dietary risk connected with pork products.

Regarding raw products not derived from liver, in the absence of reported clinical cases and/or serological data, analysis can only be based on an evaluation of exposure factors. The manufacturing process for these products does not include any step likely to inactivate HEV. Consequently, the risk appears to be tied to the frequency and level of contamination of the raw ingredients (muscle or fat). Given the stage of viraemia associated with its dissemination in the body and the presence of HEV found in muscle, it is likely that HEV can be identified in this type of preparation. Nevertheless, the quantitative data to assess their impact are lacking. Some of these data are in the process of being acquired during experimental infection of pigs. In addition, the Group suggests an investigation be undertaken on the quantitation of genomes of HEV in various raw delicatessen products.

In conclusion, the measures that could be implemented (information on at-risk individuals as defined at the beginning of the section) would only raise, where appropriate, a precautionary principle; their impact on public health is not demonstrable thus far. The expert assessment group believes, however, that the data for refining this assessment could be available within a year.

Products that are cooked or to be cooked

The impact of cooking on the virus hazard can only be assessed from data based on its infectious nature. The viral genome, which can be quantified by molecular biology techniques, does not allow the infectious nature of the virus to be confirmed. Yet, there are no permissive cells allowing HEV replication. Resistance to the treatments can therefore only be assessed approximately, by extrapolation from known data for other cultured viruses, or based on experiments conducted on animals.

Thus, for the hepatitis A virus (HAV), recognised as being a highly heat-resistant virus, numerous studies have converged to consider that cooking to the core at 90°C for two minutes will reduce the viral titre by over 4 log units (7) (18) (76) (21). This is observed in matrices as diverse as cream, seafood and strawberry puree (with 28% sugar). However, the matrix effect is important. For example, it has been shown that the sugar content (21) and fat content (7) can have a preservative effect. Thus, one of the most significant values of $D_{90^{\circ}\text{C}}$ (the time required for inactivation of 1 log unit at 90°C) described to date is three minutes (21). It was described for HAV in strawberry puree with 52% sugar. Unfortunately, no studies of inactivation have been conducted on liver specimens to assess the effect of this matrix on the inactivation of HAV, and HEV levels in pig liver are still too imprecise to estimate the conditions for total inactivation of the virus. However, it is acknowledged that cooking to the core for 5 min. at 100°C eliminates the HAV hazard (1).

Emerson *et al.* (24) suggest greater resistance of HAV by comparison with HEV even if, in the opinion of the authors, the experimental methods can be debated.

When referring only to the data concerning HEV, the studies by Feagins *et al.* (27) have shown, by bioassay, that the virus found in the liver could be infectious for the pig but that reaching a core temperature of 71 °C in diced pig liver of 0.5 to 1cm² by frying it at 191 °C for 5 minutes or cooking in boiling water for 5 minutes inactivates the virus present due to natural contamination. Conversely, incubation at 56 °C for one hour was insufficient for total inactivation of the hepatitis E virus. However, these results are difficult to interpret because the initial level of contamination was unknown. Consequently, this information is partial because the methodology does not enable verification of how this result can be extended to livers having various levels of contamination. Nevertheless, these results reinforce the hypothesis that a treatment of five minutes at a core temperature of 100 °C is effective for eliminating the virus.

A protocol aimed at quantifying the conditions for thermal inactivation can be developed based on the only reliable test of infectivity currently available: bioassay by inoculation of pigs. Under these conditions, the study of inactivation parameters, although not presenting any methodological difficulty, will be costly, but could be conducted by French laboratories working on the subject.

According to the current state of knowledge, products eaten cooked appear to pose a lesser risk than their raw counterparts, even more so when they are cooked at a high core temperature for long periods of time. The data are nonetheless inadequate for proposing practical methods of effective cooking.

Risk management procedures:

Considering the frequency of contamination in pigs, obtaining unaffected herds would be an unrealistic objective, even in the medium term. Analysis of raw ingredients (e.g.: liver mixtures) of the foods concerned seems to be a possibility. As it is not possible to culture the hepatitis E virus in cell lines, detection of HEV is primarily molecular. The detection techniques and their limits, and the methods for interpreting the results, were described in section 3.a. “Methods for detecting the hepatitis E virus”.

c. Risks to the environment: contamination by slurry

Given the significant faecal shedding of HEV, this section considers methods for managing pig slurry, applicable treatments and risks of survival of HEV in it.

Methods for managing pig slurry

A study by the Central Office for Statistical Surveys and Studies (SCEES) (91) has shown that 94% of pig finishing houses and 85% of gestation stalls for pregnant sows are on slatted floors. Pig waste is therefore mainly produced in the form of slurry. However, a significant percentage of animals are raised on litter (straw, sawdust, chips), which also leads to the production of manure.

At the end of 2005, 378 treatment stations handling livestock effluents were listed in France with 85% just for the Brittany area (60). These units mainly perform biological waste treatment using activated sludge and to a lesser extent, composting of slurry on straw or green waste (75 and 15% of the units in operation, respectively). Considering the objectives of waste reduction, the number of stations to be established would eventually be between 400 and 500 units in western France. The treatment processes lead to a high diversity of products that are divided among the following categories:

- **Raw waste:** slurry, manure (uncomposted)
- **Solid treatment by-products:** Phase-separated refuse, composted manure on straw or green waste and dehydrated slurry/sludge.
- **Liquid treatment by-products:** biological sludge, waste water, aerated slurry.

Practices for managing effluents from pig farms are highly diverse and range from spreading fresh untreated manure, to chemical treatment in exceptional cases of confirmed epizootic disease. The treatments actually carried out in real conditions are implemented primarily to reduce the discharge of nitrogen and phosphorous and not for the purpose of cleaning up *vis à vis* a potential biological risk. This is why the data on the effectiveness of these types of treatment on the survival of pathogens are very fragmented and often non-specific, based on bacteria, parasites and virus indicators (generally enterovirus for the latter). Effluents from pig production are by and large (90%) treated in liquid systems (slurry) with an average of 6-10 months storage. Ten percent of slurry is treated aerobically (see below), 10% in phased separation and the remaining 80% does not undergo treatment. Ten percent of farms treat their solid waste (manure), and most of this manure (90%) is treated in composting; the remaining 10% are not treated (Anne Marie Pourcher, CEMAGREF [French Agricultural and Environmental Engineering Research Centre], direct communication).

Various types of applicable treatment

Various types of treatment can be applied to slurry: physical treatment (spreading fresh untreated material, storage before spreading, mechanical phased separation, lagooning, dehydration, heat treatment), biological treatment (aerobic, composting, anaerobic digestion = methanation) and chemical treatments (primarily lime). Detailed information on these different treatments is presented in **Annex 4**.

Survival of HEV in slurry

No study has been conducted to determine the survival of HEV in pig production waste or the effect of storage or treatment (chemical, heat). Generally, non-enveloped viruses transmitted by orofaecal route are resistant in the environment (parvovirus, enterovirus, norovirus) and in slurry (for these swine viruses). The data available on HEV only cite the infectious nature of the samples of waste found positive by RT-PCR (50) and confirm possible contamination of the environment. In France, the identification of HEV by gene amplification was carried out on several kinds of piggyery effluents:

- 1) raw slurry in livestock buildings,
- 2) effluent after 5-6 weeks storage in a homogenisation pit (anaerobic but regularly replenished with fresh slurry) then mechanical solid and liquid phase separation with liquid phase treatment in an aeration reactor for the elimination of nitrogen (40 days), and
- 3) sludge stored from 4-6 months after the second stage of mechanical separation and nitrogen treatment.

Quantification by real time RT-PCR was performed on the positive samples. The results show titres of 10^5 to 10^6 GE per gram of raw slurry, 10^4 to 10^5 GE per gram of slurry after aeration (40 days) and $<10^1$ GE per gram of sludge. Two positive samples at 10^5 and 10^6 GE per gram were inoculated intravenously in pigs to confirm the presence of infectious virus. The inoculated animals presented HEV faecal shedding and seroconversion. These inocula corresponded to raw slurry sampled at pig farms.

This study was conducted on a limited number of slurry samples but the presence of 10^5 GE of HEV per gram in a sample after four months of storage suggests that the virus may survive long-term in an unfavourable environment.

There are no data on the possible contamination of the environment by HEV following spreading of slurry: groundwater, food watered with contaminated water or food grown in contaminated soil. In the study by Kasorndorkbua *et al.* in the United States (50), HEV was not found in the surface water near contaminated pig farms. However, farming and manure spreading conditions in France are different. The effect of the spreading method and in particular ploughing in the slurry is to be determined.

There is a paradox between the presence of HEV in large amounts in slurry and the epidemiological data: the geographic distribution of human cases does not correspond to areas of maximum density of pigs. Although hypotheses can be proposed (almost exclusive consumption of bottled water in areas of intense pig production, absence of karst terrain), this paradox underscores our incomplete understanding of HEV transmission factors.

Spreading fresh slurry could pose a risk of environmental contamination. However, in view of the available epidemiological data, the risk of transmission to humans by this route could only affect a very limited number of the cases at most. Additional studies would be needed in order to more effectively answer to this point.

Conclusions and recommendations

The hepatitis E virus is characterised by its wide asymptomatic distribution in pig production and by the existence of human cases in proportionately low numbers, sometimes severe and much more rarely lethal. Many unknown factors remain, particularly when considering the following points:

- The role of pigs as reservoirs in human contamination, their probable involvement being proven nevertheless only in rare cases;
- The routes of transmission to humans from this reservoir;
- The relative importance of factors involved in clinical expression in the infected individuals (dose, specific mutations linked to tropism of the host, individual susceptibility factors);
- Survival of the virus in different environments and, as applicable, its part in human contamination;
- Methods for detecting the virus: A standardised method for detecting the HEV genome in food matrices is desirable for establishing reliable investigation and management measures, and the means for its validation should be implemented.

Information should be provided to population groups exposed to potential risks, especially individuals at risk from severe forms, and greater awareness should be promoted among health professionals.

These are the data that AFSSA is able to provide in answer to the questions raised in the Request.

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Key words

Cooking, Figatelli, liver, hepatitis E, pig, risks, sausage, drying, hepatitis E virus, inactivation, slurry, detection methods.

The Director-General of AFSSA

Marc Mortureux

Annexes

Annex 1: Decision to create an emergency collective expert assessment group and modification decision

AGENCE FRANÇAISE DE SECURITÉ SANITAIRE DES ALIMENTS

**Décision n°2009/04/293
portant création du groupe d'expertise collective d'urgence « Risque de contamination humaine par le virus de l'hépatite E via l'ingestion de figatelles »**

La Directrice générale de l'Agence française de sécurité sanitaire des aliments,

Vu le code de la santé publique, et notamment ses articles L.1323-4 et R.1323-22 ;

Vu l'arrêté du 4 août 2006 portant nomination des membres des comités d'experts spécialisés de l'Agence française de sécurité sanitaire des aliments ;

Vu l'arrêté du 17 octobre 2006 relatif aux comités d'experts spécialisés placés auprès de l'Agence française de sécurité sanitaire des aliments ;

Vu l'arrêté du 27 décembre 2006 modifiant l'arrêté du 17 octobre 2006 relatif aux comités d'experts spécialisés placés auprès de l'Agence française de sécurité sanitaire des aliments ;

Vu la décision du 27 octobre 2006 portant nomination à des comités d'experts spécialisés de l'Agence française de sécurité sanitaire des aliments ;

Vu le règlement intérieur de l'Agence française de sécurité sanitaire des aliments,

DECIDE :

Article premier. Il est créé sur proposition de la Directrice générale et en concertation avec la présidente du comité d'experts spécialisé « Microbiologie » un groupe d'expertise collective d'urgence dénommé « **Risque de contamination humaine par le virus de l'hépatite E via l'ingestion de figatelles** » chargé d'évaluer le risque de contamination humaine par le virus de l'hépatite E (VHE) par ingestion de figatelles (saucisses crues à base de foie de porc) (saisine 2009-SA-0101).

Article 2. Le groupe mentionné à l'article premier est composé des membres suivants :

- Membres du CES « Microbiologie » :
 - M. Christophe GANTZER (Faculté de pharmacie de Nancy)
 - M. Pascal GARRY (IFIP Maisons Alfort)
 - M. Bernard PICOCHÉ (ADRIA Normandie)
 - Mme Véronique VAILLANT (InVS Saint-Maurice)
- Personnalités scientifiques :
 - M. Marc ELOIT (ENV Alfort)
 - M. Jacques IZOPET (CHU Toulouse)
 - Mme Elisabeth NICAND (CNR VHE Paris)
 - Mme Nicole PAVIO (Afssa Lerpaz Alfort)
 - Monsieur Nicolas ROSE (AFSSA Ploufragan)

Article 3. M. Marc ELOIT est nommé président du groupe mentionné à l'article premier.

Article 4. Les conclusions du groupe seront émises sous la forme d'un avis avant le 30 avril 2009.

Article 5. La coordination scientifique du groupe mentionné à l'article premier est assurée par l'Unité d'évaluation des risques biologiques de la Direction de l'évaluation des risques nutritionnels et sanitaires.

Article 6. La présente décision sera publiée dans le *Bulletin officiel* de l'Agence française de sécurité sanitaire des aliments.

Fait à Maisons-Alfort, le **23 AVR. 2009**

La Directrice générale de l'Agence française de
sécurité sanitaire des aliments

Pascale BRIAND

A handwritten signature in black ink, appearing to read 'P. Briand', with a horizontal line underneath it.

FRENCH FOOD SAFETY AGENCY

Decision no. 2009/04/293

concerning the establishment of an emergency collective expert assessment group on the 'Risk of human contamination by the hepatitis E virus due to consumption of Figatelli'

The Director-General of the French Food Safety Agency,

Considering the Public Health Code, and Articles L. 1323-4 and R. 1323-22 specifically;

Considering the 4 August 2006 Order concerning the appointment of members to the Scientific panels of the French Food Safety Agency;

Considering the 17 October 2006 Order regarding the Scientific panels set up with the French Food Safety Agency;

Considering the 27 December 2006 Order amending the 17 October 2006 Order regarding the Scientific panels set up with the French Food Safety Agency;

Considering the 27 October 2006 decision regarding appointment to the Scientific panels of the French Food Safety Agency;

Considering the internal by-laws of the French Food Safety Agency,

HAS DECIDED:

Article 1. Upon the proposal of the Director-General in consultation with the Chairperson of the Scientific Panel on 'Microbiology', a '**Risk of human contamination by the hepatitis E virus due to the consumption of Figatelli**' emergency collective expert assessment group shall be set up, responsible for assessing the risk of human contamination by the hepatitis E virus (HEV) due to the consumption of Figatelli (raw pork liver sausages) (Request 2009-SA-0101).

Article 2. The group mentioned in Article 1 shall be made up of the following members:

- Members of the Scientific Panel on 'Microbiology':

Mr. Christophe GANTZER (Nancy Faculty of Pharmacy)

Mr. Pascal GARRY (IFIP [French Pig Institute] Maisons-Alfort)

Mr. Bernard PICOCHÉ (ADRIA [Association for Research and Development in the Food Industry] Normandy)

Ms. Véronique VAILLANT (InVS [French Institute for Public Health Surveillance] Saint-Maurice)

- Key scientific figures:

Mr. Marc ELOIT (ENV [National Veterinary College] Alfort)

Mr. Jacques IZOPET (CHU [University Hospital] Toulouse)

Ms. Elisabeth NICAND (CNR VHE [HEV National Reference Centre] Paris)

Ms. Nicole PAVIO (AFSSA LERPAZ Alfort)

Mr. Nicolas ROSE (AFSSA Ploufragan)

Article 3. Mr. Marc ELOIT shall be named Chairman of the group listed in Article 1.

Article 4. The conclusions of the group shall be issued as an Opinion before 30 April 2009.

Article 5. Scientific coordination of the group listed in Article 1 shall be provided by the Biological risk assessment unit of the Department for the evaluation of nutritional and health risks.

Article 6. This Decision shall be published in the Official Bulletin of the French Food Safety Agency.

Issued at Maisons-Alfort on 23 April 2009

Director-General of the French Food Safety Agency

Pascale BRIAND

[Signature]

AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES ALIMENTS

Décision n°2009/08/428

Relative au groupe d'expertise collective d'urgence « Risque de contamination humaine par le virus de l'hépatite E via l'ingestion de figatelles »

La Directrice générale adjointe de l'Agence française de sécurité sanitaire des aliments,

Vu le code de la santé publique, et notamment ses articles L.1323-1, L.1323-4, R.1323-16 et R.1323-22 ;

Vu l'arrêté du 17 octobre 2006 relatif aux comités d'experts spécialisés placés auprès de l'Agence française de sécurité sanitaire des aliments, modifié par l'arrêté du 27 décembre 2006;

Vu la décision du 21 juillet 2009 portant nomination aux comités d'experts spécialisés de l'Agence française de sécurité sanitaire des aliments ;

Vu le règlement intérieur de l'Agence française de sécurité sanitaire des aliments,

DECIDE :

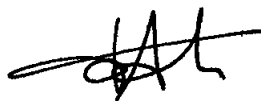
Article premier. L'intitulé du groupe d'expertise collective d'urgence dénommé « Risque de contamination humaine par le virus de l'hépatite E via l'ingestion de figatelles » institué par la décision n° 2009/04/293 du 23 avril 2009 (saisine n°2009-SA-0101¹) est modifié en « virus de l'hépatite E » :

Article 2. La durée du mandat du groupe d'expertise collective d'urgence « virus de l'hépatite E » est prolongée jusqu'au 1^{er} septembre 2009, ceci notamment afin de permettre au groupe de répondre à la saisine n°2009-SA-0146².

Article 3. La présente décision sera publiée dans le *Bulletin officiel* de l'Agence française de sécurité sanitaire des aliments.

Fait à Maisons-Alfort, le 25 AOUT 2009

La Directrice générale adjointe de l'Agence française de sécurité sanitaire des aliments



Valérie BADUEL

¹ demande d'avis relatif au risque de contamination humaine par le virus de l'hépatite E (VHE) par ingestion de figatelli (saucisses crues à base de foie de porc).

² demande d'avis sur les méthodes de détection du virus de l'hépatite E et sur le comportement du virus dans le lisier de porc, lors de la cuisson, du séchage, du salage ou du fumage des produits à base de foie de porc

FRENCH FOOD SAFETY AGENCY

Decision no. 2009/08/428

Regarding the ‘Risk of human contamination by the hepatitis E virus due to the consumption of Figatelli’ emergency collective expert assessment group

The Deputy Director-General of the French Food Safety Agency,

Considering the Public Health Code and Articles L. 1323-1, L.1323-4, and R.1323-16 and R.1323-22 specifically;

Considering the 17 October 2006 Order regarding the Scientific panels set up with the French Food Safety Agency, amended by the 27 December 2006 Order;

Considering the 21 July 2009 Decision regarding appointment to the Scientific panels of the French Food Safety Agency;

Considering the internal by-laws of the French Food Safety Agency,

HAS DECIDED:

Article 1. The title of the ‘Risk of human contamination by the hepatitis E virus due to consumption of Figatelli’ emergency collective expert assessment group established by Decision no. 2009/04/293 on 23 April 2009 (Request no. **2009-SA-0101**¹) shall be amended to ‘Hepatitis E virus’:

Article 2. The term of office of the ‘Hepatitis E virus’ emergency collective expert assessment group shall be extended to 1 September 2009, specifically to enable the group to answer Request no. **2009-SA-0146**².

Article 3. This Decision shall be published in the Official Bulletin of the French Food Safety Agency.

Issued at Maisons-Alfort on 25 August 2009

Deputy Director-General of the
French Food Safety Agency

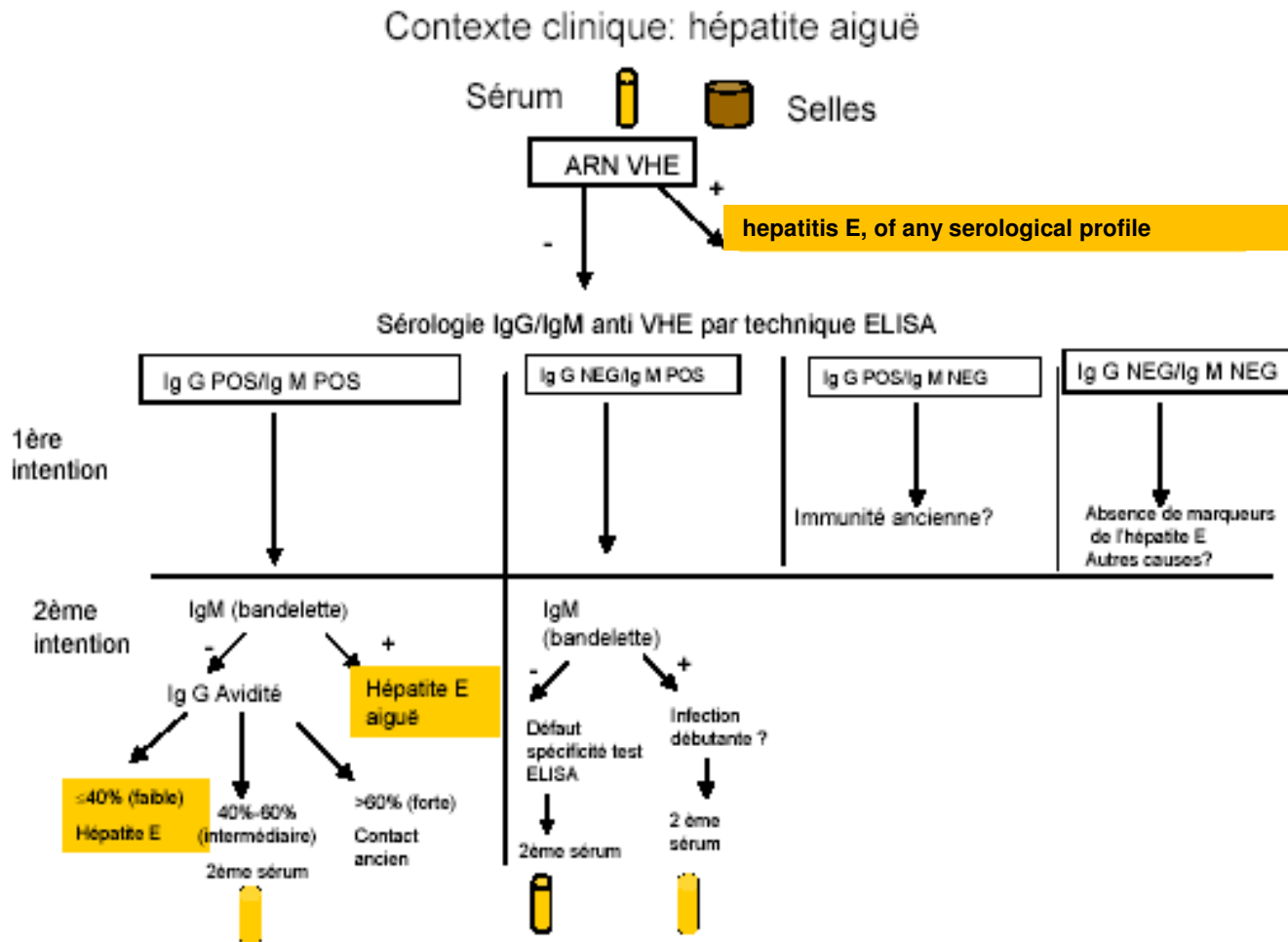
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Valérie BADUEL

¹ Request for an Opinion on the risk of human contamination by the hepatitis E virus (HEV) due to consumption of Figatelli (raw pork liver sausage).

² Request for an Opinion on methods for detecting the hepatitis E virus, and the behaviour of the virus in pig slurry, and during cooking, drying, salting or smoking of products made from pig liver

Annex 2: Algorithm for interpreting biological profiles for hepatitis E, 2007 (source NRC)



Contexte clinique	Clinical background
Hépatite aiguë	Acute hepatitis
Sérum	Serum
Selles	Stool
ARN VHE Hépatite E, quels que soient les profils sérologique	HEV RNA Hepatitis E, of any serological profile
Sérologie IgG/IgM anti VHE par technique ELISA	Anti-HEV IgG/IgM serology by enzyme-linked immunosorbent assay (ELISA) technique
IgG POS/IgM POS	IgG POS/IgM POS
IgG NEG/IgM POS	IgG NEG/IgM POS
IgG POS/IgM NEG	IgG POS/IgM NEG
IgG NEG/IgM NEG	IgG NEG/IgM NEG
1ère intention	First line
Immunité ancienne ?	Prior immunity?
Absence de marqueurs de l'hépatite E Autres causes ?	Absence of hepatitis E markers Other causes?
2ème intention	Second line
IgM (bandelette)	IgM (strip)
IgG Avidité	IgG avidity

Hépatite E aiguë	Acute hepatitis E
≤40% (faible) Hépatite E	≤40% (low) hepatitis E
40%-60% (intermédiaire)	40%-60% (intermediate)
>60% (forte)	>60% (high)
2ème sérum	2 nd serum
Contact ancien	Prior contact
Defaut spécificité test ELISA	ELISA lack of specificity test
Infection débutante?	Early stage of infection?

Annex 3: Technical notice regarding estimates of consumption of pork, wild boar or venison meat products**Background and objectives**

Following a meeting of the 'Risk of human contamination by the hepatitis E virus due to the consumption of Figatelli' emergency collective expert assessment group, the following question was asked: *“What information is available in the INCA [Individual and National Study on Food Consumption] database to support the reasoning among the expert assessment group members about the problem – the presence of HEV in meat products from pigs, wild boar and deer?”*

To offer the best support for the expert assessment group's analysis, it was agreed that the following information be provided:

- Average amount and standard deviation across the general population, average amount and standard deviation among consumers only, and consumer rates for pork, wild boar and venison products
- The available information as to form (fresh, canned, frozen and other) and source (commercial, homemade and other) of the products
- Consumption habits for pork meat, bacon chunks and raw sausages, and cooking habits for pork meat and sausages
- Available information on cooking and the cuts of pork meat eaten

The purpose of this notice is to present this information.

Materials and methods

The food consumption data come from the INCA 2 study, which was conducted in 2006-07 involving 4,079 people, aged 3-79 years, who were divided into two sub-samples: 1,455 children aged from 3-17 years and 2,624 adults aged from 18-79 years. Participants were selected in the 1999 population census and based on new home construction between 1999 and 2004, according to a three-stage sampling plan stratified by the size of the town and the region.

A weight was assigned to each individual in the two samples (3-17 years and 18-79 years) in order to ensure their representativeness on a national level. Furthermore, in order to guarantee the validity of the estimates, underreporting individuals were excluded from the analyses, which therefore applied to 1444 children and adolescents and 1918 adults aged from 18-79, who were non-underreporting.

Collection of food consumption data

The food survey method was a seven-day food record. Each day the food was divided into three main meals (breakfast, lunch, and dinner) and three between-meal snacks (between breakfast and lunch, between lunch and dinner, and after dinner until breakfast the following day). For each main meal, participants had to describe the place where it was taken, with whom it was taken, and the time the meal started and ended. This information was not recorded for the snacks taken between meals. The participant then had to describe in detail the food and beverages consumed at each meal or snack.

The participant was required to write down, in a column provided for this purpose, the food name and brand, when known. Then, the participant was to estimate the amount consumed using a photograph manual of portion sizes, or household measurements, or weight and volume units. The participant also had to write down the number of units or pieces consumed.

Lastly, for each food or beverage, the participant was to indicate whether it involved a product that was:

- reduced fat, reduced sugar, fortified, dietetic
- fresh, canned, frozen or other (form of the product)

- commercial, made by the participant or a family member, or from another source (product source)

Collection of information on eating habits

Using a self-administered age-appropriate questionnaire, each person also reported additional information on their eating habits. Included in this information are two questions on consumption of raw meat and method of cooking. These two questions were only included in the questionnaire administered to adults (18-79 years).

- The question about eating particular foodstuffs (that were meant to be eaten cooked) without cooking them, was the following:

Do you sometimes eat the foods below without cooking them⁵?

Circle only one answer per line

	One or more times per week	One to three times per month	Less than once per month	You never eat it raw	You never eat it cooked or raw	You don't know
Raw bacon chunks (in pasta, salad, as a snack) 1 2 3 4 5 6
Raw packaged sausages (frankfurters, etc.) ⁶ 1 2 3 4 5 6
Raw pork meat 1 2 3 4 5 6

- The question about the degree of cooking was the following:

When you eat these meats cooked, indicate the usual degree of cooking...⁷

Circle only one answer per line

	You never eat it	Very rare	Rare	Medium	Well done	Very well done
Pork (escalope, chop, roast, etc.) 1 2 3 4 5 6
Pork sausage 1 2 3 4 5 6

⁵ This question was also asked in the survey for other foods such as other types of meat (beef, poultry, and horse) as well as fish and eggs.

⁶ NB: Frankfurters are actually cooked products

⁷ This question was also asked in the survey for other meats: beef, veal, horse, lamb, and poultry.

Results

Amounts of food eaten

The following food groups were first composed and then analysed:

- Pork meat (except delicatessen meats)
- Pork delicatessen meats
- Pork offal
- Total pork meat
- Wild boar meat (except delicatessen meats)
- Wild boar delicatessen meats
- Wild boar offal
- Total wild boar meat
- Venison meat (except delicatessen meats)
- Venison delicatessen meats
- Venison offal
- Total venison meat

To compose them, identification was conducted using the following nomenclature groups from INCA2: Meat, Poultry and Game, Offal and Meat Products (delicatessen meats).

Pork meat-based products were identified using INCA2 nomenclature headings that clearly indicate a product made from pork: for example: cooked pork kidneys, raw ham, etc.). For products that may be made from pork or other meats (potted meat), the food headings entered by the survey participants made it possible to distinguish when the food was clearly made from meat other than pork. Ultimately, 15,219 occasions were identified when pork meat-based products were eaten.

Wild boar or venison meat-based products were identified using the food headings entered by the individual survey participants. Ultimately, 86 occasions were identified when wild boar meat products were eaten, 46 occasions of roe deer (*chevreuil*) meat product consumption, and 17 occasions of red deer (*cerf*) meat products being consumed.

The following information was taken from each of the food groups listed previously, in children and adults, by gender and age categories:

- average amount of the product consumed compared to the whole population (i.e., consumers and non-consumers) as well as the associated standard deviation;
- average amount of product consumed by consumers only, as well as the associated standard deviation;
- the consumer rates that correspond to the ratio between the number of consumers and the whole population.

The amounts are expressed in grams per day.

Table 1. Average amount (grams/day) and standard deviation, for the whole population and consumers only, and consumer rates for pork, wild boar and venison products in children by gender

	Garçon (684 individus)					Fille (760 individus)					Ensemble (1444 individus)				
	Ensemble Population		Seuls Consommateurs		Taux de conso	Ensemble Population		Seuls Consommateurs		Taux de conso	Ensemble Population		Seuls Consommateurs		Taux de conso
	Moy	ET	Moy	ET		Moy	ET	Moy	ET		Moy	ET	Moy	ET	
Viande de porc (hors charcuteries)	8,47	13,74	19,60	14,79	43,3	6,69	10,53	15,90	10,82	43,2	7,61	12,32	17,83	13,18	43,2
Charcuteries à base de porc	22,42	22,09	26,74	21,59	84,8	17,98	16,45	21,67	15,69	84,3	20,27	29,68	24,30	19,15	84,6
Abats de porc	0,03	0,88	16,24	13,09	0,4	0,03	0,70	13,67	6,54	0,3	0,03	0,80	14,89	10,26	0,3
Viande de porc totale	30,92	27,35	35,38	26,42	88,7	24,70	20,67	28,56	19,60	88,6	27,91	24,54	32,10	23,63	88,6
Viande de sanglier (hors charcuteries)	0,12	2,42	35,99	22,92	0,7	0,09	0,91	7,94	2,79	0,8	0,11	1,85	14,22	16,14	0,8
Charcuteries à base de sanglier	0,02	0,36	7,14	0,00	0,1	0,01	0,16	3,26	0,35	0,4	0,01	0,28	5,36	1,95	0,3
Abats de sanglier	0,04	1,00	27,00	0,00	0,1	0,01	0,33	10,29	0,00	0,1	0,02	0,76	20,16	8,22	0,1
Viande de sanglier totale	0,17	3,09	29,72	27,83	0,9	0,11	0,98	7,39	3,08	1,3	0,14	2,32	13,82	18,21	1,1
Viande de cerf et chevreuil (hors charcuteries)	0,05	0,65	7,28	3,09	0,6	0,05	0,75	8,58	4,23	0,5	0,05	0,70	7,88	3,71	0,6
Charcuteries à base de cerf et chevreuil	0,01	0,22	3,57	0,00	0,3	0,01	0,32	6,77	3,55	0,3	0,01	0,27	4,54	2,45	0,3
Abats de cerf et chevreuil	0,03	0,93	28,57	0,00	0,1	0,03	0,78	18,57	0,00	0,1	0,03	0,86	22,47	4,88	0,1
Viande de cerf et chevreuil totale	0,09	1,48	8,82	11,37	0,9	0,10	1,13	10,07	5,50	0,9	0,10	1,32	9,40	9,16	0,90

Garçon (684 individus)	Boys (684 individuals)
Fille (760 individus)	Girls (760 individuals)
Ensemble	Combined group (1444 individuals)
Ensemble population	Whole population
Seuls consommateurs	Consumers only
Taux de conso	Consumption rates
Moy	Mean
ET	SD
Viande de porc (hors charcuteries)	Pork meat (other than delicatessen meats)
Charcuteries à base de porc	Pork delicatessen meats
Abats de porc	Pork offal
Viande de porc totale	Total Pork meat
Viande de sanglier (hors charcuteries)	Wild Boar meat (other than delicatessen meats)
Charcuteries à base de sangliers	Wild Boar delicatessen meats
Abats de sanglier	Wild Boar offal
Viande de sanglier totale	Total Wild Boar meat
Viande de cerf et chevreuil (hors charcuteries)	Venison meat (other than delicatessen meats)
Charcuteries à base de cerf et chevreuil	Venison delicatessen meats
Abats de cerf et chevreuil	Venison offal
Viande de cerf et chevreuil totale	Total Venison meat

Source: AFSSA, INCA2 Study, 2006-07

Table 2. Average amount (grams/day) and standard deviation, for the whole population and consumers only, and consumer rates for pork, wild boar and venison products in children by age

	3-10 ans (570 individus)					11-14 ans (450 individus)					15-17 ans (424 individus)				
	Ensemble Population		Seuls Consommateurs		Taux de conso	Ensemble Population		Seuls Consommateurs		Taux de conso	Ensemble Population		Seuls Consommateurs		Taux de conso
	Moy	ET	Moy	ET		Moy	ET	Moy	ET		Moy	ET			
Viande de porc (hors charcuteries)	6,81	10,79	15,45	11,43	44,7	9,12	14,22	21,06	14,69	44,4	7,77	13,25	20,33	14,28	39,9
Charcuteries à base de porc	19,34	16,65	22,69	15,79	86,3	22,11	20,97	25,49	20,51	85,3	20,37	24,45	27,28	24,74	81,4
Abats de porc	0,01	0,36	9,64	0,00	0,2	0,02	0,71	20,57	0,00	0,2	0,07	1,44	17,30	13,74	0,7
Viande de porc totale	26,17	20,71	29,55	19,61	90,0	31,25	26,70	34,82	25,89	89,3	28,22	29,78	35,53	29,27	86,1
Viande de sanglier (hors charcuteries)	0,01	0,36	11,43	0,00	0,2	0,16	1,42	8,13	6,18	1,1	0,28	3,68	31,47	23,11	1,2
Charcuteries à base de sanglier	0,02	0,38	5,50	1,96	0,5	0,00	0,09	3,57	0,00	0,2	0,00	0,00	0,00	0,00	0,0
Abats de sanglier	0,00	0,00	0,00	0,00	0,0	0,00	0,00	0,00	0,00	0,0	0,12	1,65	20,16	8,22	0,5
Viande de sanglier totale	0,03	0,52	6,62	2,92	0,7	0,16	1,42	7,98	6,13	1,3	0,40	4,74	35,20	27,73	1,4
Viande de cerf et chevreuil (hors charcuteries)	0,02	0,35	5,36	1,70	0,4	0,11	1,05	8,64	3,80	0,9	0,06	0,82	10,18	3,63	0,5
Charcuteries à base de cerf et chevreuil	0,01	0,21	3,57	0,00	0,4	0,00	0,00	0,00	0,00	0,0	0,03	0,49	6,62	3,53	0,5
Abats de cerf et chevreuil	0,03	0,74	18,57	0,00	0,2	0,00	0,00	0,00	0,00	0,0	0,07	1,46	28,57	0,00	0,2
Viande de cerf et chevreuil totale	0,06	0,85	7,00	5,54	0,9	0,11	1,05	8,64	3,80	0,9	0,16	2,27	16,13	15,99	0,9

3-10 ans (570 individus)	3-10 years (570 individuals)
11-14 (450 individus)	11-14 years (450 individuals)
15-17 (424 individus)	15-17 (424 individuals)
Ensemble population	Whole population
Seuls consommateurs	Consumers only
Taux de conso	Consumption rates
Moy	Mean
ET	SD
Viande de porc (hors charcuteries)	Pork meat (other than delicatessen meats)
Charcuteries à base de porc	Pork delicatessen meats
Abats de porc	Pork offal
Viande de porc totale	Total Pork meat
Viande de sanglier (hors charcuteries)	Wild Boar meat (other than delicatessen meats)
Charcuteries à base de sangliers	Wild Boar delicatessen meats
Abats de sanglier	Wild Boar offal
Viande de sanglier totale	Total Wild Boar meat
Viande de cerf et chevreuil (hors charcuteries)	Venison meat (other than delicatessen meats)
Charcuteries à base de cerf et chevreuil	Venison delicatessen meats
Abats de cerf et chevreuil	Venison offal
Viande de cerf et chevreuil totale	Total Venison meat

Table 3. Average amount (grams/day) and standard deviation, for the whole population and consumers only, and consumer rates for pork, wild boar and venison products in adults by gender

	Homme (776 individus)					Femme (1142 individus)					Ensemble (1918 individus)				
	Ensemble Population		Seuls Consommateurs		Taux de conso	Ensemble Population		Seuls Consommateurs		Taux de conso	Ensemble Population		Seuls Consommateurs		Taux de conso
	Moy	ET	Moy	ET		Moy	ET	Moy	ET		Moy	ET	Moy	ET	
Viande de porc (hors charcuteries)	15,19	20,13	27,95	19,73	53,0	8,47	13,86	20,82	14,65	40,5	11,67	17,46	24,73	17,97	45,5
Charcuteries à base de porc	34,97	30,86	39,22	30,02	88,7	22,84	20,08	26,89	19,12	85,0	28,61	26,48	32,90	25,79	86,5
Abats de porc	0,10	1,85	28,65	14,40	0,5	0,16	1,93	20,42	7,45	0,9	0,13	1,89	22,70	10,54	0,7
Viande de porc totale	50,26	38,80	54,41	37,47	91,8	31,48	25,78	35,52	24,62	88,8	40,41	33,95	44,71	23,91	90,0
Viande de sanglier (hors charcuteries)	0,36	3,35	27,83	10,70	1,5	0,20	2,25	18,17	11,45	1,3	0,27	2,83	23,12	12,08	1,4
Charcuteries à base de sanglier	0,09	1,34	13,35	9,28	0,9	0,04	0,62	7,76	3,50	0,7	0,06	1,03	10,76	7,72	0,8
Abats de sanglier	0,00	0,00	0,00	0,00	0,0	0,01	0,40	18,57	0,00	0,1	0,00	0,29	18,57	0,00	0,1
Viande de sanglier totale	0,45	3,61	23,43	12,02	2,3	0,25	2,42	15,47	11,18	2,0	0,34	3,04	19,58	12,28	2,1
Viande de cerf et chevreuil (hors charcuteries)	0,16	2,01	19,41	10,16	1,2	0,07	0,90	8,49	4,66	0,7	0,12	1,53	13,58	9,46	0,9
Charcuteries à base de cerf et chevreuil	0,03	0,41	5,87	0,75	0,5	0,03	0,78	7,98	9,16	0,7	0,03	0,63	6,90	6,48	0,6
Abats de cerf et chevreuil	0,00	0,00	0,00	0,00	0,0	0,00	0,00	0,00	0,00	0,0	0,00	0,00	0,00	0,00	0,0
Viande de cerf et chevreuil totale	0,19	2,05	14,47	10,40	1,7	0,11	1,19	8,32	6,46	1,4	0,15	1,66	11,29	9,13	1,5

Homme (776 individus)	Men (776 individuals)
Femme (1142 individus)	Women (1142 individuals)
Ensemble	Combined group (1918 individuals)
Ensemble population	Whole population
Seuls consommateurs	Consumers only
Taux de conso	Consumption rates
Moy	Mean
ET	SD
Viande de porc (hors charcuteries)	Pork meat (other than delicatessen meats)
Charcuteries à base de porc	Pork delicatessen meats
Abats de porc	Pork offal
Viande de porc totale	Total Pork meat
Viande de sanglier (hors charcuteries)	Wild Boar meat (other than delicatessen meats)
Charcuteries à base de sangliers	Wild Boar delicatessen meats
Abats de sanglier	Wild Boar offal
Viande de sanglier totale	Total Wild Boar meat
Viande de cerf et chevreuil (hors charcuteries)	Venison meat (other than delicatessen meats)
Charcuteries à base de cerf et chevreuil	Venison delicatessen meats
Abats de cerf et chevreuil	Venison offal
Viande de cerf et chevreuil totale	Total Venison meat

Source: AFSSA, INCA2 Study, 2006-07

Table 4. Average amount (grams/day) and standard deviation, for the whole population and consumers only, and consumer rates for pork, wild boar and venison products in adults by age

	18-34 ans (442 individus)					35-54 ans (826 individus)					55-79 ans (650 individus)				
	Ensemble Population		Seuls Consommateurs		Taux de conso	Ensemble Population		Seuls Consommateurs		Taux de conso	Ensemble Population		Seuls Consommateurs		Taux de conso
	Moy	ET	Moy	ET		Moy	ET	Moy	ET		Moy	ET	Moy	ET	
Viande de porc (hors charcuteries)	10,70	18,33	26,58	20,30	38,2	12,67	18,14	25,35	18,36	48,9	11,36	15,96	22,95	15,76	46,2
Charcuteries à base de porc	25,23	24,68	31,61	23,61	79,6	29,83	27,76	33,28	27,29	88,1	29,88	26,19	33,19	25,54	89,1
Abats de porc	0,02	0,62	16,71	0,00	0,2	0,09	1,22	15,20	5,37	0,6	0,26	2,85	28,28	10,28	1,2
Viande de porc totale	35,96	33,30	43,12	31,95	83,9	42,59	36,11	45,72	35,45	91,8	41,49	31,76	44,73	30,70	91,8
Viande de sanglier (hors charcuteries)	0,30	3,05	24,55	12,46	1,6	0,30	3,22	26,99	14,58	1,3	0,22	2,13	18,38	6,18	1,4
Charcuteries à base de sanglier	0,03	0,39	5,15	1,77	0,9	0,03	0,63	11,12	2,63	0,5	0,13	1,55	12,83	9,05	1,1
Abats de sanglier	0,02	0,56	18,57	0,00	0,2	0,00	0,00	0,00	0,00	0,0	0,00	0,00	0,00	0,00	0,0
Viande de sanglier totale	0,35	3,12	18,85	13,39	2,7	0,33	3,28	23,60	14,52	1,8	0,35	2,71	17,18	8,42	2,2
Viande de cerf et chevreuil (hors charcuteries)	0,07	0,71	6,71	2,32	0,7	0,17	2,08	18,22	11,29	1,1	0,10	1,29	14,65	6,50	0,8
Charcuteries à base de cerf et chevreuil	0,03	0,43	5,32	1,05	0,7	0,02	0,47	7,57	7,33	0,4	0,05	0,86	7,98	8,30	0,9
Abats de cerf et chevreuil	0,00	0,00	0,00	0,00	0,0	0,00	0,00	0,00	0,00	0,0	0,00	0,00	0,00	0,00	0,0
Viande de cerf et chevreuil totale	0,10	0,83	6,17	2,05	1,4	0,19	2,13	16,36	11,44	1,5	0,14	1,55	11,56	8,10	1,7

18-34 ans (442 individus)	18-34 years (442 individuals)
35-54 (826 individus)	35-54 years (826 individuals)
55-79 (650 individus)	55-79 (650 individuals)
Ensemble population	Whole population
Seuls consommateurs	Consumers only
Taux de conso	Consumption rates
Moy	Mean
ET	SD
Viande de porc (hors charcuteries)	Pork meat (other than delicatessen meats)
Charcuteries à base de porc	Pork delicatessen meats
Abats de porc	Pork offal
Viande de porc totale	Total Pork meat
Viande de sanglier (hors charcuteries)	Wild Boar meat (other than delicatessen meats)
Charcuteries à base de sangliers	Wild Boar delicatessen meats
Abats de sanglier	Wild Boar offal
Viande de sanglier totale	Total Wild Boar meat
Viande de cerf et chevreuil (hors charcuteries)	Venison meat (other than delicatessen meats)
Charcuteries à base de cerf et chevreuil	Venison delicatessen meats
Abats de cerf et chevreuil	Venison offal
Viande de cerf et chevreuil totale	Total Venison meat

Source: AFSSA, INCA2 Study, 2006-07

Information on the form and source of meat products

Table 5. Information regarding the form and source of pork, wild boar or venison products

	Etat						Origine				
	Frais	En conserve	Surgelé	Autre	Non spécifié	Total	Industriel	Fait Maison	Autre	Non spécifié	Total
Viande de porc (hors charcuteries)	74,7%	0,5%	8,8%	11,7%	4,3%	100,0%	27,1%	37,5%	30,5%	4,9%	100,0%
Charcuteries à base de porc	78,3%	3,7%	2,1%	13,9%	2,1%	100,0%	62,3%	8,7%	24,5%	4,5%	100,0%
Abats de porc	86,2%	1,5%	2,3%	3,8%	6,2%	100,0%	14,7%	45,7%	33,4%	6,2%	100,0%
Viande de porc totale	77,3%	2,8%	4,0%	13,2%	2,7%	100,0%	52,2%	16,9%	26,2%	4,6%	100,0%
Viande de sanglier (hors charcuteries)	62,7%	3,5%	18,8%	13,6%	1,4%	100,0%	2,0%	75,3%	18,9%	3,8%	100,0%
Charcuteries à base de sanglier	43,3%	27,1%	0,0%	21,9%	7,6%	100,0%	28,7%	38,0%	25,7%	7,6%	100,0%
Abats de sanglier	100,0%	0,0%	0,0%	0,0%	0,0%	100,0%	0,0%	33,2%	66,8%	0,0%	100,0%
Viande de sanglier totale	61,4%	6,9%	15,1%	14,2%	2,3%	100,0%	6,0%	67,7%	22,1%	4,2%	100,0%
Viande de cerf et chevreuil (hors charcuteries)	54,5%	0,9%	31,4%	11,5%	1,8%	100,0%	10,8%	56,4%	32,8%	0,0%	100,0%
Charcuteries à base de cerf et chevreuil	33,7%	47,6%	3,8%	15,0%	0,0%	100,0%	48,8%	36,2%	15,1%	0,0%	100,0%
Abats de cerf et chevreuil	100,0%	0,0%	0,0%	0,0%	0,0%	100,0%	0,0%	100,0%	0,0%	0,0%	100,0%
Viande de cerf et chevreuil totale	53,0%	12,4%	22,0%	11,4%	1,2%	100,0%	19,3%	54,9%	25,7%	0,0%	100,0%

Etat	Form
Origine	Source
Frais	Fresh
En conserve	Canned
Surgelé	Frozen
Autre	Other
Non spécifié	Not specified
Total	Total
Industriel	Commercial
Fait Maison	Homemade
Autre	Other
Non spécifié	Not specified
Total	Total
Viande de porc (hors charcuteries)	Pork meat (other than delicatessen meats)
Charcuteries à base de porc	Pork delicatessen meats
Abats de porc	Pork offal
Viande de porc totale	Total Pork meat
Viande de sanglier (hors charcuteries)	Wild Boar meat (other than delicatessen meats)
Charcuteries à base de sangliers	Wild Boar delicatessen meats
Abats de sanglier	Wild Boar offal

Viande de sanglier totale	Total Wild Boar meat
Viande de cerf et chevreuil (hors charcuteries)	Venison meat (other than delicatessen meats)
Charcuteries à base de cerf et chevreuil	Venison delicatessen meats
Abats de cerf et chevreuil	Venison offal
Viande de cerf et chevreuil totale	Total Venison meat

Source: AFSSA, INCA2 Study, 2006-07

Particular attention should be paid to the term “homemade” for the source of the product. Basically, in the case of the categories “Pork meat” and “Pork offal”, this concept is closely associated with the fact that people cook the meat themselves, whereas with the category “Pork delicatessen meats” it will be closely associated with the fact that the individuals have prepared the product. With products made from game (wild boar and venison), this concept is not only closely associated with the homemade product, but also with the source of this game (hunting).

Eating habits: raw food and method of cooking

Only the adults (2624 individuals) were asked questions about these two items. The number of individuals who consumed pork was 2332. Among these consumers, 977 were men (41.9%) and 1355 were women (58.1%). Among these women, 25 were pregnant: the INCA2 study included a total of 28. The very low number in this population category means that these results should be interpreted with caution.

Consumption of raw food:

Figure 1. Eating habits in adults who consume raw foodstuffs

Source: AFSSA INCA2 Study, 2006-07

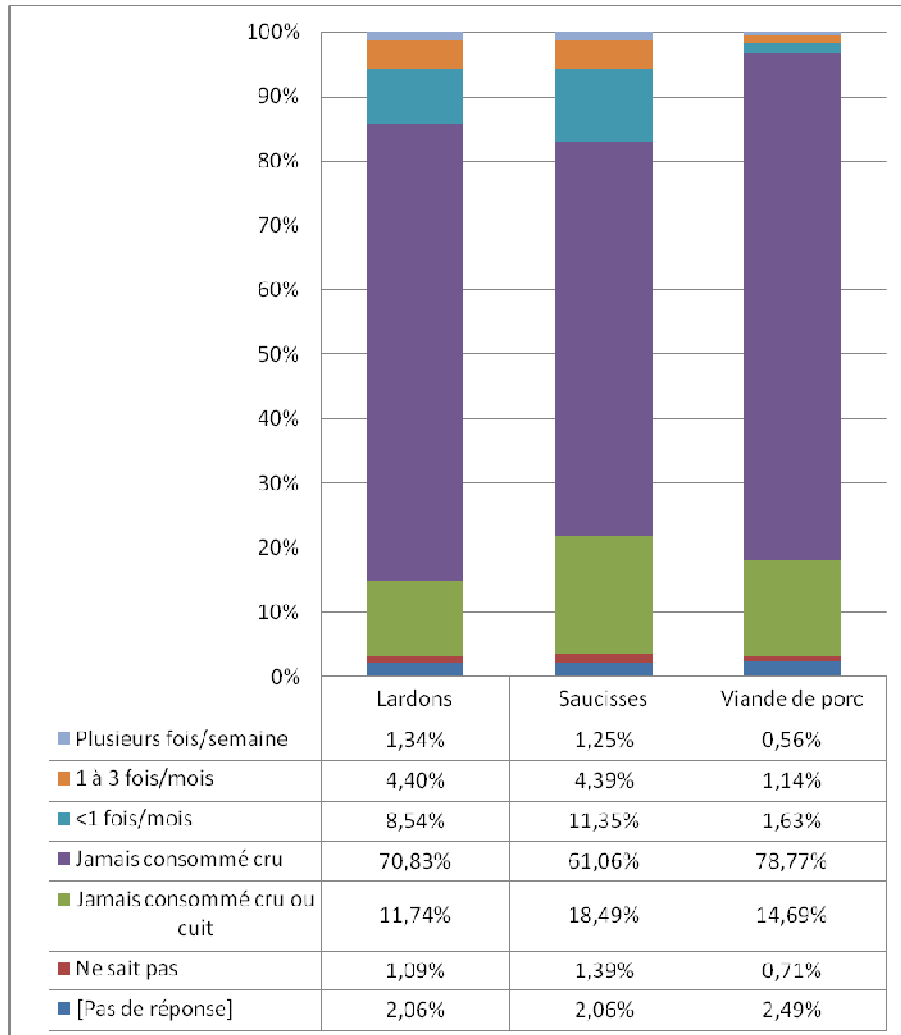
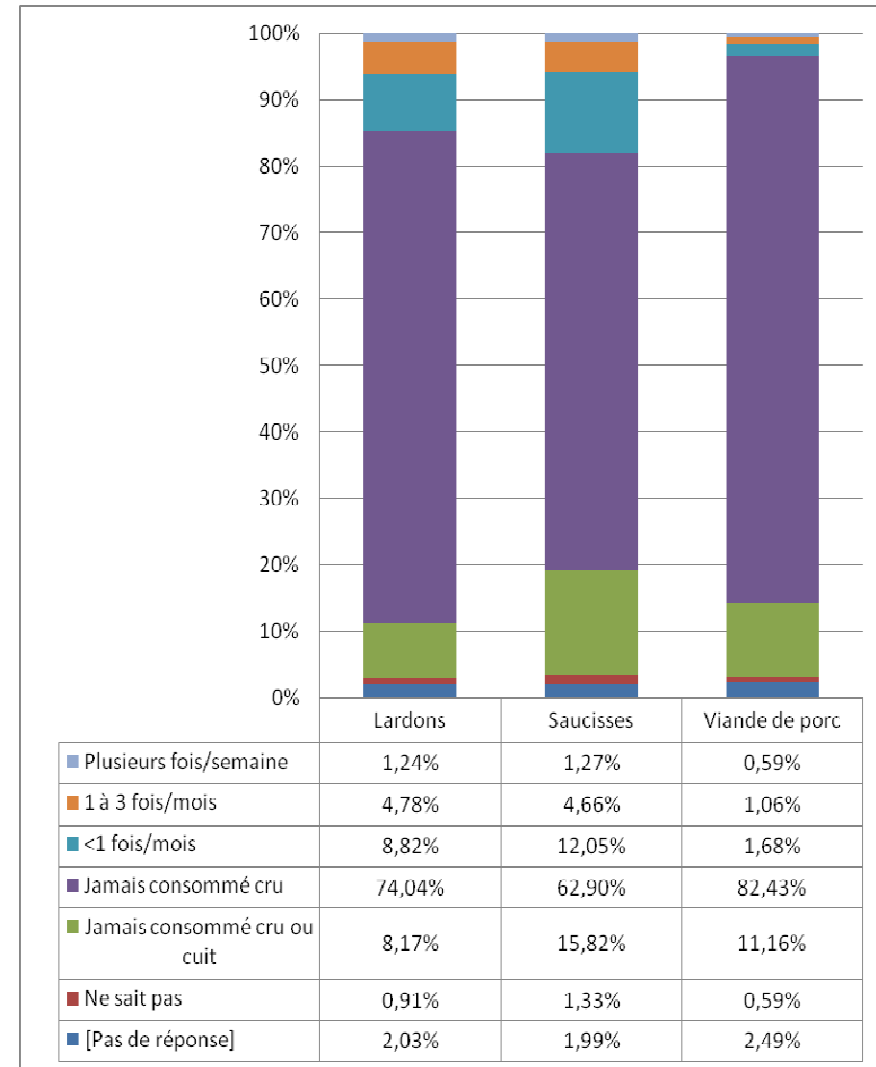


Figure 2. Eating habits in adults who consume raw foodstuffs and eat pork

Source: AFSSA, INCA2 Study, 2006-07



Lardons	Bacon chunks
Saucisses	Sausages
Viande de porc	Pork meat
Plusieurs fois/semaine	Several times/week
1 à 3 fois/mois	1 to 3 times/month
<1 fois/mois	Less than once/month
Jamais consommé cru	Never eaten raw
Jamais consommé cru ou cuit	Never eaten raw or cooked
Ne sait pas	Does not know
Pas de réponse	No answer

Table 6. Eating habits in men, women and pregnant women who consume raw foodstuffs and eat pork

	Hommes consommateurs de porc (977 individus)			Femmes consommatrices de porc (1355 individus)			Femmes enceintes consommatrices de porc (25 individus)		
	Lardons	Saucisses	Viande de porc	Lardons	Saucisses	Viande de porc	Lardons	Saucisses	Viande de porc
[Pas de réponse]	2,2%	1,9%	2,7%	1,9%	2,1%	2,3%	0,0%	0,0%	0,0%
Ne sait pas	1,5%	1,2%	0,9%	0,3%	1,5%	0,3%	0,0%	0,0%	0,0%
Jamais consommé cru ou cuit	7,9%	12,8%	9,5%	8,4%	18,8%	12,8%	1,7%	0,0%	1,7%
Jamais consommé cru	72,2%	64,6%	82,8%	75,9%	61,2%	82,1%	94,0%	66,5%	98,3%
<1 fois/mois	8,8%	12,9%	2,0%	8,8%	11,2%	1,3%	0,0%	23,9%	0,0%
1 à 3 fois/mois	6,0%	4,9%	1,4%	3,6%	4,4%	0,7%	3,1%	9,6%	0,0%
Plusieurs fois/semaine	1,4%	1,7%	0,7%	1,0%	0,8%	0,5%	1,2%	0,0%	0,0%

Source: AFSSA, INCA2 Study, 2006-07

Hommes consommateurs de porc (977 individus)	Men who eat pork (977 individuals)
Femmes consommatrices de porc (1355 individus)	Women who eat pork (1355 individuals)
Femmes enceintes consommatrices de porc (25 individus)	Pregnant women who eat pork (25 individuals)
Lardons	Bacon chunks
Saucisses	Sausages
Viande de porc	Pork meat
(Pas de réponse)	(No answer)
Ne sait pas	Does not know
Jamais consommé cru ou cuit	Never eaten raw or cooked
Jamais consommé cru	Never eaten raw
<1 fois/mois	< once/month

1 à 3 fois/mois	1 to 3 times/month
Plusieurs fois/semaine	Several times/week

Table 7. Eating habits in adults who consume raw foodstuffs and who eat pork, by age

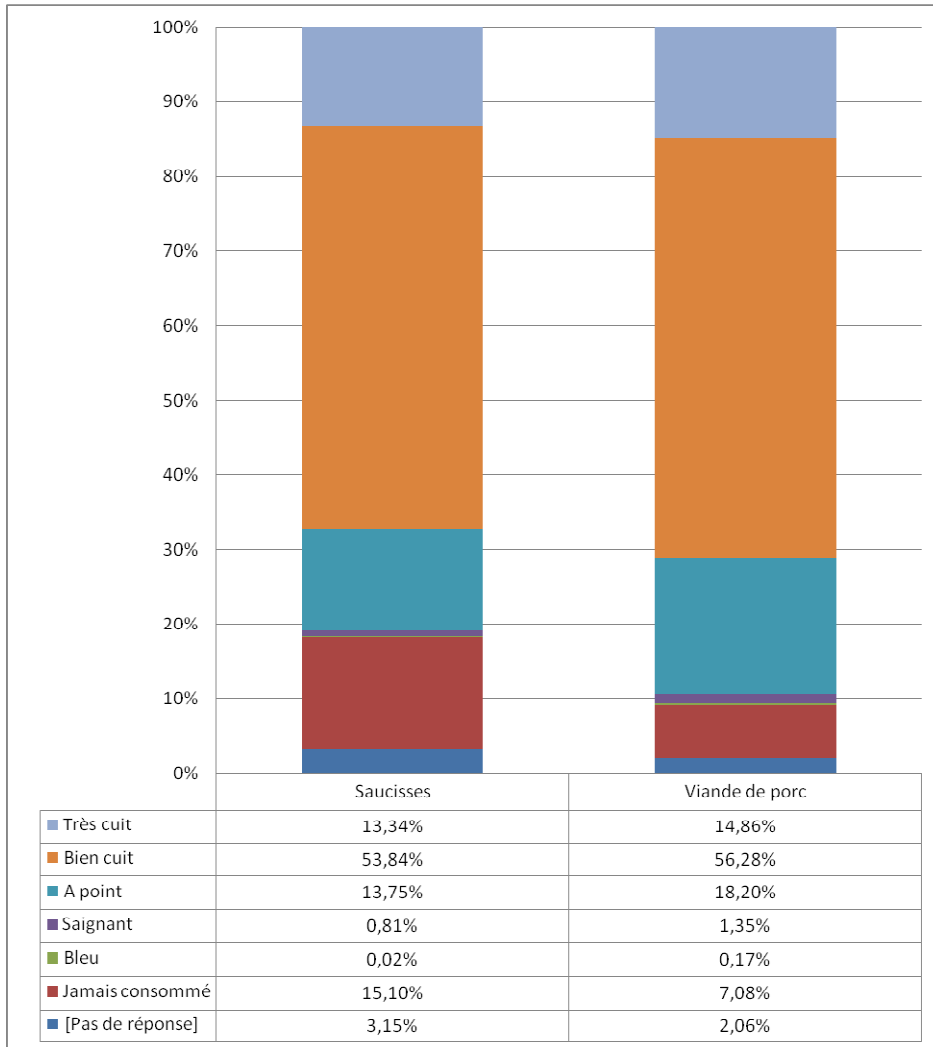
	18-34 ans consommateurs de porc (562 individus)			35-54 ans consommateurs de porc (1037 individus)			55-79 ans consommateurs de porc (733 individus)		
	Lardons	Saucisses	Viande de porc	Lardons	Saucisses	Viande de porc	Lardons	Saucisses	Viande de porc
[Pas de réponse]	0,9%	1,1%	1,1%	1,6%	1,6%	2,4%	3,5%	3,1%	3,8%
Ne sait pas	1,4%	0,6%	0,5%	1,0%	1,9%	0,6%	0,4%	1,3%	0,6%
Jamais consommé cru ou cuit	6,0%	13,5%	8,8%	6,4%	13,9%	9,7%	11,9%	19,9%	14,7%
Jamais consommé cru	73,7%	58,6%	83,4%	75,3%	62,6%	84,7%	73,0%	66,9%	79,1%
<1 fois/mois	9,0%	15,9%	3,8%	9,9%	14,4%	1,2%	7,4%	6,1%	0,4%
1 à 3 fois/mois	7,2%	8,2%	0,9%	4,7%	4,5%	1,2%	2,9%	1,9%	1,0%
Plusieurs fois/semaine	1,7%	2,0%	1,4%	1,1%	1,2%	0,1%	1,0%	0,7%	0,5%

Source: AFSSA, INCA2 Study, 2006-07

18-34 ans consommateurs de porc (562 individus)	Pork consumers aged 18-34 (562 individuals)
35-54 ans consommateurs de porc (1037 individus)	Pork consumers aged 35-54 (1037 individuals)
55-79 ans consommateurs de porc (733 individus)	Pork consumers aged 55-79 (733 individuals)
Lardons	Bacon chunks
Saucisses	Sausages
Viande de porc	Pork meat
(Pas de réponse)	(No answer)
Ne sait pas	Does not know
Jamais consommé cru ou cuit	Never ate raw or cooked
Jamais consommé cru	Never ate raw
<1 fois/mois	< once/month
1 à 3 fois/mois	1 to 3 times/month
Plusieurs fois/semaine	Several times/week

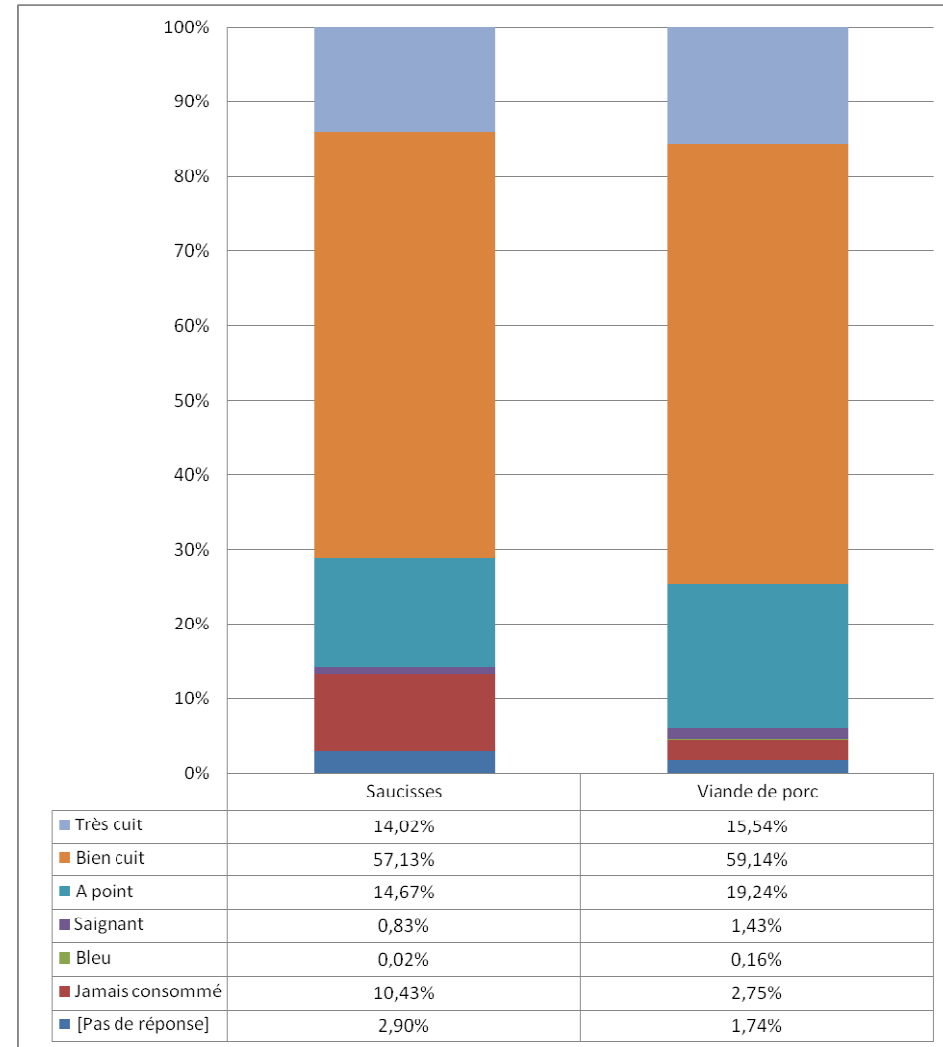
- Method of cooking:

Figure 3. Cooking habits of adults



Source: AFSSA, INCA2 Study, 2006-07

Figure 4. Cooking habits of adults who eat pork



Source: AFSSA, INCA2 Study, 2006-07

Saucisses	Sausages
Viande de porc	Pork meat
Très cuit	Very well cooked
Bien cuit	Well cooked
A point	Medium
Saignant	Rare
Bleu	Very rare
Jamais consommé	Never consumed
[Pas de réponse]	No answer

Table 8. Cooking habits in men, women and pregnant women who eat pork

	Hommes consommateurs de porc (977 individus)		Femmes consommatrices de porc (1355 individus)		Femmes enceintes consommatrices de porc (25 individus)	
	Saucisses	Viande de porc	Saucisses	Viande de porc	Saucisses	Viande de porc
[Pas de réponse]	3,0%	1,5%	2,8%	2,0%	1,2%	0,0%
Jamais consommé	8,0%	2,5%	12,8%	3,0%	7,9%	1,7%
Bleu	0,0%	0,3%	0,0%	0,0%	0,0%	0,0%
Saignant	1,4%	2,2%	0,3%	0,7%	0,0%	0,0%
A point	19,6%	23,4%	9,9%	15,2%	18,7%	24,7%
Bien cuit	56,4%	58,0%	57,9%	60,2%	66,0%	67,4%
Très cuit	11,6%	12,1%	16,3%	18,9%	6,2%	6,2%

Source: AFSSA, INCA2 Study, 2006-07

Hommes consommateurs de porc (977 individus)	Men who eat pork (977 individuals)
Femmes consommatrices de porc (1355 individus)	Women who eat pork (1355 individuals)
Femmes enceintes consommatrices de porc (25 individus)	Pregnant women who eat pork (25 individuals)
Saucisses	Sausages
Viande de porc	Pork meat
[Pas de réponse]	[No answer]
Jamais consommé	Never consumed
Bleu	Very rare
Saignant	Rare
A point	Medium
Bien cuit	Well cooked
Très cuit	Very well cooked

Table 9. Cooking habits in adults who eat pork, by age

	18-34 ans consommateurs de porc (562 individus)		35-54 ans consommateurs de porc (1037 individus)		55-79 ans consommateurs de porc (733 individus)	
	Saucisses	Viande de porc	Saucisses	Viande de porc	Saucisses	Viande de porc
[Pas de réponse]	2,9%	1,4%	1,8%	1,7%	4,1%	2,0%
Jamais consommé	12,9%	4,2%	9,8%	2,2%	9,0%	2,1%
Bleu	0,0%	0,4%	0,1%	0,1%	0,0%	0,1%
Saignant	1,8%	3,1%	0,7%	1,1%	0,2%	0,4%
A point	19,0%	27,0%	12,9%	15,9%	13,2%	16,7%
Bien cuit	52,4%	51,8%	59,2%	62,7%	58,8%	61,1%
Très cuit	11,1%	12,0%	15,5%	16,3%	14,7%	17,6%

Source: AFSSA, INCA2 Study, 2006-07

18-34 ans consommateurs de porc (562 individus)	Pork consumers ages 18-34 (562 individuals)
35-54 ans consommateurs de porc (1037 individus)	Pork consumers ages 35-54 (1037 individuals)
55-79 ans consommateurs de porc (733 individus)	Pork consumers ages 55-79 (733 individuals)
Saucisses	Sausages
Viande de porc	Pork meat
[Pas de réponse]	[No answer]
Jamais consommé	Never consumed
Bleu	Very rare
Saignant	Rare
A point	Medium
Bien cuit	Well cooked
Très cuit	Very well cooked

Information on cooking and the cuts of pork meat consumed

Based on the food headings entered by the individual survey participants, some information can be found on the cooking of meat and the cuts of meat consumed. However, this information should be interpreted with caution: specifically, the food headings written down by the participants are not harmonised (there are misspellings, food in singular and plural forms, etc.) and the level of accuracy may vary considerably from one individual to another, making any analysis based on this information difficult.

The food headings do not contribute useful information on the cooking of pork meat: in 91% of cases, there was no information at all. For the most part, it was the method of cooking that was indicated (barbecue, frying pan, oven, etc.), but this does not provide information on the level of cooking itself.

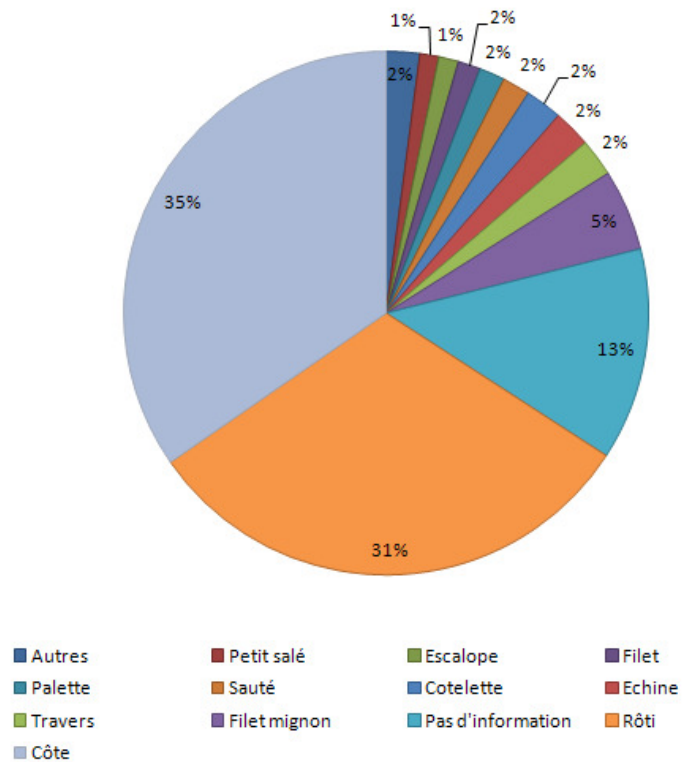
Table 10. Information on cooking pork meat consumed in INCA2

Level of cooking	Percentage
Very well done	0.07
Medium	0.15
Fried	0.36
Well done	1.12
Grilled	7.07
No information	91.23

Source: AFSSA, INCA2 Study, 2006-07

A close examination of the food headings reveals additional information on the cuts of pork meat consumed. There is no specific information on the cut for 13.08% of the meat consumed. The cuts most often listed for pork meat are the “chops” (34.61%) and the “roast” (31.24%).

Figure 5. Information on the cuts of pork meat consumed in INCA2



Source: AFSSA, INCA2 Study, 2006-07

Autres	Other
Petit salé	Salt pork
Escalope	Escalope
Filet	Tenderloin
Palette	Shoulder
Sauté	Sautéeing pork
Cotelette	Chop
Echine	Collar
Travers	Spare rib
Filet mignon	Filet mignon
Pas d'information	No information
Rôti	Roast
Côte	Loin chop

The "Other" category includes: Kebab (0.83%), Knuckle (0.54%), pork slivers (0.36%), Carbonade [grilled pork loin] (0.14%), Cheeks (0.07%), Steak (0.04%), Stuffed tenderloin (0.04%) and Jowls (0.04%).

Annex 4: Description of the various types of treatments applicable to slurry

Various types of treatments may be applied to slurry:

i. Physical treatments

Spreading fresh untreated manure: This is the treatment most likely to contaminate food or drinking water, although agricultural soils are inhospitable to the survival of bacteria (85), (40). The data on viruses are not known.

Storage prior to spreading: Storing with no intervention. This corresponds to accumulating effluents in a manure pit or on a platform for solid waste. One study (2) has shown that Brittany now has storage capacity for an average period of 7.6 months. Thus 85% of the volume can now be stored for at least six months.

Mechanical phase separation: This usually consists of conducting a phase separation between the solid and liquid portions of the slurry by sedimentation, filtration or dehydration. Phase separation is carried out using special equipment (screw compactor, sludge dewatering centrifuge, etc.) operating according to various physical parameters (temperature, airflow, pressure, gravity).

Lagooning: This extensive storage method enables the treatment of raw slurry after phase separation upstream (by sedimentation, screw compaction, etc.). The ponds are shallow (1.0 to 1.2 m) but have a large surface area.

Dehydration: Various dehydration processes are currently commercially available. They may involve drying by raising the temperature, by recirculating air extracted from piggeries or even by percolation/evapotranspiration in drying beds planted with reeds.

Heat treatment: This involves increasing temperatures to between 70 and 90°C. This method of treatment is not really feasible with livestock due to the investment and operating costs.

ii. Biological treatments

Biological treatment by aerobic means: Aerobic biological stabilisation involves the consumption of oxygen by microorganisms responsible for the breakdown of organic matter (bacteria and fungi). The exothermic reaction observed during the degradation of this organic matter by biochemical oxidation may be exploited for sanitation purposes. However, the increase in temperature will be particularly effective in composts, whereas it remains much lower in the liquid phase. Given the constraints of nitrogen reduction, the aerobic biological treatment of pig slurry has greatly progressed in recent years, representing more than 90% of the 350 stations currently operating. Among them, biological treatment by activated sludge has become the dominant model, accounting for three-quarters of the units in operation and more than 80% of the slurry treated. Composting slurry on straw or green waste comes in second place, with 16% of facilities and only 6% of treated slurry (60). In practice, biological treatment by activated sludge involves a reactor dwell time of 46 days on average (from 30 to 60 days maximum, (59)). Temperature, oxidative stress, ammonia concentration, and bacterial competition are the primary parameters bringing about a reduction in the number of pathogens by the aeration technique (36). In 1979, Derbyshire and Brown (22) showed inactivation of enteroviruses and adenoviruses after 21 days of oxygenation of pig slurry in the laboratory. Inactivation of enteric viruses was 98% in the sludge at the end of treatment (aerobic + sedimentation treatment). In actual practice, aerobic treatment takes place in the psychrophilic and mesophilic stages (0-30°C). Some authors suggest combining it with temperature regulation to increase the effectiveness of purification.

Biological treatment by composting: Due to their physico-chemical characteristics, solid effluents (manure, separation phase refuse, slurry composts on straw and green waste) have a more reliable composting capability. The emission of heat accompanying the aerobic respiration of organic materials may also be used to purify them, given sufficient time. The time/temperature combination is thus one of the main criteria for compost purification. When temperatures rise above 55°C for a sufficient period of time, there is a rapid reduction in bacteria. This has not been documented for

viruses. Effluents can be composted in reactors that are able to reach high temperatures in a few days. These are generally tunnels or cylinders that can operate either uninterrupted or intermittently. Still, these composting reactors are expensive solutions in terms of equipment and require energy to mix and convey the material.

Anaerobic biological treatment (methanation). Slurry contains fermentable organic material that can be methanised. The general principle consists in introducing the product into an airtight digestion tank where conditions are maintained with temperatures (30-60°C), agitation and dwell times favourable to the development of an active anaerobic biomass. The purifying effect of these processes is improved with sequential or continuous feed, as this avoids temperature fluctuations. The methanation factors influencing pathogen reduction are essentially treatment time and temperature: 10 days at 55°C, 15 days at 35°C, and 60 days at 20°C. But the level of dry matter, concentration of NH₄⁺ and VFA [volatile fatty acids], and pH also have a role in decontamination.

iii. Chemical Treatments

Lime is the primary chemical product used for purification. Its alkaline pH and resulting exothermic reaction are the main factors reducing bacteria, viruses and parasites. These effects combined with the release of ammonia have an even more lethal effect. The main problem is the ammonia emission that occurs with this treatment. However, in effluents where the concentration of ammonia nitrogen has been reduced beforehand, and in biological sludge or mature compost, the use of this chemical process for additional purification may be expedient. Other compounds have purifying properties and could be used to treat effluents, these include urea, zeolite and bentonite, hydrogen peroxide and some disinfectants. They may be applied in exceptional cases for epizootic outbreaks and when storage is impossible. However, these may not be considered as sustainable solutions for environmental reasons.