The Director General

Maisons-Alfort, May 18, 2017

NOTICE

the National Food Safety Agency, environment and labor

on the detection of shigatoxin-producing *E. coli* **(STEC) considered highly pathogenic in the minced beef industry**

ANSES implements independent and pluralistic scientific expertise.
ANSES mainly contributes to ensuring health safety in the areas of the environment, work and food and to assessing the health risks they may entail.

It also contributes to ensuring, on the one hand, the protection of the health and well-being of animals and the health of plants and, on the other hand, to the evaluation of the nutritional properties of food.

It provides the competent authorities with all the information on these risks as well as the expertise and technical scientific support necessary for the development of legislative and regulatory provisions and the implementation of risk management measures (article L.1313- 1 of the public health code).

Its opinions are published on its website.

On 17 May 2016, ANSES received a request from the Directorate General for Food (DGAL) for an opinion on the detection of shigatoxin- producing *E. coli* (STEC) considered highly pathogenic in the minced meat sector bovine.

1. CONTEXT AND PURPOSE OF THE REFERRAL

The current procedures for managing the STEC hazard implemented by the DGAL are essentially based on the opinions of ANSES relating to referral 2010-SA-0031:

- of May 27, 2010 relating to the relevance of a revision of the definition of pathogenic STEC, specified by Afssa's opinion of July 15, 2008,

- of 11 January 2011 relating to the revision of the definition of typical major enterohaemorrhagic E. *coli* (EHEC), the quantitative assessment of the risks associated with these bacteria at different stages of the food chain, according to the different modes of consumption of steaks minced meat, and consideration of the hazard of enteropathogenic *E. coli* (EPEC) in food.

Since the publication of these opinions, changes have been observed, in particular concerning the preventive control procedures put in place by the professional operators and the verification methods (sampling plan defined within the framework of self-checks). Studies and work have also continued in order to supplement scientific knowledge in this area. ANSES Opinion No. 2013-SA 0223 of 6 May 2014 on the definition of a sampling plan for the detection of *E. coli* O157:H7 in the context of self-checks in the minced beef sector distinguished three levels of distribution of bacteria in a scrum: homogeneous, moderately homogeneous and heterogeneous. In order to specify the degree of homogeneity of the scrums, a study was coordinated by the Livestock Institute (IDELE), making it possible to characterize the parameter "b" relating to the distribution of the contamination of the strains of STEC in a scrum. .

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Finally, discussions were initiated at the end of 2013 by the European Commission, with a view to drafting guidelines to harmonize, within the Member States, the management measures implemented during the detection of STEC in food. Several proposals were discussed, but none succeeded due to the lack of consensus on the level of risk associated with the different profiles of STEC strains.

With a view to updating the STEC management measures by the DGAL, ANSES received the following questions:

1°/ Does the definition of STEC strains to be considered as potentially highly pathogenic in the Agency's opinion of 27 May 2010 need to be updated in the light of recent French, European and international epidemiological data?

2°/ The Agency's opinion of 11 January 2011 specifies that, "in the case of isolation in the laboratory of an AEEC1 strain belonging to one of the 5 major typical EHEC serogroups in an enrichment broth in which an *stx* gene has been detected, it was not possible in the current state of knowledge to conclude as to the absence or presence in the food of an STEC which could be highly pathogenic". Does the evolution of scientific knowledge on the mechanisms of acquisition and loss of *stx* genes in *E. coli* and on the genetic and phenotypic characterization of AEEC strains now make it possible to specify the risk associated with the isolation of these strains? in food?

3°/ What data are available on the concentrations of STEC (cfu per g) in food and water at the origin of the various epidemics that have occurred in the world? To what extent can they be used to specify the sampling plans for ground beef with regard to epidemic risk control?

4°/ Based on the conclusions of the IDELE study on the homogeneity of scrums:

- Is a sampling plan based on the analysis of a 25 g sample (n=1, m=absence in 25g) capable of detecting a STEC concentration of 1 cfu/g in the mix?

- A sampling plan of type n=3, c=0, m= absence in 25 g or n=1, m= absence in 75 g (with an analysis method whose performance for this test portion would be validated as equivalent to a test sample of 25 g) can it be considered as enabling the detection of a STEC concentration of 0.1 cfu/g in a fray?

- What is the difference in risk level between systematic detection in 25 g (n=1, m=absence in 25 g), and systematic detection in 75 g (n=3, c=0, m=absence in 25 g or n=1, m=absence in 75 g)?

5°/ In the context of a minced meat production establishment having a health control plan deemed satisfactory and which carries out, for the purpose of verifying the effectiveness of the preventive control measures implemented, systematic screening for STEC (O157:H7 or other serotype according to the analytical methods used) on each scrum, what would be the estimated level of risk (prevention of clustered and epidemic cases) if management measures (for the serotype(s) causing the object of a systematic analysis) were implemented only on the products resulting from the fray detected positive, (without action on the framing frays which gave a result of negative autocontrol, as planned today in the technical instruction DGAL /MUS/2015-888)?

6°/ Taking into account the representativeness of the data available at the French level and according to an integrated and preventive approach to health control, would a strategy including checks on raw materials be likely to optimize the cost/effectiveness ratio of the plans for self-checks, which, to date, mostly only target finished products? If so, what protocols would be suitable (matrix, n, m, analysis methods)?

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[1] AEEC: "Attaching and erasing *E. coli*"

2. ORGANIZATION OF EXPERTISE

The expertise was carried out in compliance with standard NF X 50-110 "Quality in expertise – General requirements of competence for an expertise (May 2003)".

The collective expert appraisal was carried out by the Specialized Expert Committee (CES) "Assessment of biological risks linked to food" (BIORISK) on the basis of an initial report drawn up by a group of rapporteurs. The expertise was based on:

- French and European epidemiological data;

- the information transmitted by the NRL *E. coli,* including *E. coli* producing shigatoxins, on the contamination of food by STEC;

- recent literature (cf. bibliographical references);

- the information provided by the professional federations (hearing of September 9, 2016) and manufacturers (questionnaire) of the minced beef sector.

The model developed by ANSES in the context of request 2013-SA-0223 has been modified to take into account the data and information provided by professionals and the development of scientific knowledge (in particular the study of IDELE on the evaluation of the degree of homogeneity of mixed minced meats).

ANSES analyzes the links of interest declared by the experts before their appointment and throughout the work, in order to avoid the risk of conflicts of interest with regard to the points dealt with in the context of the expert appraisal. In this context, one expert did not take part in the work and deliberations on this referral. The experts' declarations of interest are published on the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES BIORISK

3.1. Critical review of the definition of major typical EHECs

3.1.1. Reminder of definitions and concepts on the pathogenicity of EHEC

ÿ **EHEC/STEC**

Based on the clinical signs observed in patients, the strains of pathogenic *E. coli* are grouped into pathovars (or pathotypes) including **EHEC** *(enterohemorrhagic E. coli).*

In humans, EHECs are responsible for various disorders ranging from mild watery diarrhea to hemorrhagic colitis that can progress to serious forms: hemolytic-uremic syndrome (HUS), mainly in young children, or thrombotic microangiopathy (MAT) in adults.

EHECs are characterized by the production of **shigatoxins** (Stx) (formerly called Verotoxins Vtx), encoded by the *stx* genes carried by bacteriophages. These toxins lead to the death of target cells by stopping protein synthesis and induce lesions of the vascular endothelium, mainly intestinal, renal and cerebral. Any strain *of E. coli* possessing an *stx* gene is called **STEC** for " *shigatoxin-producing E. coli* " (formerly called VTEC).

Two major types of shigatoxins, Stx1 and Stx2, and numerous variants have been identified (Afssa 2008). The type of variant would reflect both the origin of the strains, their phylogeny, but also their pathogenicity.
Epidemiological studies have shown that Stx2 is more often associated with more severe disease in humans than Stx1.

The mere presence of the *stx* gene is not enough to trigger a pathology in humans. The majority of EHEC strains induce so-called "attachment and effacement" lesions of the cells of the mucosa of the distal ileum and the colon, in particular through the intermediary of a membrane protein, intimin . This protein is encoded by the *eae* gene carried by the chromosomal locus of erasure of enterocytes (LEE).

Several intimin variants (alpha, beta, gamma, etc.) have been identified; they would be involved in cell tropism, host specificity and therefore in the pathogenic power of EHEC (Afssa 2008).

Other strains of EHEC lack the *eae* gene and therefore do not produce an attach-and-efface lesion. These strains therefore possess other adhesion factors allowing colonization of the colonic mucosa. For example, the O104:H4 strain responsible for the epidemics in Germany and France in 2011 is characterized by the production of shigatoxin and the ability to adhere to the intestinal mucosa with an aggregative adhesion profile. This adhesion mechanism, characteristic of enteroaggregative *E. coli* strains (EAEC for *Enteroaggregative E. coli)* is due to AAF fimbriae whose expression is regulated by the *aggR gene.*

A state of knowledge on the mechanisms and determinants of the virulence of the pathotypes *of E. coli* and in particular EAEC was recently carried out by the EFSA BIOHAZ panel (EFSA BIOHAZ Panel 2015).

ÿ **Definitions of Afssa's opinions of 2008 and 2010**

Analysis of epidemiological data shows that certain EHEC serotypes isolated in humans are more frequently associated with severe disease.

The strains most frequently involved in epidemics have been defined by the Agency as "typical major EHEC" strains according to the following genetic criteria:

EHEC O157:H7 = *rfbEO157, flicH7, stx1* and/or *stx2,* eae-gamma, (OI#1222).

EHEC O26:H11 = *wzxO26, flicH11, stx1* and/or *stx2,* eae-beta, (OI#122).

EHEC O145:H28 = *ihp1O145, flicH28, stx1* and/or *stx2,* eae-gamma, (OI#122).

EHEC O103:H2 = *wzxO103, flicH2, stx1* and/or *stx2,* eae-epsilon, (OI#122).

EHEC O111:H8 = *wbd1O111, flicH8, stx1* and/or *stx2,* eae-theta, (OI#122).

The Agency underlined in its opinion the temporary nature of the proposed definition. This must be revised according to new clinical observations, the results of epidemiological investigations, the results of research projects and the development of detailed methods.

In addition, the Agency underlined that during the bacteriological examination of a food, carried out outside a clinical context in humans, it is indeed the demonstration of the various factors or markers of virulence within the same strain which makes it possible to estimate its pathogenicity. Consequently, the Agency recommended considering a strain as :

- highly pathogenic when it presents the characteristics of a major typical EHEC
    (possession of the virulence genes *stx1* and/or *stx2* and *eae* and belonging to one of the following serotypes and their non-motile derivatives: O157:H7, O26:H11, O145:H28, O103:H2 and O111:H8),

- pathogenic when it presents the characteristics of a typical EHEC (possession of the genes of
    virulence *stx1* and/or *stx2* and *eae).*

ÿ **Opinion of the EFSA BIOHAZ panel on VTEC (STEC), the concept of seropathotype and the scientific criteria allowing the evaluation of their pathogenicity (EFSA BIOHAZ Panel 2013)**

This opinion proposes a molecular approach for the assessment of the risk associated with STEC strains isolated in food. In addition to the *stx* gene , the *eae, aaic* and *aggR* genes are considered. Three risk groups are considered: I (high potential risk) to III (unknown risk). Group I includes the 5 serogroups recognized as major and O104. The addition of *aaic* and *aggR* markers and serogroup O104 is linked to the outbreak caused by the STEC/EAEC O104:H4 hybrid strain in 2011.

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[2] Island of pathogenicity (" *O Island* ") containing 4 virulence genes

EFSA emphasizes the provisional nature of this classification, which must be confirmed by epidemiological data.

Table 1. Molecular approach proposed by the EFSA BIOHAZ panel for the classification of STEC *(stx +)* (EFSA BIOHAZ Panel 2013)

			Potential risk	
Band	Genoa	Serogroups	Diarrhea	HUS / Hemorrhagic colitis
I	*eae* + or *(aaiC* and *aggR)* +	O157, O26, O103, O145, O111, O104	Pupil	Pupil
II	*eae* + or *(aaiC* and *aggR)* +	Any other serotype	Pupil	Unknown
III	*eae* – and *(aaiC* and *aggR)* -	Any other serotype	Unknown	Unknown

> **The approach adopted is to adapt the EFSA classification, based on French and European epidemiological data (2011-2015) and available data on the contamination of tanks and food by EHEC.**

3.1.2. Review of French and European epidemiological data (2011-2015)

ÿ **French epidemiological data (data from CNR and Public Health France)**

In France, the surveillance of EHEC infections has been based since 1996 on the surveillance of cases of HUS in children under 15 years of age, through the intermediary of a network of pediatric nephrology hospital-university services. Cases of HUS are notified to Public Health France. The strains are sent to the National Reference Center (CNR) for *E. coli, Shigella* and *Salmonella* (Pasteur Institute) and its associated laboratory (Robert Debré Hospital) in the event of suspected EHEC infection in children or adults. .

EHEC infection is confirmed by the CNR using the following techniques (Bruyand *et al.* 2016):

- On stool or rectal swab:

    o *In situ* gene amplification by PCR of the virulence genes *stx (stx1, stx2), eae* and *hlyA,* and genes coding for 10 of the serogroups frequent in France (O157, O121, O26, O103, O91, O145, O55, O111, O104 and O80);

    o Isolation of STEC strains and characterization of virulence factors *(stx, eae, hly, aggR)* and serogrouping;

- In the serum: by demonstration of serum antibodies directed against the lipo-polysaccharide of 9 serogroups (O26, O55, O91, O103, O104, O111, O128, O145, O157).

• *Incidence of HUS in children under 15*

During the period 2011 - 2015, 687 cases of HUS were notified (137 cases on average per year). Since 1996, the annual incidence of HUS has varied between 0.6 and 1.3 cases/100,000 children under 15 years old. These cases mainly occur sporadically. The highest annual incidence is observed in children under 3 years of age; it was 3.1 cases/100,000 children in 2015 (Bruyand *et al.* 2016). A resurgence of cases is observed in the summer period.

• *Microbiological characteristics of strains involved in HUS cases*

Microbiological or serological confirmation of EHEC infection was achieved for approximately 80% of pediatric HUS cases notified in France between 2011 and 2015.

Table 2 presents the distribution of EHEC serogroups involved in pediatric HUS cases between 2011 and 2015. A decrease in the proportion of serogroup O157 in favor of other serogroups is observed (from 34% in 2011 to 17% in 2015). Serogroup O26 is the second isolated serogroup.

The O80 serogroup emerged in France in 2010, and represents the third serogroup in frequency. In 2015, serogroup O80 was the most isolated serogroup in the stools of patients with HUS (33%) followed by serogroups O157 (24%) and O26 (14%). The prevalence of serogroup O104, responsible for the German and French epidemic in 2011, has clearly decreased and is only found in a few sporadic cases.

Table 2. Most frequently isolated serogroups in HUS cases in France between 2011 and 2015 **2011**

Serogroup	Number of Cases	%	Number of Cases	%	Number of Case	%	Number of Cases	%	Number of Cases	%
	2012		**2013**		**2014**		**2015**			
O157	56	34.6	50	34.5	48	31.6	37	31.6	19	17.1
O26	7	4.3	13		21	13.8	10	8.5	16	14.4
O80	0		6	9	10	6.6	10	8.5	15	13.5
O121	2	0	10	4.1	7	4.6	4	3.4	2	1.8
O103	5	1.2	0			0.7	2	1.7	2	1.8
O91		3.1	2	6.9	1			0.9	0	0
O145	1	0.6	5	0	0		1	1.7	3	2.7
O111	5	3.1	7	1.4	0	0 0	2	1.7	4	3.6
O104	2	1.2	0		7		2			0.9
O55	4 0	2.5 0	2	3.4 4.8 0 1.4	3 0	4.6 2 0	0 6	0 5.1	1 4	3.6

During the period from 2011 to 2015, the CNR and the associated laboratory isolated 910 strains of EHEC. The virulence profile most often found among the EHEC strains is the profile associating the *stx2 eae hlyA* genes (44.2% of the strains). Regarding the strains responsible for HUS, 95% of the strains isolated between 2011 and 2015 possessed the virulence gene *stx2* (alone or in association with *stx1)* and 85% of the strains isolated possessed the virulence genes *stx1* and/or *stx2* and *eae* . (see table 3)

Table 3. Virulence profile of EHEC strains isolated from HUS cases in France between 2011 and 2015 (excerpt from ECDC TESSY database)

Virulence profile	Proportion of strains				
	2011	2012	2013	2014	2015
stx1-,stx2+,eae+	65	68	71	76	78
stx1-,stx2+,eae-	21	11	15	12	14
stx1+,stx2+,eae+	8	15	11	7	5
stx1+,stx2-,eae+	3	4	1	2	4
stx1-,stx2-,eae+	2	1	2	0	0
stx1+,stx2-,eae-	1	0	1	1	0
stx1+,stx2+,eae-	1	0	0	0	0

• *Focus on the emergence of the O80:H2 serotype*

E. *coli* serotype O80:H2, a pathotype that has been emerging in France for about ten years, have several particularities. It is a "hybrid" pathotype possessing the characteristics of EHEC but also virulence factors of extra-intestinal *E. coli* with clinical repercussions (bacteraemia). As a result, the therapeutic attitude recommended in a case of HUS is called into question (Soysal *et al.* 2016).

Molecular characterization of this serotype, affiliated to phylogenetic group A, revealed that in addition to the attributes of EHEC strains *(stx2, eae, hlyA),* it possesses an extra-intestinal virulence plasmid. This plasmid is similar to pS88, a ColV plasmid implicated in strain virulence of bacteremia and neonatal meningitis (Soysal *et al.* 2016)

The O80:H2 serotype has a particular geographical distribution, with a clear predominance in Rhône-Alpes, a French region spared by the major O157 serotype.

According to current knowledge, this serotype has been rarely found in other European countries (a few strains in Spain and Switzerland). Investigations are currently being carried out to identify the source of the serotype (investigation of sporadic cases, research as part of surveillance plans).

• *Epidemics and grouped cases identified*

Between 2011 and 2015, four foodborne outbreaks were detected in France by the surveillance system (see Table 4).

Table 4. Outbreaks of foodborne EHEC infections identified in France between 2011 and 2015

Year	Region	Serogroup / serotype Virulence profile	Number of cases (including SHU)	Source
2011	Bordeaux	O104:H4	15	Fenugreek sprouts
		stx2 aggR	(9)	
	North of the France	O157:H7 sorbitol+	18	Minced beef
		stx2 eae hlyA	(18)	
2012	South West	O157:H7	6	Ground beef steak
		stx2 eae hlyA	(4)	
2013	West	O157: H	8	Raw milk cheese
		stx2 eae hlyA	(5)	

In addition to epidemics, 51 outbreaks of clustered cases at EHEC were identified during the period. A focus of grouped cases is defined by Public Health France as the identification of an EHEC infection by stool analysis or serology in at least one person (with or without clinical symptoms) in the entourage of a case of HUS. These grouped cases mainly occur in the family setting.

ÿ **European epidemiological data**

European surveillance of EHEC infections has been coordinated by the European Center for Disease Control (ECDC) since 2007. The member states of the European Economic Area (the European Union (EU), Norway, Iceland and Switzerland) transmit data on EHEC infections identified in their country each year.

EHEC infections are notifiable in the majority of EU countries, Iceland and Norway. In six countries, the notification of infections is based on a voluntary surveillance network (Belgium, France, Italy, Luxembourg, Spain) or a specific system (United Kingdom). Surveillance coverage is national in the majority of countries with the exception of France and Belgium.

Since 2012, an increase in the incidence of EHEC infections has been observed in several EU countries. Table 5 presents the 10 main serogroups involved in cases of EHEC infections between 2012 and 2015.

Serogroup O157 remains predominant in EHEC infections declared in Europe between 2012 and 2015, even if a decrease in their proportion is observed. This serogroup represents 42% of confirmed human infections in 2015 compared to 55% in 2012 (EFSA and ECDC 2015b, 2016). O26 represents the second serogroup isolated in Europe (15% in 2015).

Serogroups O157, O26, O103, O111 and O145 represent 70% of human EHEC infections identified at European level during the period 2012-2015 (EFSA and ECDC 2015b, 2016). Between 2012 and 2015, only 30 cases of infections linked to serogroup O104 were reported in Europe (including 6 linked to serotype O104:H4) (EFSA and ECDC 2015b, a, 2016).

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Related referrals No. 2013-SA-0223; 2010-SA-0031

Table 5. Most frequently isolated serogroups in cases of EHEC infections between 2012 and 2015 in Europe (EFSA and ECDC 2015a, 2016)

Serogroup	2012		2013		2014		2015	
	Number of	%	Number of Cases	%	Number of Cases	%	Number of Cases	%
O157		54.9	1828	48.9	1692	46.3	1510	41.7
O26		11.6	476	12.7	444	12.2	537	14.8
NST1		3.8	298		315	8.6	430	11.9
O103		6.4	160	8	193	5.3	171	4.7
O91		3.6	94	4.3	105	2.9	114	3.1
O145		3.1	96	2.5	105	2.9	95	2.6
O146		1.6	75		83	2.3	74	2
O128			41	2.6	47	1.3	49	1.4
O-rough2			41	2	55	1.5	45	1.2
O111		1 1 1.8	78	1.1 1.1 2.1	54	1.5	12	1.2

[1] Cases 1981 417 136 231 131 112 59 37 37 66 NNT: not serotypable

[2] O-rough: O-rough strains do not have the O chains of the lipopolysaccharide

3.1.3. Contamination of animal reservoirs and food by EHEC

ÿ **Prevalence in the animal reservoir**

The overall prevalence of adult cattle shedding EHEC belonging to the five serotypes (O157:H7, O26:H11, O145:H28, O103:H2 or O111:H8) has been estimated in France at 1.8%: 4.5% young dairy cattle, 2.4% young beef cattle, 1.8% dairy cows, 1% beef cows (Bibbal *et al.*
2015). The search for genetic markers characteristic of the epidemic strain O104:H4 in the faeces of 1,468 cattle did not identify animals carrying this strain, suggesting that the French cattle herd is not a reservoir of this type. strain (Auvray *et al.* 2012). Likewise, this

strain could not be demonstrated in cattle during various studies in other countries, for example in Germany, Spain and the United States (Cabal *et al.* 2015, Paddock *et al.*
2013, Shridhar *et al.* 2016, Wieler *et al.* 2011).

ÿ **Prevalence of isolated strains in food in France**

Since 2005, monitoring plans (PS) have been implemented annually by the DGAL on the following products:

- frozen ground beef (VHS) at production (2007, 2011, 2012, 2013);
- chilled ground beef (VHR) for distribution (2006, 2009, 2015, 2015);
- beef ores (ground meat) (2008, 2013);
- raw milk cheeses to production (2005; 2007,2009, 2014).

The strains sought are:

- strains possessing the *stx (stx1* and/or *stx2)* and *eae* virulence genes and belonging to one of the five serotypes O157:H7, O26:H11, O145:H28, O103:H2 or O111:H8 (strains known as " top 5"),

- and since 2012, for ground beef only, strains possessing the *stx (stx1* and/or *stx2)* and *eae* virulence genes and belonging to either serogroup O45 or serogroup O121, targeted by US regulations.

The PS results (see Appendix 2) show a low prevalence of contamination in minced meat (between 0.3 and 0.5%) and raw milk cheeses (less than 0.9%) (Loukiadis et al . 2012, Loukiadis, Mazuy-Cruchaudet, and Ferre 2012, Loukiadis and Mazuy-Cruchaudet, 2013, 2014, Sergentet, Mazuy Cruchaudet, and Ruez 2015, Loukiadis, Mazuy-Cruchaudet, et al. 2017) . Of the 57 "top 5" strains isolated from beef in the context of PS, 40% belong to serotype O157:H7. THE

The most frequently found serotypes are then, in order of importance, O26:H11 (28%), O103:H2 (25%), O145:H28 (2%) and O111:H8 (2%). No strain of serotypes O45 or O121 has been found in beef in France. The majority of strains isolated in raw milk cheese belong to serotype O26:H11 (68%).

ÿ **Genetic characteristics of strains isolated from food in France**

A research project carried out within the framework of an agreement between ANSES, VetAgro Sup and the Robert Debré hospital, aimed to establish a national assessment of the characteristics of the STEC strains isolated between 2011 and 2012 in humans, in food, animals and the environment (Mariani-Kurkdjian *et al.* 2014).

A collection of 270 strains of human and non-human origin representative of the strains circulating in France was characterized genetically (search for virulence markers, typing by PFGE, MLST and MLVA) and phenotypically (antibiotic resistance, in particular the study of the minimum concentration azithromicin inhibitor). This study had highlighted a great diversity of strains circulating in France between 2011 and 2012. All the strains studied were sensitive to azythromicine, which is the treatment recommended by the High Council for Public Health during diarrhea at EHEC in communities. .

Comparative analysis of the virulence profiles of strains of human and food origin does not show any significant difference, which confirms that the strains present in food can be pathogenic for humans.

The results obtained confirm that the EHEC O157:H7 strains, whatever their origin, possess a greater number of virulence genes than the other strains belonging to the serotypes most frequently involved in epidemics (O26:H11, O103:H2, O145:H28, O111:H8, O121:H19). Strains belonging to other less frequent serotypes possess significantly fewer virulence genes than the preceding ones.

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Conclusion

The analysis of French and European epidemiological data highlights the following points:

- Serogroup O157 remains the majority in cases of infections declared in Europe, but its proportion is decreasing, particularly in France (from 34% in 2011 to 17% in 2015);

- We note the emergence, in France only, of the O80:H2 serotype, which represents the third serogroup isolated in 2015. This serotype, found in the HUS of children, presents a hybrid virulence both intestinal and extra-intestinal ;

- The vast majority of EHEC strains isolated in HUS cases display the virulence characteristics described in the Agency's previous opinions *(stx1* and/or *stx2, eae).* The virulence profiles of strains of human origin and food strains are similar;

- Since 2012, serogroup O104 has remained a minority in HUS cases declared in Europe (30 cases between 2012 and 2015). Moreover, the O104:H4 strain, responsible for the German and French epidemic in 2011, was not detected in the bovine reservoir in France or in other countries.

Any strain *of E. coli* isolated from humans or food should be considered as EHEC, if it has the following virulence genes:

- *stx1* and/or *stx2*

- *eae* or other gene(s) encoding an adhesion system in the human digestive tract.

Certain EHEC serotypes are more frequently associated with severe disease (HUS). In view of French epidemiological data, it is proposed to include serogroup O80 in group I of high-risk EHEC (Table 6).

The CES BIORISK stresses, however, that the source of contamination of the O80:H2 strain should be identified before any introduction of this serotype in the list of EHEC strains to be searched for in the context of self-checks. Similarly, given the available data on contamination of the bovine reservoir, testing for the O104:H4 serotype in products of bovine origin does not seem relevant.

Table 6. Classification of *stx*+ strains according to their public health risk in France

Band	Genoa	Serogroups	Risk	
			Diarrhea	HUS / Hemorrhagic colitis
I	*stx*+ *eae* + or *(aaiC* and *aggR)*+	O157, O26, O103, O145, O111, O104, O80	pupil	pupil
II	*stx* +*eae* + or *(aaiC* and *aggR)*+	any other serotype	pupil	potential
III	*stx* + *eae*- and *(aaiC* and *aggR)*-	any other serotype	potential	potential

3.2. Evaluation of the pathogenicity of *stx-eae+* strains belonging to one of the major serotypes and isolated from a culture broth positive for *stx*

3.2.1. Context

The *stx* genes are carried by mobile elements, temperate bacteriophages having their genome inserted (we speak of "Stx prophage") in the EHEC chromosome (ANSES opinion no. 2010-SA-0031).

Due to the presence of an Stx prophage in their chromosome, EHECs are qualified as "lysogenic" bacteria. In the presence of certain stresses or an inducing agent such as mitomycin C, induction of the Stx prophage can occur, which triggers a lytic cycle (Figure 1; steps 1 to 4). During this cycle, the prophage genome excises itself from the bacterial chromosome and multiplies (replication).

New phage particles are then produced, then released into the external environment thanks to the lysis of the bacteria. These phages can infect new bacteria, and make them lysogenic via the insertion of phage DNA into the bacterial chromosome, this is called the lysogenic cycle (Figure 1; steps 5 to 8).
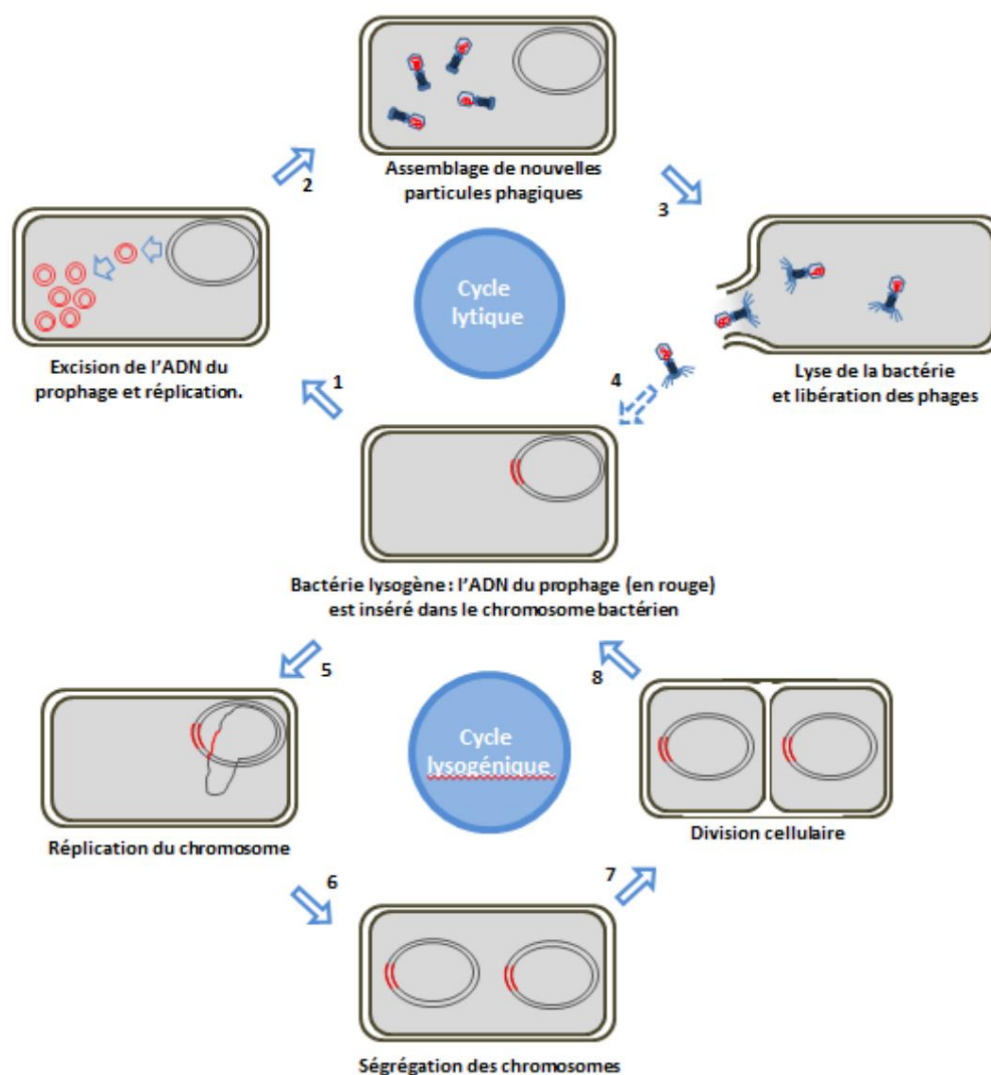

Figure 1. Diagram of the lytic and lysogenic cycles of temperate bacteriophages (adapted from (Bonanno 2016))

It has been shown that strains *of E. coli,* displaying all the genetic characteristics of EHEC except the *stx genes,* could be isolated from cattle, humans and food products. Such *stx-eae+* strains are EPEC3 (enteropathogenic *E. coli).*

The implementation of the method for the detection of EHEC in foods during official analyzes sometimes leads to the isolation of *stx-eae+* strains from an enrichment broth of a food in which an *stx* gene has been detected by PCR. These strains could be the witness of the presence of an EHEC in the food, from which they would derive after the loss of their Stx prophage, either in the food, or during their isolation.

ANSES in its opinion of 11 January 2011 indicated that it was not possible to conclude in the current state of knowledge on the absence or presence of an EHEC in the food. The Agency recommended the acquisition of additional scientific knowledge concerning the mechanisms of acquisition and loss of *stx* genes in *E. coli* and the genetic and phenotypic characterization of these strains.

3.2.2.Update of knowledge

The mechanism of the transformation of an EHEC into an EPEC via a loss of the Stx prophage remains poorly understood. It is likely that when triggering a lytic cycle, some bacterial cells initiate a partial lytic cycle which aborts before the lysis step, for example after the phage DNA excision step (Figure 1, step 1), leading, at the time of cell division, to obtaining cells without Stx prophage, qualified as " stx-negative".

Genetically, EHEC-derived EPEC strains (via a loss of Stx prophages) cannot be distinguished from non-EHEC-derived EPEC strains. This is shown by the results of a French study, during which 123 strains *of E. coli* O26, of human and food origin, including stx-positive (n=66) and stx-negative (n=57) strains , were compared at the genetic level by different methods, in particular by high-throughput PCR, MLVA and PFGE (and genomic sequencing for 12 of them) (Neto *et al.* 2012). If two major categories of strains have been identified genetically, we find both stx-positive strains and stx-negative strains in each of the categories, therefore without the possibility of distinguishing these two types of strains by genetic markers. .

• *Lytic cycle*

Concerning the induction of Stx prophages, it has been shown since the publication of the ANSES opinion of 11 January 2011 that Stx prophages have a "spontaneous" induction capacity (i.e. in the absence of inducing agent) higher than that of classical lambdoid prophages (devoid of *stx genes),* and that the difference in stability of Stx prophages and classical lambdoid prophages within the bacterial chromosome results from variations in the intracellular concentration of the repressor phage, named cI, involved in switching between lysogenic and lytic cycles (Colon *et al.* 2016). Moreover, in the presence of an inducing agent, a fraction of the STEC population does not trigger a lytic cycle and thus escapes bacterial lysis, which allows the survival of the STEC population (Imamovic et al. 2016) . This phenomenon is linked to a modulation of the induction of Stx prophages by the RpoS protein, qualified as a "master regulator" of the stress response (Imamovic *et al.* 2016).

Other work confirmed that Stx prophages from STEC O26:H11 strains were inducible *in vitro* in culture broth (i) in the presence of an inducing agent (mitomycin C), with concomitant lysis of the majority of bacterial cells, but also (ii) in the absence of an inducing agent (i.e. say spontaneously), but at a lower rate, i.e. not leading to the lysis of the majority of cells in the population (Bonanno *et al.* 2015, Bonanno, Petit, *et al.* 2016) . The enrichment step of the STEC detection method is also able to induce Stx prophages from STEC O26:H11 strains, however without causing lysis of the majority of cells in the population (Bonanno 2016, Bonanno , Cherchame, *et al.* 2016). It should be noted, however, that when the selective substances used in this step were tested individually with respect to the induction of Stx prophages, none could be identified as being at the origin of this phenomenon (Bonanno et al . *para.*

2017).

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[3] These strains were called "AEEC" in ANSES Opinion No. 2010-SA-0031.

When different enrichment media from the XP CEN ISO/TS 13136 technical specification and alternative methods were tested for their ability to induce Stx prophages from milk and cheese samples artificially contaminated with EHECs belonging to the five major serotypes, induction of Stx prophages was demonstrated, regardless of the enrichment method used (Bonanno, Cherchame, *et al.* 2016). However, if the phenomenon of induction of Stx prophages of STEC O26:H11 could be observed *in vitro,* this does not seem to lead to a high production of EPEC (stx-negative) O26:H11 strains because such strains n could not be isolated *in vitro* under the conditions tested, despite multiple attempts (Bonanno 2016).

Evaluation of the influence of certain physico-chemical parameters related to cheese manufacturing processes (such as temperature and concentration of hydrogen peroxide, salt and lactic acid) on the induction of Stx prophages from STEC O26:H11 has made it possible to demonstrate an induction of these prophages in the presence of H2O2 and NaCl, at levels that do not, however, lead to lysis of the majority of cells in the population (Bonanno *et al.* 2017). The production of Stx phages in cheeses during a manufacturing process carried out using milk inoculated with STEC O26:H11 was also demonstrated for 20% of the samples tested (10 out of 48) (Bonanno et al . 2017).

• *Lysogenic cycle*

Regarding the acquisition of Stx prophages by EPEC O26:H11 strains (called the lysogenization process), this could not be observed *in vitro* (Bonanno, Petit, *et al.* 2016). This result is in agreement with other attempts at lysogenization (including some carried out using O26:H11 strains) which have shown that this phenomenon was not systematic (Bielaszewska *et al.* 2007, Schmidt, Bielaszewska, and Karch 1999). Moreover, difficulties in obtaining stable lysogenic strains have also been reported in other studies carried out using various strains *of E. coli (E. coli* O157:H7 stx-negative, EPEC and EAEC) (Muniesa *et al.* 2004, Tozzoli *et al.* 2014). Note, however, that the acquisition of Stx prophages by commensal *E. coli* strains has been demonstrated *in vitro and in vivo,* and that the contribution of this phenomenon to an increase in shigatoxin production *in vivo* cannot be ruled out (Toth *et al* 2003, Goswami *et al* 2015, Iversen *et al* 2015).

Conclusion

The induction of Stx prophages causing the production of phage particles and the lysis of part of the EHEC population is possible *in vitro* in enrichment broths and in food matrices. However, the transformation of EHEC into EPEC during this induction phenomenon would be a rare event which remains poorly understood at present. The acquisition of an *stx* prophage by an *E. coli* (which would lead to the obtaining of an EHEC) also seems to constitute a rare event or one which does not result in the stable maintenance of the phage genome in the bacterial chromosome.

Without confirmation by strain isolation, obtaining a positive broth for *stx* and one of the intimin *(eae)* types of the 5 main EHEC serotypes is a sign of the potential presence of EHEC. If the isolated strain is *stx-,* it cannot be considered as an EHEC.
Nevertheless, the interpretation of the results must take into account the epidemiological context in which the sample was taken as well as the sensitivity and specificity of the analytical methods used.

ANSES opinion

Request No. 2016-SA-0121

Related referrals No. 2013-SA-0223; 2010-SA-0031

3.3. Review of the literature on recent epidemics with quantification data in foods and verification of consistency with the dose-response relationship

Foodborne outbreaks associated with EHEC (particularly serotype O157:H7) can involve a wide variety of products. In the past, beef products, vegetable products, as well as dairy products (Farrokh *et al.* 2013, Callejón *et al.* 2015, CDC 2016) have been implicated. Ground beef seems to be particularly affected. For example, over the period 1998-2014, more than 100 epidemics were reported in the USA (CDC 2016).

Despite the large number of foci of infection, it seems that few studies have focused on the quantification of EHEC in the foods involved. These data are nevertheless useful for estimating the dose-response relationship, supplying data to quantitative risk assessment models and measuring the performance of sampling plans.

This synthesis aims to take stock of the data concerning the levels of contamination in food products at the origin of epidemics (in particular beef-based products) and to verify whether these data make it possible to provide elements of answer to the question relating to the levels of contamination "resulting in an epidemic risk".

3.3.1. Contamination levels in products causing outbreaks

Table 7 reports data on the levels of contamination of meats involved in outbreaks associated with *E. coli* O157. The counts in raw products (before cooking) do not provide information on the doses ingested by consumers.

Some of these epidemics were used to construct the various dose-response relationships currently available in the literature. In these cases, the investigations carried out during epidemics have also made it possible to estimate the dose ingested by consumers (by integrating the quantity of food ingested and the effect of meat preparation practices).

Table 7. Contamination levels in beef foods implicated in outbreaks **Year Levels Samples Reference**

	Food	Characteristic *of E. coli* pathogenic	Country		Levels	Samples	Number of cases/Number of presentations	Reference
1	Ground beef steak	O157	USA	1992-93	1.5 MPN/g (0.3 – 15 MPN/g)	76 steaks from the same batch (before cooking)	398/5634	(Tuttle *et al.* 1999, Strachan *et al.* 2005)
2	Frozen ground beef	O157:H7, *stx1, stx2*	Japan	2004	1.45 MPN/g	Steaks from the same batch cooked similarly to consumers	3/6	(Hara-Kudo and Takatori 2011)
3	Beef meat	O157:H7, *stx2*	Japan	2004	23 MPN/g	Steak(s) from the same batch	-/-	
4	raw beef liver	O157:H7, *stx2*	Japan	2006	0.04 to 0.18 cfu/g	Leftover liver kept in the freezer	3/3	
5	Chopped steak fresh frozen	O157:H7	France	2007	5.9 cfu/g	22 steaks from the same batch (before cooking)	16/2155	(Delignette Muller and Horned 2008)
6	Frozen ground beef seasoned with garlic and pepper	O157 *stx1, stx2, eae*	Canada	2012	3.1 to 11.5 MPN/140g steak	12 steaks from the same batch	8 cases/-	(Gill and Huszczynski 2016)

- Not reported

These products have markedly different levels of contamination. Figure 2 shows contamination levels in ground beef before cooking.
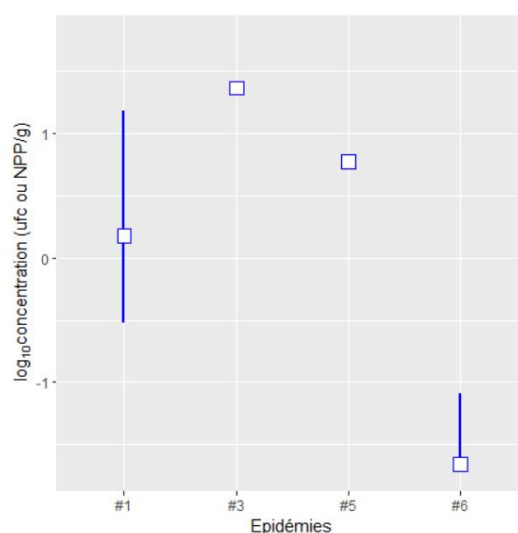
Figure 2. Concentrations of *E. coli* O157 in raw minced meat implicated in different epidemics (cf. Table 7 for the description of the epidemics). (ÿ) median (ÿ) range of contamination levels.

EHEC concentrations (mainly O157) have been characterized for other food categories implicated in epidemics (Table 8): other meat products, vegetables and dairy products. Contamination levels are also very variable from 0.3 cfu/g up to nearly 100 cfu/g (Table 8). Some of the data concerning these foods have been used with those from epidemics involving beef to construct dose-response relationships. The most recent epidemics do not make it possible to confirm the existing dose-response relationships, the number of people exposed in these epidemics not being clearly known.

Table 8. Contamination levels in various foods of animal origin implicated in outbreaks

Food	Characteristic *of E. pathogenic coli*	Country	Year	Levels	Samples	Number of cases/Number of presentations	Reference
Salami	O157	USA	1994	0.3-0.4 cfu/g	Other bundles in the bundle	17/2778	(Strachan *et al.* 2005, Teunis, Ogden, and Strachan 2008)
Deer jerky	O157	USA	1995	3-93 cfu/g	Stay	10/12	
Salad and fish sauce	O157	Japan	1996	4-18 cfu/100g	Meal samples	Coproculture e 208/828 in children 7/43 in adults	
Melon	O157	Japan	1997	43 cfu/g	Meal samples	32/71 (adults and children)	
Cheese	O157	Big Brittany	1997	5-10 cfu/g	-	2/360	
Ice cream	O145:H28, O26:H11	Belgium	2007	2.4 cfu/g, 0.03 cfu/g	Ice cream from the same batch	1/8, 2/11, 2/?	(Buvens *et al.* 2011)
Gouda	O157:H7	Canada	2013	1-10 MPN/g (Company A) 0.4-4 MPN/g (Company B)	Gouda analyzed several weeks after consumption (levels at time of consumption estimated)	29/?	(Gill and Oudit 2015)

3.3.2.Assessment of dose-response relationships

The objective of a dose-response relationship is to establish a link between the level of microbial exposure (total ingested dose of microorganisms) and the probability of occurrence of an effect. We can look at different effects: infection, illness and death. Most of the dose-response models published are mechanistic and are based on two biological hypotheses (Jaloustre 2011): (1) the absence of a threshold, i.e. a minimum number of cells of the pathogen in below which no harmful effects are observed; (2) the minimum infectious dose (MID). Two reasons have led to the preference for non-threshold models: (i) the contradiction, for certain epidemics, between the low doses ingested and the classically advanced DMIs and (ii) the hypothesis, supported by the understanding of the infectious mechanisms, according to which a single cell can be enough to infect a host thanks to its capacity for multiplication.

Several dose-response relationships are available (Strachan *et al.* 2005, Teunis, Ogden, and Strachan 2008, Delignette-Muller and Cornu 2008, Perrin *et al.* 2015). They have all been established from data collected for serotype O157. These relationships are distinguished by the nature of the predicted effects: probability of infection for two of the models (Strachan *et al.* 2005, Teunis, Ogden, and Strachan 2008), probability of haemolytic-uremic syndrome for the other two (Delignette-Muller and Cornu 2008, Perrin *et al.* 2015).

These last two models are also distinguished by the introduction of the sensitivity of the populations in the calculation of the probability of the effect. They are based on data from the same outbreak. The first model proposes to separate children into two distinct categories ([0.5 years] and [5-10 years]), the second model improves the consideration of age by integrating it into the model as a continuous variable in calculating the probability of disease.

Conclusion

Meat preparation practices, the quantity of food ingested, the size of the contaminated batches, the sensitivity of the people exposed are all factors that contribute (along with the levels of contamination) to the occurrence of cases of EHEC infection associated with a production batch.

Recent data do not make it possible to call into question or improve the dose-response relationship which best takes into account the population of interest in this referral, ie children under 15 years of age.

There is no threshold concentration below which there would never be an epidemic. For a given sensitive population (for example children under 15) whose annual consumption would be known as well as the preparation practices of the foods considered and therefore the exposure to the hazard, it is nevertheless possible to estimate:

• the annual number of cases and its uncertainty,

• if an acceptable risk (or appropriate level of health protection – Appropriate Level of Protection/ALOP) is indicated by the risk manager, a dose such that the probability of not respecting this acceptable risk does not exceed 1% or any other value set by the risk manager, and the uncertainty around this probability.

In the rest of this report, the expertise will focus on the prevention of HUS cases in their entirety (sporadic cases and epidemics).

3.4. Modeling the contamination of raw materials and minced meat mixes and evaluating the impact of management measures on reducing the risk of HUS

3.4.1. Description of the minced meat sector

Since 2006, animals brought to the slaughterhouse have been classified according to their state of cleanliness from A to D (A: clean animal; D: very dirty animal) according to an interprofessional grid based on taking into account the dirt visible on the animal leather. During the various stages of slaughter, the carcasses, initially sterile, can be contaminated on the surface, particularly during the skinning and evisceration operations. This is why a certain number of good practices and control measures have been implemented and included in the interprofessional control plans. Thus during pre-skinning and dressing operations, in addition to hand hygiene and decontamination of equipment, carcasses contaminated by hides must be identified and soiled quarters marked. An appropriate treatment is applied for so-called "spot" stains, that is to say localized and small-sized stains without flow. The treatment consists of trimming the soiled piece (trimming with or without steam treatment).
Trimming and any treatment with steam are carried out on the line. In the event of extensive contamination, the carcasses are managed off-rate. For the manufacture of chilled minced meat (VHR) soiled carcasses are excluded. For the manufacture of frozen minced meat (VHS) only the soiled quarters are excluded.

Similarly, in the event of faecal or ruminal contamination of carcasses, management is done according to the size of the soiling: trimming with or without steam treatment for spot soiling, exclusion of quarters or the carcass for soiling stretches.

The non-excluded carcass quarters are then dispatched or sent to the cutting workshops. The cutting step consists of deboning the quarters and cutting the meat into pieces. The different pieces obtained are sorted according to their characteristics and their destination.

Pieces of meat intended for the manufacture of minced meat constitute minced meat
(HPV, formerly called "ore"). The term "HPV" refers to a mixture of muscles with adjoining tissues, possibly cut into large pieces or a mixture of muscles and freedmen4
(maximum 50%) obtained on the same production day. A **unit of HPV** consists of selected pieces of meat whose composition depends on the desired fat content (5%, 15% or 20%). The pieces may come from several carcasses. The number of carcasses used per HPV unit as well as the size of an HPV unit are highly variable.

The maximum storage times for meat after slaughter for the production of minced beef are six days for refrigerated meat and 15 days for boneless and vacuum-packed meat (Regulation (EC) no. 853/2004). For refrigerated minced meats, the HPVs from deboning and cutting are kept in cold rooms (between 0 and 1°C) for one to two days before being used. Frozen ground meats can be made from chilled or frozen HPV with a variable chilled/frozen ratio depending on meat availability and production rate.

A minced meat **mix** is defined here as a set of microbiologically similar products representing the contents of one or more mixers during a defined period of production; this or these mixers can contain materials from one or more grinders. The scrum thus defined serves as a basis, in the companies' health control plan, for the management of microbiological non-conformities.

Although there is no microbiological criterion defined in Regulation (EC) No 2073/2005 for STEC in minced meat, the DGAL recommends taking into account EHEC belonging to the 5 major serotypes ("STEC considered as highly pathogenic") as a hazard and to implement microbiological self-checks to verify the effectiveness of the control measures.
The minimum sampling recommended by the technical instruction DGAL/SDSSA/2016-353 is as follows: (i)

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[4] Pieces of skeletal muscle of any size recognized as fit for human consumption, together with their tissues greasy and conjunctive residuals obtained after trimming.

for HSV: systematic search in each scrum (n=1, search in 25g) for serotype O157:H7 and at least one analysis per week (n=1, search in 25g) for the four other serotypes; (ii) for ORVs: search for serotype O157:H7 in a scrum at least once a week (n=1, search in 25g) by modifying the sampling day to cover all days of the week. It may nevertheless be relevant to increase this frequency in the case of workshops with a high production tonnage having a high number of scrums produced daily, or in specific situations (obtaining a non-compliant result, management of a STEC alert). On the other hand, for sensitive products (minced meat to be eaten raw of the "tartar" type or range intended for children), the frequency of analysis must be increased.

Following a confirmed positive result, additional analyzes must be carried out by the manufacturer. Appendices 2 and 3 of the technical instruction DGAL/MUS/2015-888 detail the analysis methods and the sampling plan to be carried out. These additional analyzes concern the positive detected scrum (m) as well as the scrums made before (m-1, m-2, etc.) and after it (m+1, m+2, etc.). Apart from the context of a human case, these additional analyzes are carried out according to two possible sampling methods chosen by the manufacturer: (i) the number of samples analyzed (n) is variable and less than 30 (case no. 1); (ii) n is equal to 30 (case 2) (n=29 on the positive detected scrum at the origin of the additional analyses).

Whatever the sampling chosen, if the analysis results are non-compliant for the scrums m-1 and m+1, additional analyzes are carried out on the surrounding scrums (m-2 and m+2) and so on until compliant results are obtained (application of the cascade) or a cleaning/disinfection operation of the production line has been implemented. In general, withdrawal or recall must be applied to all scrums that have given at least one non-compliant result (presence within 25g) following additional checks.

ÿ **Review of responses to the questionnaire intended for establishments producing ORVs and VHS**

A questionnaire drawn up by ANSES was sent to VHR and VHS producers via the professional federations. Information was collected on the following points:
   - Management of raw materials (HPV): definition of the batch, number of units of HPV entering into the composition of a mix, description of the analyzes carried out (method, sampling plan, frequency);

   - The analyzes carried out on the scrums or the finished products: method, sampling plan, frequency, type of analysis (pool or individual), definition of a presumptive positive result), the results with possible confirmation from the NRL;
   - The analyzes carried out on the surrounding scrums.

Responses were received from 22 minced meat manufacturing sites, including 10 VHR manufacturing sites, 8 VHS manufacturing sites and 4 VHR and VHS manufacturing sites. Between 2013 and 2015, the average annual volume represented by these 22 sites was 131,764 tonnes, of which 79% was the standard VHS range and 30% the standard VHR range, the "tartar" and "children's" ranges representing around 1% of production. total ground meat (Figure 3).
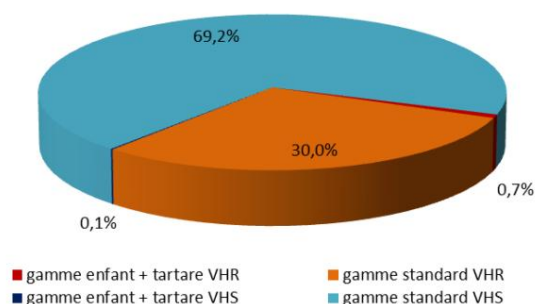
Volume de production (2013-2015)

Legend: ■ gamme enfant + tartare VHR   ■ gamme standard VHR
■ gamme enfant + tartare VHS   ■ gamme standard VHS

Figure 3. Percentage of average annual production volume in VHR and VHS according to the range

• *HPV management*

For each site surveyed, the minimum, maximum and modal values (the most frequent values) were entered for (i) the size of the HPV units, (ii) the number of HPV units entering into the composition of a scrum and (iii) the size of a scrum.

According to the results of this questionnaire, the size of the HPV units used for the manufacture of VHS varies between 50 kg and 9000 kg with a mode varying between 750 and 6000 kg depending on the workshops. For the manufacture of VHR, the HPV units used have a size between 10 kg and 2000 kg with a modal value varying between 90 kg and 1500 kg depending on the workshops.

The number of HPV units used to make a melee also varies depending on the workshops and the type of minced meat produced. In VHS, a scrum consists of 1 to 50 units of HPV, the most frequent values being between 2 and 10. In VHR, the number of HPV units entering into the composition of a scrum varies from 1 to 22 , the most frequent values being between 1 and 5.

The size of a melee is also very variable depending on the type of manufacture and the workshops. According to the results of the questionnaire, it varies between 200 kg and 2000 kg in the case of VHS production, the values most often observed being between 600 kg and 1500 kg; in VHR the size of the scrums varies between 15 kg and 2600 kg, the most frequent values being between 30 kg and 1100 kg. • *Analyzes carried out on scrums or finished products*

According to the results of the questionnaires, between 2013 and 2015, 140,920 STEC research analyzes were carried out in mixed or finished products. While the overall number of analyzes increases each year (2013: 37,724 analyses, 2014: 43,582 analyses; 2015: 59,614 analyses), this is not the case for each of the product ranges. Indeed, only the standard range and in particular in VHS is concerned (Figure 4).
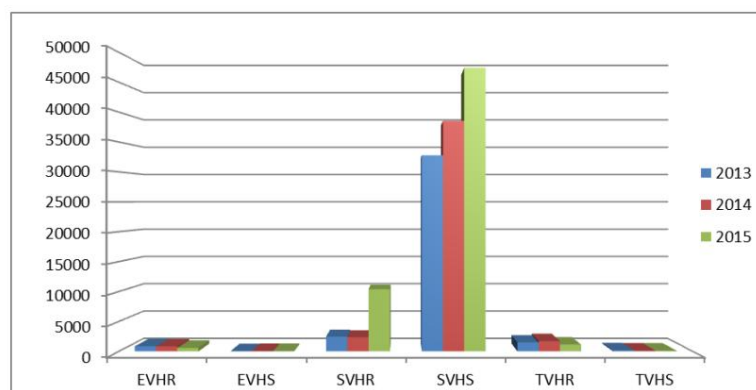

Figure 4. Evolution of the number of STEC research analyzes by product range between 2013 and 2015 (EVHR: VHR children's range, EVHS: VHS children's range, SVHR: VHR standard range SVHS: VHS standard range, TVHR: VHR tartar range , TVHS: Tartar VHS range)

Among these analyses, 84% were carried out in the standard VHS range against 10% in the standard VHR range. 77.2% of all the analyzes concerned serotype O157:H7 only, 5% the search for serotypes belonging to the TOP 5 (O157:H7, O26:H11, O103:H2, O145:H28, O111:H8) and 22.3% search for TOP 7 serotypes (TOP 5+ O45:H2 and O121:H19).

The evolution of the types of analyzes carried out between 2013 and 2015 (search for serotype O157:H7, search for serotypes belonging to the TOP 5, search for serotypes belonging to the TOP 7) according to the product range is represented in figure 5. The proportion analyzes targeted on the O157:H7 serotype remains relatively stable and between 63% and 67% in the standard VHS range. Very few analyzes concern the search for the TOP 5 since they represent, all ranges combined, less than 1% of annual analyses. On the other hand, the search for the TOP 7 represents approximately 20% of the annual analyzes and is essentially carried out in the standard VHS range, although in 2015 it was also used for certain manufactures of the refrigerated standard range (Figure 5).
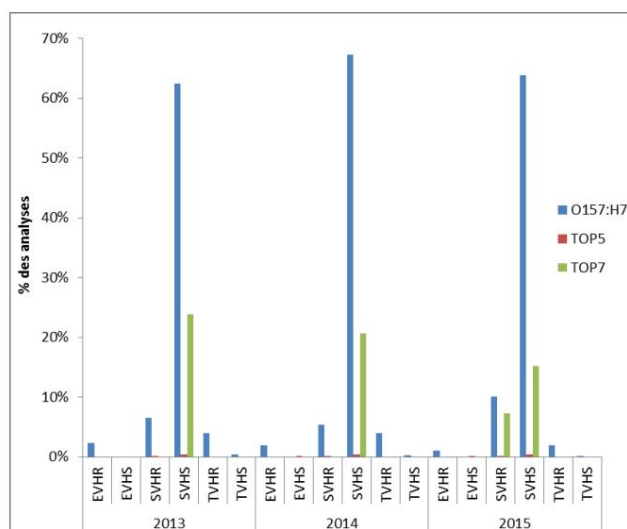
Machine Translated by Google

Figure 5. Evolution of STEC serotypes screened for during self-checks according to product ranges between 2013 and 2015 as a percentage of analyzes carried out annually.

Not all presumptive positive samples are subject to confirmatory analysis by the NRL. The management of a presumptive positive sample is generally similar to that of a confirmed positive sample:
withdrawal, recall or redirection.

The rate of presumptive positive results is less than 0.5% for all ranges between 2013 and 2015 with significant variability between product ranges. Indeed, in the "children's" range, which represents 1.6% of annual analyses, no presumptive positive result was declared between 2013 and 2015. Similarly, for the frozen tartare range, no presumptive result was obtained following the 342 analyzes carried out from 2013 to 2015. On the other hand, for the chilled tartar range, which represents 3% of the total analyses, the percentage of presumptive positive samples which was 0% in 2013 increased in 2014 (0.29 %) and 2015 (0.44%) although the number of analyzes remained stable and it was still a search for *E. coli* O157:H7. Among the 10 presumptive samples observed between 2013 and 2015 in this range, 2 samples were sent to the NRL for confirmation and none of them were confirmed (Figure 7).
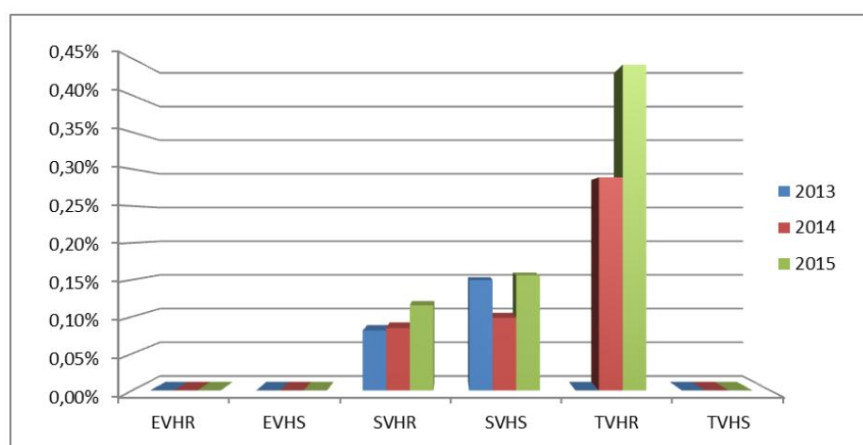

Figure 6. Evolution of the percentage of presumptive positive results by product line between 2013 and 2015
NB: The definition of a presumptive positive result differs according to the analytical method used.

In the standard refrigerated range, the average rate of presumptive positives over the three years is 0.09% (Figure 6). In 2013 and 2014, all of the presumptive samples obtained with the VHR standard were sent to the NRL for confirmation of the result; the observed confirmation rate is about 50%. In 2015, 25% of presumptive positive samples in the VHR standard range were sent to the NRL (n=3) none of the samples were confirmed positive (Figure 7).

Concerning the frozen standard range, the average rate of presumptive positives is 0.13% (Figure 6). The percentage of presumptive positive samples sent to the NRL is 84% in 2013, 60% in 2014 and 53% in 2015. The confirmation rate of these samples for the three years is 51%, 48% and 56% respectively (Figure 7).
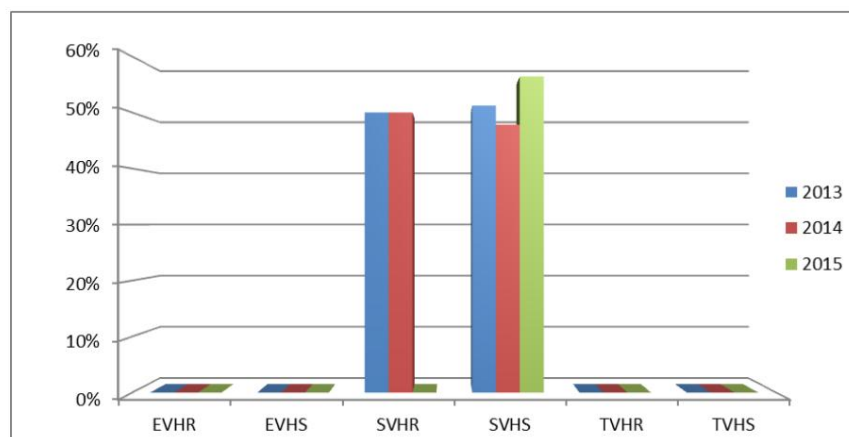

Figure 7. Evolution of the confirmation rate of presumptive positive samples sent to the NRL by product range

• *Analysis of framing scrums*

According to the questionnaires completed by 22 minced meat manufacturing workshops, not all of them carry out additional analyzes on the supervising scrums. Indeed, only 14 declare that they do these analyses, including 6 VHR manufacturing workshops (among the 10 questioned), 6 VHS manufacturing workshops (among the 8 questioned) and 2 VHS and VHR manufacturing workshops (out of the 2 questioned) . It should be noted that some workshops systematically destroy or direct towards other productions (cooking) the m+1 and m-1 scrums surrounding a positive scrum without analyzing them.

According to the questionnaires, on all the manufacturing sites, between 2013 and 2015, 77 scrums were the subject of additional analyzes (75 scrums from the standard VHS range and 2 scrums from the standard VHR range). Of these scrums analysed, 45 were found to be non-compliant following additional analyses. For 29 of the 77 scrums detected positive, the surrounding scrums were detected non-compliant at levels up to m-3 or m+3.

3.4.2. Modeling the contamination of raw materials (HPV) and meat scrambles chopped

The model developed in this opinion is based on various published models (Cassin *et al.* 1998, Cummins *et al.* 2008, Smith, Fazil, and Lammerding 2013, Anses 2014, 2015) and simulates the different steps in the manufacture of ground beef from the slaughter of cattle until the finished products leave the factory and then their consumption. The objective of the model is to estimate and compare the effectiveness of different sampling plans and management measures proposed by the DGAL on reducing the probability of occurrence of cases of HUS linked to the consumption of minced beef from Beef. The structure of the model and the different calculation steps are those presented in the ANSES opinions of 2014 and 2015.
In addition, changes were made following new data and new questions asked by the DGAL.

The originality of this model consists in taking into account (i) the different types of dirt that can contaminate the carcasses used in the manufacture of minced meat, (ii) the distribution of HPV units between the frays (Figure 8) and (iii) so-called "framing" scrums.

As with the previous models, in the absence of data to characterize the distribution of the levels of contamination of HPV and minced meat mixes by STEC in France, these are simulated using a model mathematics based on levels of contamination in cattle faeces.

The input parameters of the model are presented in appendix 3.

To answer question 4 of the referral, the sampling plans assessed in this opinion consist of analyzing for each scrum:

ÿ Plan n°1: 1 sample of 25g (n=1, m=absence in 25g);

ÿ Plan n°2: 3 samples of 25g (n=3, c=0, m=absence in 25g);

ÿ Plan n°3: 1 sample of 75g (n=1, m=absence in 75g).

To answer question n°5, the making of melee from different HPV units as well as the concept of framing melee are modeled. Two scrum analysis scenarios are evaluated and compared in terms of HUS risk reduction and proportion of positive scrums detected:

ÿ Scenario 1: systematic analysis of scrums according to a defined sampling plan;

ÿ Scenario 2: systematic analysis of the scrums according to a defined sampling plan followed by a reinforced analysis of the surrounding scrums (scrums surrounding a scrum initially detected positive).

The modifications made to the models presented in Opinion 2013-SA-0223 (ANSES 2014) are detailed in the following paragraphs.
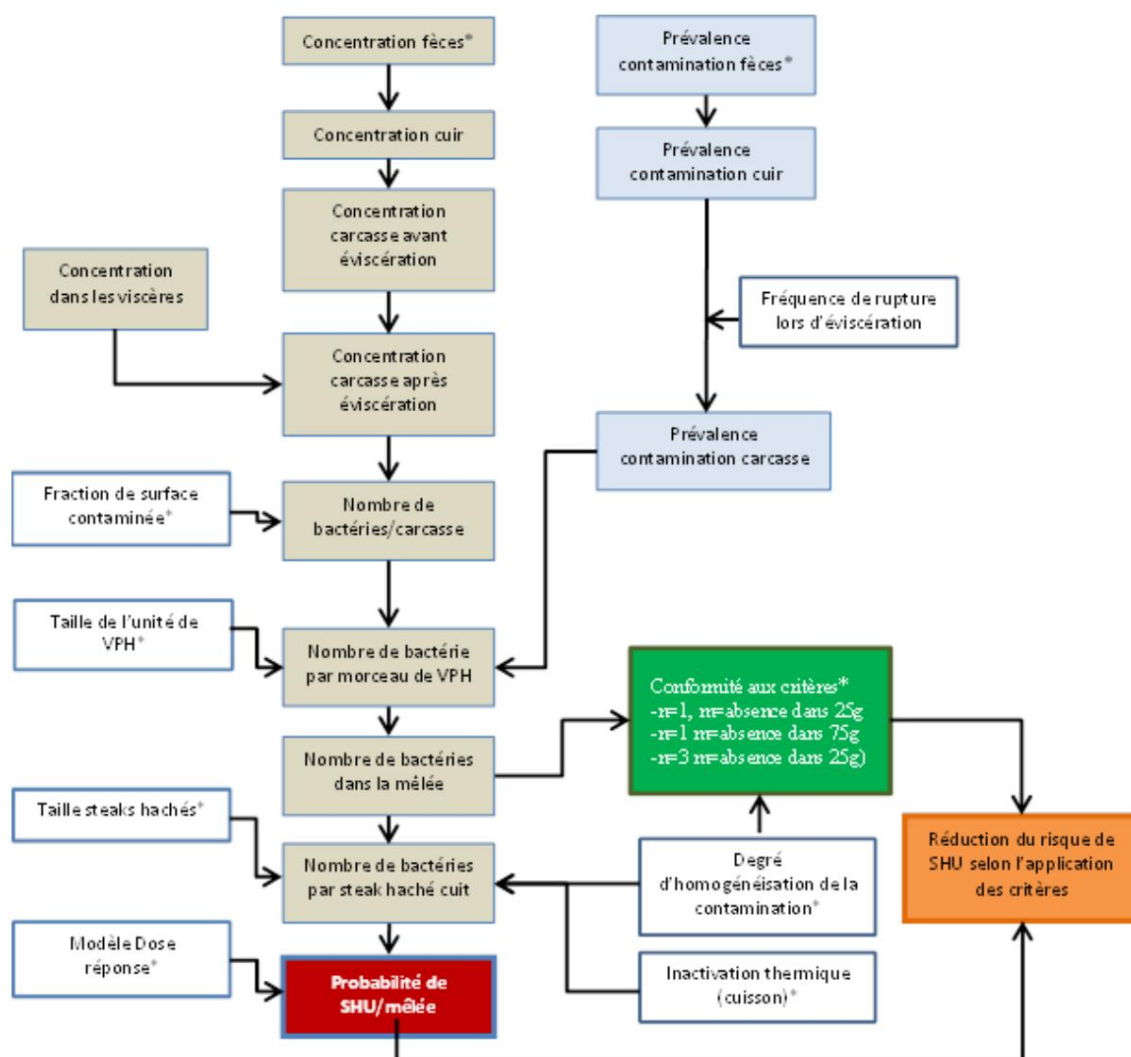

Figure 8. General structure of the model adapted from the model in the 2014 ANSES opinion
Blue boxes: prevalence of contamination in EHEC; gray boxes: quantity/concentration of contamination; red/green/orange boxes: model outputs. Parameters marked with the symbol are modified from the model of the 2014 opinion (see Table appendix 3).

ÿ **Prevalence of excretory animals (contamination of faeces)**

The prevalence of excretory animals was estimated thanks to a study conducted in six slaughterhouses in France between October 2010 and June 2011 (Bibbal *et al.* 2015). A total of 1318 animals belonging to the four main categories of cattle (young dairy cattle (JBL), young beef cattle (JBV), dairy cows (VL), beef cows (VV)) were sampled in six different slaughterhouses. The prevalence of the five major serotypes (O157:H7, O26:H11, O103:H2, O111:H8 and O145:H28) was estimated in the faeces of these animals. Among the animals sampled, 2.4% ([1.8%; 3.5%]; 95% confidence interval) excrete one of the five major EHEC serotypes. Taking into account the proportion of animals belonging to the different categories, the overall prevalence of shedding adult cattle is estimated at 1.8%. By grouping the analyzes into two distinct periods, October to February, and March to June, the prevalence observed in spring was 3.3 times higher than that observed in winter ([1.5; 7.3]; confidence interval at 95%), namely 1.2% in winter and 3.9% in spring. The model used in this advice considers both periods of excretion unlike the model of the 2014 advice in which only the period of high excretion was taken into account.

Given the absence of demonstration of a possible difference in virulence between the five major serotypes, the model developed in this opinion uses the prevalence of these five serotypes for the risk assessment. Thus, in the model, the prevalence of faecal contamination for the period of low excretion (autumn/winter) and the period of high excretion (spring/summer) are respectively PfLow,= 1.2% *and* Pfhigh =3.9 %.

ÿ **Concentration in faeces**

As with prevalence, the level of faecal contamination varies depending on the sampling period (Stephens, McAllister, and Stanford 2009). In addition, it is assumed that the variability of EHEC contamination in faeces is the same for all five major serotypes.

The study by Stephens, McAllister, and Stanford (2009) presents the distribution of O157:H7 contamination levels in cattle whose samples were found positive with a detection method at two periods of the year: autumn-winter and spring-summer. The data is presented as an interval. The R package fitdistrplus was used to fit a Normal distribution on these data at the log10 scale. Thus, the distribution law used for the contamination of faeces during periods of low excretion *(Cflow)* follows a normal law with a mean of 1.59 log10 cfu/g and a standard deviation of 1.45 and that used for the contamination of faeces in period of high excretion *(Cfhigh)* follows a normal law with a mean of 2.44 log10 cfu/g and a standard deviation of 1.98.

ÿ **Contamination of carcasses by hides before evisceration**

During skinning, carcass contamination can occur through direct contact with the leather or indirectly through dust escaped from the leather, the hands or the utensils used. The prevalence of carcasses contaminated with *Pc* leather is described in the model in the 2014 ANSES opinion (ANSES 2014).

The EHEC concentration *(Cc)* of soiling from leather is described in the 2014 ANSES opinion and depends on the EHEC concentration on the leather *(Ccuir)* and the transfer fraction from the leather to the surface of the leather. carcass *(FTcuir)* defined in the table of model input parameters (Appendix 3).

In this opinion, the model used considers two types of dirt observed in slaughterhouses: so-called "spot" dirt not exceeding 3 cm in diameter and circumscribed dirt whose surface corresponds to the size of a hand (Cartier 2009) ; the latter will be designated later by the term 5
"hand" defilement . In this study, 70 carcasses with localized soiling from leather on a slaughter line were observed. 80% of the stains observed correspond to "spots" and 20% to "hand" stains (Cartier 2009). Extensive contaminations, that is to say of a size greater than the surface of the palm of a hand, are not taken into account by the model. In effect,

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5 The control plan for pathogenic *E. coli* for minced meat and refrigerated and frozen minced meat preparations produced by Culture Viande (January 2016) defines "spot" contamination as "localised, of reduced size (of size less than or equal to a palm of the hand) in the absence of flow and which can be trimmed with a single gesture by the line operator". This definition therefore encompasses the "spot" type and "hand" type stains defined in this notice.

these contaminations are subject to strict management measures resulting in the exclusion of the cuts concerned from the production of chilled and frozen minced meat.

In the model, the proportion of stains "spots" *Pspot* is fixed at 80%. The surface of these stains *Sspot* is fixed at 7 cm2 corresponding to a spot of 3 cm in diameter. The proportion of "hand" stains by the *Pmain* leather is set at 20% with a *Smain* surface set at 150 cm2 .

The number of stains present on a contaminated carcass *(Nstains)* follows a Poisson distribution *(mstains)* with *mstains* the average number of stains per carcass. In the absence of data on this average number, it is considered an uncertain parameter of the model. The simulations are therefore carried out for several values of *msoil* between 1 and 20 with a default value of 10, which makes it possible to estimate the impact of this parameter on the results of the model. The number of spots *Nspot* is estimated for each contaminated carcass and follows a Binomial distribution of parameters *Nspots* and *Pspot.* The total contamination surface of a carcass *a* is defined by:

$$a = N_{spot} \times S_{spot} + \left(N_{souillures} - N_{spot}\right) \times S_{main}$$

Equation 1

With: *a* the contamination surface of the carcass (cm2)
    Nspot the number of "spot" type stains on the carcass
    Nstain the total number of stains on the carcass
    Sspot the surface of a "spot" type stain (cm2)
    Smain the surface of a "hand" type stain (cm2)

The model takes into account visual checks throughout the slaughter chain and considers a sensitivity (or probability) of detecting spots *(Sespot)* and "hand" stains *(Semain).* In the absence of data on these sensitivity levels, these parameters are considered as uncertain parameters of the model for which several values are therefore considered with a default value *Sespot* =0.5 and *Week* =0.8.

The dirt detection sensitivity integrates the effectiveness of the treatment (trimming and/or water vapour). In other words, all of the contamination coming from the soils detected is eliminated. It is therefore the undetected dirt that is responsible for the contamination of the meat. The number of undetected spots and hand stains noted respectively *Nspot_remaining* and *Nmain_remaining* vary from one contaminated carcass to another and follow a binomial probability distribution *(1-Sespot)* and *(1-Week)* and size *Nspot* and *(Nsouillures -Nspot).*

The contamination surface of the carcasses after inspection and treatment of the detected dirt is noted *acorr :*

$$a_{corr} = N_{spot\_restant} \times S_{spot} + N_{main\_restant} \times S_{main}$$

Equation 2

With: *acorr* the contamination surface of the carcass after control and treatment (cm2)
    Nspot_remaining the number of "spot" type stains remaining on the carcass after treatment
    Nmain_remaining the number of "hand" type stains remaining on the carcass after treatment
    Sspot the surface of a "spot" type stain (cm2)
    Smain the surface of a "hand" type stain (cm2)

ÿ **Contamination of carcasses following an evisceration accident**

Contamination of carcasses by evisceration accident (perforation or poor preparation of the digestive tract) is modeled as described in the 2014 ANSES opinion, assuming that the concentration of EHEC in the viscera is the same as that in the faeces *(Cf).* However, the maximum value of the quantity of contents of the digestive tract on the carcass *(q)* remaining after trimming and possible treatment is lowered from 50 g to 20 g in order to be closer to field observations.

ÿ **Quantity of bacteria per contaminated carcass after slaughter**

At the end of the slaughter, a number *Contc* of carcasses is contaminated by EHEC through the leather and/or the contents of the viscera. For each of the contaminated carcasses, the contamination surface of the

carcass and the number of EHECs on the carcass are estimated. The total area of a standard carcass *(TSA)* is assumed to be 32,000 cm2 .

• *Number of bacteria from leather contamination*

The number of bacteria contaminating the carcass from leather *(Oh)* is equal to:

$$O_h = a_{corr} \times 10^{Cc}$$ Equation 3

With: *Oh* the number of EHEC per contaminated carcass from leather (log10 cfu)
  acorr the contamination surface of the carcass after control and treatment (cm2)
  Cc the concentration of EHEC on the surface of a carcass following contamination by leather (log10 cfu/ cm2)

• *Number of bacteria from contamination by evisceration accident*

The number of bacteria contaminating the carcass from the contents of the viscera *(Ov)* is equal to:

$$O_v = q \times 10^{Cf}$$ Equation 4

With: *Ov* the number of EHEC per carcass contaminated by viscera (log10 cfu)
  q the amount of viscera content soiling the carcass (g)
  See EHEC concentration in faeces and viscera (log10 cfu/g)

• *Bacteria count from leather contamination and evisceration accident*

The quantity of bacteria per contaminated carcass *(Oc)* resulting from contamination by hides and viscera is then equal to:

$$O_c = O_h + O_v$$ Equation 5

With: *Oc* the number of EHEC per carcass contaminated by the leather and by the viscera (log10 cfu)
  Ov the number of EHEC per carcass contaminated with viscera (log10 cfu)
  Oh the number of EHEC per contaminated carcass from leather (log10 cfu)

ÿ **Contamination of HPV units**

The number of contaminated carcasses per unit of HPV *(K)* is described in the 2014 ANSES opinion for units of HPV composed of meat from 60 animals. For each of the *K* contaminated carcasses, a series of calculations is carried out to determine the number of bacteria per piece and per carcass as described in the 2014 ANSES opinion, thus making it possible to estimate the expected total number of bacteria per unit of HPV (N).

ÿ **Contamination of scrums**

The composition of scrums from HPV units is modeled according to two scenarios. The first scenario makes it possible to estimate the risk of HUS and to test the different scrum sampling plans based on the total number of bacteria in the scrum and the scrum homogeneity coefficient (b) considering that *a* scrum is made from a unit of HPV. This scenario therefore makes it possible to answer question 4.

The second scenario, more complex in the modeling of the use of HPV units for the production of scrums, makes it possible to answer question no. 5 on the assessment of the levels of contamination of surrounding scrums (see section 3.4.6).

The model considers that a scrum consists of a unit of HPV of equal size *(MM= MVPH=* 1000 kg). The grinding and mincing operations do not allow a completely homogeneous distribution of the bacteria present on the meat of the HPV unit. The degree of homogenization achieved following the various operations depends on the proportion of contaminated HPV pieces and their distribution in the HPV unit. If the chopping led to a perfectly homogeneous melee unit, then all the chopped steaks

would present the same average level of contamination (ÿ, number of bacteria per HPV unit divided by the mass of the melee unit: ÿ=N/T). However, mincing does not lead to perfect homogeneity in ground meat. The distribution of contamination can be described using a Gamma-Poisson probability law as described in the ANSES opinion (2014). According to this distribution, the number of bacteria per gram of ground beef follows a Poisson law of parameter and this parameter () follows a Gamma law (Nauta, 2005). The probability of having *x* bacteria in a 100 g ground beef is calculated from the following formula:

$$P_{(x)} \quad = \quad \frac{(\ddot{y}bx \quad)}{xb\ddot{y}()} \ddot{y} \quad \frac{b^{b\ddot{y}}}{(\ddot{y} \ \ddot{y} \ b)^{b\ddot{y}}} \qquad \text{Equation 6}$$

With: *b* the coefficient measuring heterogeneity (when *b* tends to infinity, the model is equivalent to a Poisson law), and the

average concentration in 100 g of minced meat randomly sampled from a melee unit.

A recently published study by IDELE presents experimental results of contamination dispersion in 25 kg scrums for different levels of initial contamination of the scrum and for 100% chilled or chilled/frozen mix scrums (Loukiadis, Bièche- Terrier, *et al.* 2017).

According to the results observed in this study, the value of parameter *b* does not seem to be significantly influenced either by the levels of contamination tested or by the type of mixture and is between 1.0 and 1.6 with an average of 1, 3 which corresponds to a moderately homogeneous distribution of bacteria in the fray. It should be noted that the concentrations observed in the frays are much lower than the expected theoretical concentrations. The percentage of bacterial cells recovered varies between 4% and

27%. Two hypotheses can be put forward: either the initial population has become non-cultivable or it is not dispersed by the grinding-mixing process and remains locally fixed at one or two points in the fray. The first hypothesis supposes an experimental bias due to the strain used in the IDELE study. A fraction of the inoculated population would have difficulty surviving or growing on the selective media used after cold stress or because of competition with the microflora already present in raw beef (Lu et al. 2011, *Loukiadis ,* Bièche-Terrier, *et al.* 2017). The second hypothesis assumes the presence of one or more undetected clusters containing the rest of the population.

This could be due to the attachment of the inoculated cells to the surface of the meat and the inability of the manufacturing process to detach and disperse these bacteria.

It is impossible in the current state of knowledge to favor one of the two hypotheses. Conventional sampling plans are not able to detect this fraction and their effectiveness can only be assessed for the part of contamination that will be mixed in during the grinding process. Taking this uncertainty into account leads to a range of b values between 0.5 and 2.

In conclusion, in the modeling approach adopted to answer the questions on sampling, it was considered that all of the contamination brought by the HPV batches was distributed in the mix according to the values of b *between* 0.5 and 2.

3.4.3. Calculation of detection probabilities with scrum sampling plans according to scrum contamination scenario 1

ÿ **Plan n°1: n=1, m=absence in 25g**

The probability of detecting the presence of EHEC in a scrum *i* with a sample of 25g, *P(D25)i ,* is obtained by:

$$P_{(D25)}{}_{i} \quad = \quad 1 \quad \ddot{y}\frac{b}{\ddot{y}\ddot{y} \ \ddot{y} \ b\ddot{y}}\ddot{y}^{b} \qquad \text{Equation 7}$$

With ÿ' the average amount of EHEC in a sample of 25g of minced meat taken
randomly in a melee;
b the coefficient measuring the heterogeneity (when *b* tends to infinity, the model is equivalent to a Poisson law).

ÿ **Plan n°2: n=3, c=0, m=absence in 25g**

The probability of detection of the presence of EHEC in a scrum *i* in at least one 25g sample among *n* 25g samples taken P *(Dn)i* is obtained by:

$$P(D_n)_i = 1 - (1 - P(D_{25}))^n$$

Equation 8

ÿ **Plan n°3: n=1, m=absence in 75g**

The probability of detecting the presence of EHEC in a scrum *i* with a 25g sample, *P(D75)i ,* is obtained by:

$$P(D75)_i ÿ 1 ÿ \frac{b}{ÿ ÿ ÿ b ÿ} ÿ^b$$

Equation 9

With ÿ" the average quantity of EHEC in a sample of 75g of minced meat taken randomly in a scrum.

3.4.4. HUS risk estimation

The parameters needed to estimate the risk of HUS for consumers of hamburger patties (parameters of survival of EHECs to heat treatment, consumption data, dose-response relationship) are those presented in the 2015 ANSES report (ANSES 2015).

The survival parameters of EHEC to heat treatment are given for three types of cooking (rare, medium and well done) and four categories of consumers according to age: children under 5 years old, children from 5 to 10 years old, children from 10 to 15 years old and people over 18 years old.

The efficiency of cooking for the destruction of EHEC is expressed in number of decimal reductions *(RD)*. The proportion of cooking mode applied according to the age of the consumers as well as the number of decimal reductions expected at the end of cooking are presented in table 9.

Table 9. Proportion (%) of type of cooking applied by consumer age group and number of decimal reductions *(RD)* in the level of EHEC per ground beef by type of cooking

Cooking\age	< 5 years	5-10 years	10-15 years	> 18 years	RD	
Bleeding	10%	17%		21%	38%	0.5
Medium rare	41%	53%		54%	35%	1.6
Well done	49%	28%		24%	28%	3.9

The dose-response relationship used here is that described by Perrin *et al.* (2015). This is an exponential model whose parameter *r* varies with age. The risk, for a child under 15, of developing haemolytic uraemic syndrome following the consumption of a minced steak is obtained as follows:

$$R_{âge} = P_{âge} \times P_{dose|âge}$$

Equation 10

$$P_{dose|âge} = 1 - exp(-x \times r_{âge} \times 10^{-RD})$$

Equation 11

$$r_{âge} = 10^{-2.33} \times exp(-0.38 \times âge)$$

Equation 12

With: *Page* the proportion of minced steaks consumed by children for each of the age groups (0 to 15 years in steps of 1 year)

Pdose|age the probability of occurrence of a case of HUS knowing the dose ingested and the age of the consumer

x the number of bacteria in a serving of ground beef (dose)

rage the parameter of the exponential dose-response relationship

RD the number of decimal reductions at the end of cooking (Table 9)

The probability of occurrence of a HUS following the consumption of a minced steak from a particular mix is calculated by taking into account the variability of the intra-mix contamination and the variability of the cooking methods associated with each class of age.

The probability of occurrence of a HUS following the consumption of a minced steak (coming from any scrum) is estimated by the mean of the probabilities per scrum. This probability is called HUS risk.

3.4.5. Model results

The results presented below are valid for the parameters and assumptions of the model defined previously. The model considers in particular:

- two periods of excretion of STEC in bovine faeces for which the concentrations are not estimated from French data, contrary to the prevalence of excretory animals;

- the application of a perfect analytical method (specificity and sensitivity of 100%) for the detection of the 5 major serotypes;

- cooking methods for minced steaks according to the age groups of consumers defined on the basis of estimates made as part of the investigation of a French epidemic (Delignette-Muller and Cornu 2008);

- the dose/response relationship established for serotype O157:H7 (Perrin *et al.* 2015).

Therefore, it is highly likely that the model overestimates the risk. However, the model is not interested in the absolute value of this risk but in the risk reduction that could be obtained by different measures.

ÿ **Influence of uncertain parameters on HUS risk estimation**

Updating the model led, on the one hand, to the estimation of the range of uncertainty surrounding the value of parameter *b* and, on the other hand, to the introduction of three new parameters related to carcass contamination (*mdefilements, Sespot, Week).* At the present time, there is no study making it possible to statistically estimate the values of these three parameters, these have therefore been proposed on the basis of professional data and the opinions of experts. In order to test the influence of these model input parameters *(b, msouillures, Sespot, Semain)* on the model outputs (risk of HUS, probability of detection of contaminated scrums), simulations were carried out for a corresponding scenario at the period of high excretion *(Pfhigh, Cfhigh).* For each uncertain parameter, several values are drawn at random within its range of uncertainty. For each of its values, 100,000 iterations of the model are performed by setting the other uncertain parameters to their default value (Table 10). For each scenario, the average risk of HUS per 100 g serving is estimated for two situations. In the first situation, the reference situation, the minced meat scrums are not analysed. The model therefore considers that all the hamburgers produced are consumed. The estimated risk in this situation is called the reference risk (R0). In the second situation, the minced meat scrums are systematically analyzed (100% of the scrums analyzed) according to the sampling plan n=1, absence in 25 g. The model considers that the minced steaks from the mixes detected positive for the presence of EHEC following the analysis are not consumed (blocked batches). The estimated risk is the residual risk (Rr).

Table 10. Values of uncertain parameters chosen at random and default value

Uncertain parameter	Values	Default value
b	0.01; 0.1; 0.2; 0.5; 1; 2; 10	1
Mstaints	1 to 20 in steps of 1	10
Sespot	10% to 90% in 10% steps	50%
Week	10% to 90% in 10% steps	80%

For each uncertain parameter, the reference risk and the residual risk are estimated and represented according to the value of the uncertain parameter. The results are presented in appendix 4.

The value of parameter *b* has little influence on the reference risk level when b varies between 0.01 (distribution of heterogeneous contamination) and 10 (distribution of homogeneous contamination). On the other hand, when the frays are analysed, the heterogeneity of the contamination has a significant impact on the efficiency of the sampling and the associated level of risk. Indeed, the more the contamination is evenly distributed within the fray (b>1) the more the probability of detection with the defined sampling plan (n=1, absence in 25g) increases. Contaminated scrums therefore have a higher probability of being detected when the distribution of contamination is homogeneous, which leads to a reduction in the risk of HUS of more than 95% when b>1.

The average number of stains *(mstains)* per carcass has a significant impact on the reference risk and the residual risk, multiplying them by a factor of 5 when the number of stains is increased from 1 to 20. The number of stains also slightly influences the efficiency of the sampling: for *mstains=1,* the probability of detecting scrums is 1%; for *mstains=20,* the probability of detection is 4%. For an average number of stains per carcass of 10, the probability of detection is 3%. The associated risk reduction (between 95% and 98%) is however not directly impacted by the number of stains since different reductions are estimated for similar numbers of stains and probability of detection.

The model is not very sensitive to the probability of detection of "spot" stains. On the other hand, the probability of detection of "hand" type stains, larger in size but less frequent than spots, influences the outputs of the model. Indeed, the reference and residual risks are divided by 3 when the probability of detection increases from 10% to 90%.

ÿ **Probability of detection of EHEC according to different sampling scenarios and reduction of associated risk**

The effectiveness of three sampling plans is compared in terms of the probability of detection of melee and the percentage reduction in the average risk of HUS per 100 g serving of ground beef.

ÿ Plan n°1: 1 sample of 25g (n=1, m=absence in 25g)

ÿ Plan n°2: 3 samples of 25g (n=3, c=0, m=absence in 25g)

ÿ Plan n°3: 1 sample of 75g (n=1, m=absence in 75g)

Each of these sampling plans is tested for different contamination scenarios described in the table 11. The results obtained with 300,000 iterations are presented by sampling plan for each of the scenarios studied in table 12 and represented in figures 9 and 10.

Table 11. Contamination scenarios for which sampling plans are tested

Script	Season	Heterogeneity of intramixed contamination *(b)*	Average number of stains per carcass
S1	strong excretion	0.5	10
S2	Low excretion	0.5	10
S3	strong excretion	1.0	10
S4	Low excretion	1.0	10
S5	strong excretion	2.0	10
S6	Low excretion	2.0	10
S7	strong excretion	0.5	5
S8	Low excretion	0.5	5
S9	strong excretion	1.0	5
S10	Low excretion	1.0	5
S11	strong excretion	2.0	5
S12	Low excretion	2.0	5

It should be noted that the mean risk of baseline HUS (without sampling) during periods of low excretion is 600 times lower than the estimated risk level during periods of high excretion.

Table 12. Probability of detection of contaminated scrums and percentage reduction in the associated average HUS risk by sampling plan and by contamination scenario

Scenario Plan #2	Map 1				Map 3	
	Probability detection	**% reduction risk**	**Probability detection**	**% reduction risk**	**Probability detection**	**% reduction risk**
S1	2.68	92.57	5.37	98.15	4.60	95.93
S2	0.05	65.06	0.13	80.35	0.11	76.33
S3	2.96	95.65	5.63	98.72	5.13	97.48
S4	0.06	85.25	0.12	90.76	0.12	93.70
S5	3.14	97.05	5.80	98.76	5.48	98.43
S6	0.06	91.20	0.12	83.98	0.12	86.94
S7	1.84	93.71	3.68	98.25	3.19	94.35
S8	0.04	73.22	0.09	90.48	0.07	70.65
S9	2.02	96.89	3.89	98.87	3.56	98.11
S10	0.04	65.21	0.09	79.88	0.08	92.86
S11	2.16	97.69	3.99	98.39	3.83	98.41
S12	**0.04**	**73.48**	**0.08**	**89.97**	**0.08**	**89.38**

For all the contamination scenarios, the most effective sampling plan in terms of probability of detection is plan n°2 (n=3, c=0, absence in 25g) which allows during periods of high excretion to detect 4 % to 6% of scrums and thus reduce the average risk of HUS per serving by more than 98%.

The proportion of scrums detected positive with plan n°1 (n=1, absence in 25 g) applied to 100% of the scrums is between 0.04% and 0.06% in periods of low excretion and between 2% and 3% in periods of high excretion. The proportion of frays detected positive during periods of low excretion by this sampling plan is close to that observed with the results of self-checks by professionals: 0.09% of presumptive results (including 50% confirmed) for VHR and 0. 13% presumptive results for VHS.

It should be noted that the self-checks carried out in the VHR sector are not systematic (approximately one scrum per week analysed) and that in the vast majority of cases, in VHR as in VHS, only the O157:H7 serotype is sought. Moreover, the model assumes a perfect analytical method (sensitivity of 100%) which is not the case for the methods used in the laboratory. These elements could explain the difference observed between the simulated detection rates and the observed self-testing results. Furthermore, the simulated values are consistent with the results of the DGAL monitoring plans (VHS in 2013: 0.4% [0.05-1.45]; VHR in 2015 0.3% [0.01 - 1 .9]) when taking into account the confidence interval and an equal distribution of periods of high and low excretion.

Plan n°2, based on n=3, is the most effective in terms of risk reduction, in particular when the scrum is not homogeneous (b=0.5) and the contamination low (S2, S8). The estimated risk reduction with plan n°2 is between 80% and 90% in periods of low excretion and greater than 98% in periods of high excretion. Plans n°1 and n°3, based on n=1, are more sensitive to the value of *b* and less efficient when the contamination is heterogeneous and weak.

Overall, plan n°1 allows a risk reduction of between 65% and 85% in periods of low excretion and greater than 92% in periods of high excretion.
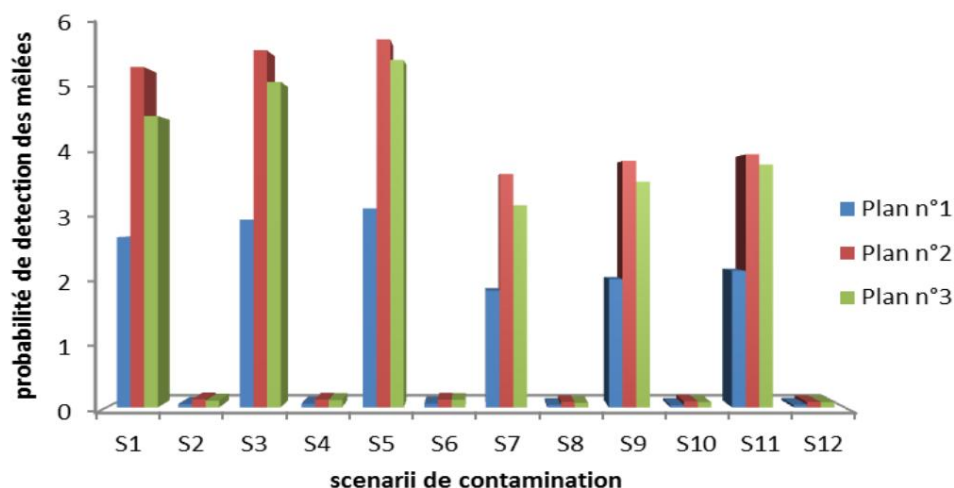
Figure 9. Probability of detection of scrums for the three sampling plans tested and the 12 contamination scenarios
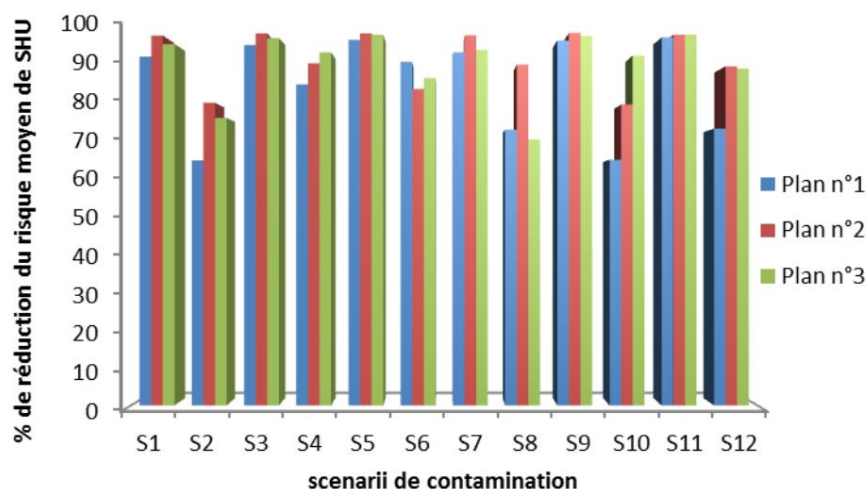

Figure 10. Percentage reduction in the average risk of HUS per 100g serving for the three sampling plans tested and the 12 contamination scenarios

According to the model, the expected proportion of detected frays ranges from 2% to 6% in periods of high shedding and from 0.04% to 0.13% in periods of low shedding. The comparison of the sampling plans shows a reduction in risk following the application of the 3 plans considered. This risk reduction is greater during periods of high excretion (92-98%) compared to periods of low excretion (57-87%).

Given the uncertainties of the model, the differences in reductions observed between the 3 plans are not significant. During periods of high excretion, the "n=1, m= absence in 25 g" plan would, if applied to all the scrums produced, reduce the risk of HUS by 10 per ground beef.

ÿ **Choice of sampling plans with respect to a performance objective and the level of consumer protection**

From the risk assessment simulation model, the average risk of HUS linked to the consumption of 100 g minced steaks is estimated according to different theoretical levels of contamination of the melee. For this, the dose-response relationship integrates the quantities consumed and the cooking habits according to the age of the consumers. The linear relationship between the log10 of the risk and that of the level of contamination of the frays in cfu/100 g is represented in figure 11.
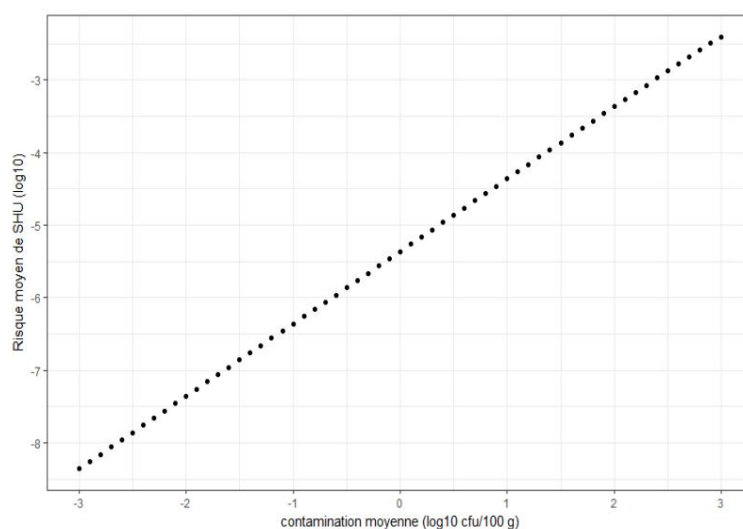

Figure 11. Average risk of HUS according to the level of contamination of the scrums (cfu/100g) (decimal logarithmic scales)

This relationship makes it possible to determine the contamination not to be exceeded in the finished product to achieve a targeted level of protection or level of risk not to be exceeded. The level of risk here is similar to the appropriate level of protection (ALOP). The level of contamination not to be exceeded in the finished product to respect the ALOP is a performance objective (PO).

By way of example, in order not to expose consumers of ground beef to a risk of more than one case per 1 million servings (i.e. log10R = -6), the performance objective to be met is 2 x 10-3 cfu /g (i.e. log10ufc/100g=-0.7) (Figure 11).

The number of 25 g samples to be analyzed to have a 95% probability of detecting scrums whose contamination exceeds the performance objective is calculated for ALOPs between 10-6 and 10-3 . This number of samples (n) depends on the heterogeneity of the distribution of the intra-mixed contamination described by the coefficient b. The number of samples to be analyzed according to the risk threshold (ALOP) not to be exceeded and b *is* presented in table 13. For example, to respect a risk level of 1 case per million servings of 100 g and therefore respect a performance objective of 2 x 10-3 cfu/g, for b=1, 50 samples of 25 g are necessary.

This relationship between the ALOP, the performance objective and the sampling plan allowing the objective to be met also makes it possible to determine the maximum level of contamination that can be detected with a probability of 95% by a sampling plan and assess the level of consumer protection linked to this plan.

Thus, a sampling plan based on the systematic analysis of scrums with n=1, absence in 25 g, for bÿ1, makes it possible to detect 95% of scrums whose contamination is greater than or equal to 0.8 cfu /g.
The estimated level of protection associated with this situation is 1 case/3000 servings.

With n=3, c=0, m=absence in 25 g, for bÿ1, the plan makes it possible to detect 95% of frays contaminated at a level greater than or equal to 0.08 cfu/g. The estimated level of protection associated with this situation is 1 case/ 30,000 servings. Plan n=1, absence in 75 g of equivalent performances.

Table 13. Number of 25 g samples to be analyzed to detect with a probability of 95% the scrums that can cause cases, for three values of b, according to the level of protection (ALOP) desired

b	0.5		1	1.5
ALOP1 (log10)	-3 -3.5 -4 -4.5 -5 -6 -3 -3.5 -4 -4	.5 -5 -6 -3 -3.5 -4 -4.5 -5 -6		
No (25g)	2 2 3 4 8 51 1	1 2 3 7 50 1		1 2 3 6 49

[1]. For example, an ALOP on a log10 scale of -3 corresponds to 1 case per 1000 servings

According to the model, a sampling plan based on the systematic analysis of scrums with n=1, absence in 25 g, for bÿ1, makes it possible to detect 95% of scrums whose contamination is greater than or equal to 1 cfu/g. The estimated level of protection associated with this situation is 1 case/3000 servings.

With n=3, absence in 25 g, for bÿ1, the plan makes it possible to detect 95% of frays contaminated at a level greater than or equal to 0.1 cfu/g. The estimated level of protection associated with this situation is 1 case/ 30,000 servings. The plan n=1, absence in 75g has equivalent performances.

These risk level results are conditional on the cooking practices considered in this model.
A cooking method more suitable for young children would significantly reduce the risk (ANSES 2015).

3.4.6. Assessment of the levels of contamination of surrounding scrums

The scrums detected positive with a simple sampling (n=1) give rise to reinforced investigations (n=30) on the surrounding scrums. If a systematic sampling of the scrums is carried out (n=1), the question arises of the relevance of carrying out a reinforced sampling on the scrums surrounding each positive scrum and in which EHEC were not detected.

In order to assess the level of risk associated with these framing scrums, a modeling of the process of making scrums from several units of HPV is proposed. This process, although simplified, is based on the data collected in the responses to the professionals' hearing questionnaires.

It is therefore assumed that scrums are made from several units of HPV. In practice, these HPV units are made up of pieces with different fat content.
Several refrigerated and frozen HPV units can also be combined in the same melee.
It is also assumed that a quantity of matter has passed from one scrum to its next (1/1000 of the mass of the scrum). For the

purposes of evaluating the status of the surrounding scrums, the following situation is retained: each scrum contains three types of HPV of a different nature (refrigerated "fat" RG, refrigerated "lean" RM, and frozen C) (Figure 12). Each scrum therefore contains between 3 and 6 different HPV units (Figure 12).

50,000 scrums produced according to this scenario were simulated from HPV units for the period of highest prevalence in cattle. The contamination levels of the flanking scrums of each scrum detected positive during the systematic control were compared to the contamination levels of the scrums distant from the positive scrums (at least 5 prior or posterior scrums).
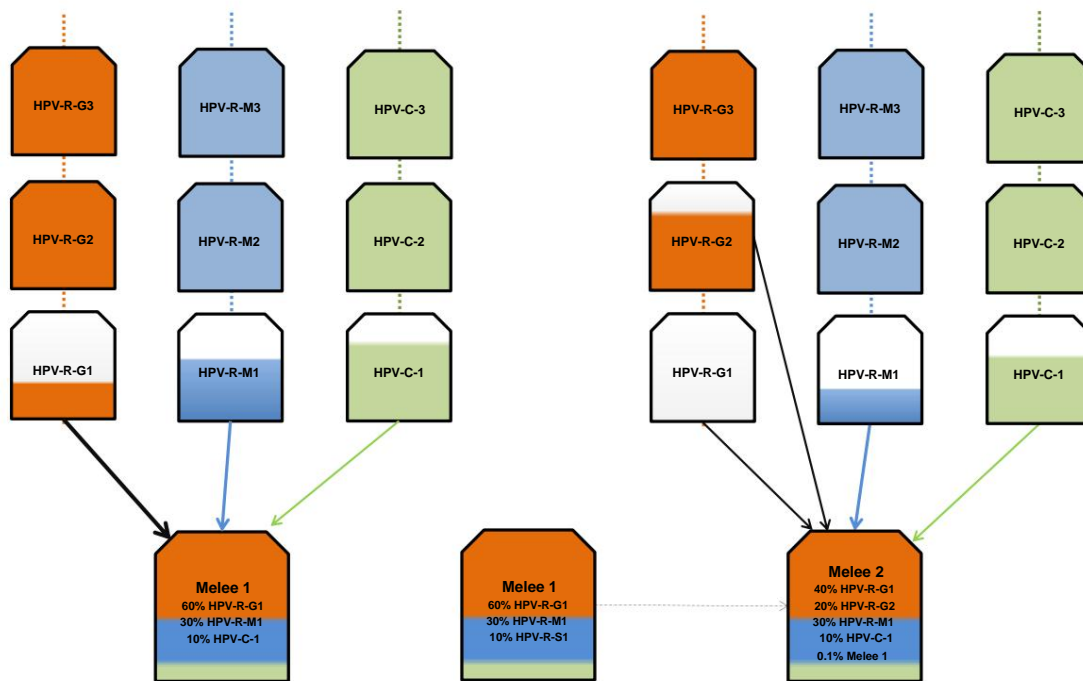
Figure 12. Description of the composition of scrums from 3 types of HPV (refrigerated "fat" RG, "lean" refrigerated RM and frozen C).

The chosen manufacturing scenario involves a mixture of several HPVs, the corollary is that one HPV unit enters into the composition of several scrums. This last point leads to a dependency of contamination levels between scrums. Figure 13 illustrates this link.
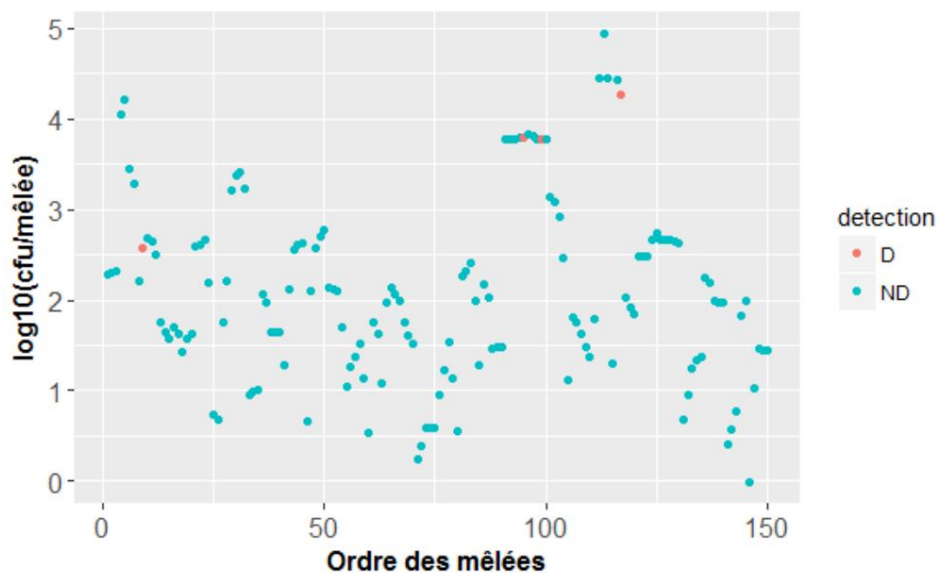

Figure 13. Contamination levels of 150 successive scrums (of 1000 kg) made from three types of HPV.
Red dots indicate positive detection of EHEC in systematic scrum sampling (n=1, m=25 g).

It can be seen that the heavily contaminated scrums are associated in pairs or in longer series. The first correspond to a contribution of contamination by a batch of "fat" or "lean" refrigerated HPVs which generally enter into the composition of two scrums (taking into account their relative share, the size of the HPVs and the scrums). The longer series (for example scrums 90 to 100 on the

figure 13) correspond to an intake by HPV-C. This HPV is included in the composition of ten scrums on average.

The scenario results in 2.7% of positive detected frays in the case of systematic sampling (period of highest excretion). It should be noted that the detection probabilities of the most heavily contaminated melee remain relatively low. Figure 14 illustrates this point. For these first 150 scrums, the detection probabilities do not exceed 0.65. Within this range of probabilities, two scrums with equivalent levels of contamination have a one in two chance of being detected.
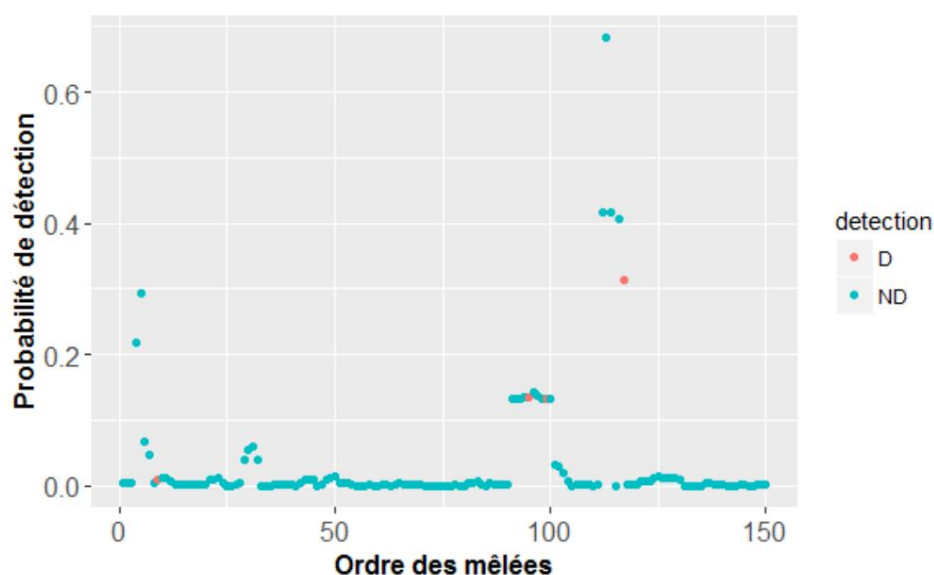
Figure 14. Probability of detection of 150 successive scrums (of 1000 kg) made from three types of HPV.
Red dots indicate positive detection of EHEC in systematic scrum sampling (n=1, m=25g).

The cumulative distribution of the number of EHECs in positive scrums is shown in Figure 15.
Given the manufacturing process in the scenario tested, the contamination levels of the directly surrounding frays are close to the frays detected positive within the framework of systematic sampling n=1, m=25 g. The levels of contamination of these flanking scrums are clearly distinguished from more distant scrums (previous or following by at least 5 scrums), for which the median contamination is 100 times lower.
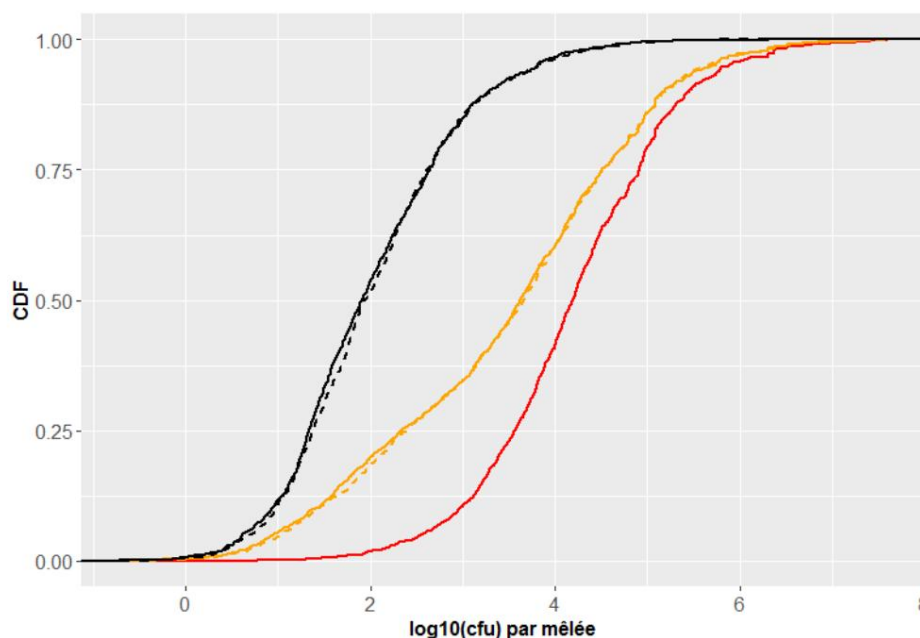
Figure 15. Cumulative distributions (CDF) of contamination levels of different scrums. Red= distribution for positive scrums under systematic sampling. Orange= distribution for surrounding scrums (directly preceding --, or following -). Black = distribution of scrums at least 5 away from positive scrums within the framework of systematic sampling.

Reinforced sampling (n=30) on the directly surrounding scrums (m-1 and m+1) shows that the probability of detection in these scrums is 66%. The results obtained by process modeling are similar to the data collected from professionals.

The sampling of surrounding scrums is therefore important to maintain for risk control.
Sampling of these surrounding scrums can only be ruled out if the supervising scrums do not share the same HPV batches and a validated cleaning-disinfection procedure guarantees the absence of cross-contamination between the scrums.

3.4.7. Effectiveness of a prevention strategy including controls on materials raw

The Agency's opinion of 6 May 2014 concludes that the application of a microbiological criterion (n=1, absence in 25g) on mixed foods is more effective than that proposed for HPVs (n=4, c= 0, m= absence in 75g). To achieve levels of risk reduction comparable to those estimated using a "Mixed" criterion, HPV analysis should involve a very large number of samples, comparable to what is recommended in the United States (60 samples of 6 .25g collected per HPV lot) (FSIS 2014).

The new knowledge acquired since 2014 is not such as to modify the conclusion of the opinion of May 6, 2014. The information collected shows a very great variability in the practices of the operators and does not make it possible to propose a protocol for the analysis of raw materials which would be applicable to all situations. In any case, an integrated approach to prevention and control of EHEC throughout the food chain (including hygiene measures and control of raw materials) should contribute to reducing the risk of HUS.

3.5. Conclusions of the CES BIORISK

The CES BIORISK issues the following conclusions in response to the questions of the referral:

ÿ *Review of the definition of potentially highly pathogenic STEC strains in Afssa's opinion of 27 May 2010*

Any strain *of E. coli* isolated from humans or food should be considered as EHEC
if it has the *stx1* and/or *stx2* and *eae* virulence genes or other gene(s) encoding an adhesion system in the human digestive tract.

Certain EHEC serotypes are more frequently associated with severe disease (HUS). The approach adopted is to update the classification proposed by EFSA's BIOHAZ panel in 2013, based on French and European epidemiological data (2011-2015). The CES BIORISK thus proposes including serogroup O80, the third serogroup isolated in the case of HUS in France, in group I of high-risk EHEC (see Table 6);

The CES BIORISK stresses, however, that the source of the O80:H2 serotype should be identified before any introduction of this serotype in the list of EHEC strains to be searched for in the context of self-checks. Similarly, in view of the data available on contamination of the bovine reservoir, testing for serotype O104:H4 in products of bovine origin does not seem relevant.

The list of five EHEC serotypes to look for in food as a priority remains valid: O157:H7, O26:H11, O103:H2, O145:H28, and O111:H8. For this research, it is recommended to use the most sensitive analytical methods.

This list may be revised according to new epidemiological data, and in particular the results of ongoing investigations concerning the source of serotype O80:H2.

ÿ *Evaluation of the pathogenicity of stx-eae+ strains belonging to one of the 5 serotypes adults and isolated from a culture broth positive for stx*

The implementation of the method for the detection of EHEC in foods during official analyzes sometimes leads to the isolation of *stx-eae+* strains from an enrichment broth of a food in which an *stx* gene has been detected by PCR. Such *stx-eae+* strains are EPEC6 *(E. coli*
enteropathogens). These strains, presenting all the genetic characteristics of EHEC except the *stx genes,* could be the witness of the presence of an EHEC in the food, from which they would derive.
after the loss of their Stx prophage, either in the food or during their isolation.

Recent work shows that the induction of Stx prophages causing the production of phage particles and the lysis of part of the EHEC population is possible *in vitro* in enrichment broths and in matrices. food. However, the transformation of EHEC into EPEC during this induction phenomenon would be a rare event which remains poorly understood at present.
The acquisition of an Stx prophage by an *E. coli* (which would lead to the obtaining of an EHEC) also seems to constitute a rare event or one which does not result in the stable maintenance of the phage genome in the bacterial chromosome.

Without confirmation by strain isolation, obtaining a positive broth for *stx* and one of the intimin *(eae)* types of the 5 main EHEC serotypes is a sign of the potential presence of EHEC. If the isolated strain is *stx-,* it cannot be considered as an EHEC. Nevertheless, the interpretation of the results must take into account the epidemiological context in which the sample was taken as well as the sensitivity and specificity of the analytical methods used.

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[6] These strains were called "AEEC" in ANSES Opinion No. 2010-SA-0031.

ÿ *Review of available data on STEC concentrations in food and water at the origin of epidemics*

Meat preparation practices, the quantity of food ingested, the size of the contaminated batches, the sensitivity of the people exposed are all factors that contribute (along with the levels of contamination) to the occurrence of cases of EHEC infection associated with a production batch.

Recent data do not make it possible to call into question or improve the dose-response relationship which best takes into account the population of interest in this referral, ie children under 15 years of age.

There is no threshold concentration below which there would never be an epidemic. For a given sensitive population (for example children under 15) whose annual consumption would be known as well as the preparation practices of the foods considered and therefore the exposure to the hazard, it is nevertheless possible to estimate:

- the annual number of cases and its uncertainty,

- if an acceptable risk (or appropriate level of health protection – Appropriate Level of Protection/ALOP) is indicated by the risk manager, a dose such that the probability of not respecting this acceptable risk does not exceed 1% or any other fixed value by the risk manager, and the uncertainty around that probability.

The expertise carried out therefore focused on the prevention of HUS cases in their entirety (sporadic cases and epidemics).

ÿ *Modeling the contamination of raw materials and minced meat mixes and evaluating the impact of management measures on reducing the risk of HUS*

The purpose of the modeling carried out was to estimate and compare the effectiveness of different sampling plans and proposed management measures on reducing the probability of occurrence of cases of HUS linked to the consumption of minced beef steaks. The model uses the prevalence of the five major serotypes for risk assessment. _____ _____

• **Effectiveness of proposed sampling plans and impact on HUS risk reduction** _____

According to the model, the expected proportion of detected frays ranges from 2% to 6% in periods of high shedding and from 0.04% to 0.13% in periods of low shedding. The comparison of the sampling plans shows a reduction in risk following the application of the 3 plans considered. This risk reduction is greater during periods of high excretion (92-98%) compared to periods of low excretion (57-87%).

Given the uncertainties of the model, the differences in reductions observed between the 3 plans are not significant. During periods of high excretion, the "n=1, m= absence of 5 serotypes in 25 g" plan would, if applied to all the scrums produced, allow the risk of HUS to be divided by 10 per ground beef.

According to the model, a sampling plan based on the systematic analysis of scrums with n=1, absence of the 5 serotypes in 25 g, for bÿ1, makes it possible to detect 95% of scrums whose contamination is greater than or equal to at 1 cfu/g. The plan n=3, absence in 25 g, for bÿ1, makes it possible to detect 95% of frays contaminated at a level greater than or equal to 0.1 cfu/g. The plan n=1, absence in 75g has equivalent performances.

To achieve the performance levels calculated in this opinion, the microbiological criterion must include the 5 major serotypes and be applied to all scrums. According to the information provided by professionals, the vast majority of the analyzes carried out relate only to the O157:H7 serotype.

• **Evaluation of the levels of contamination of the supervising scrums** _____

The modeling carried out shows that the heavily contaminated frays are associated in pairs or in longer series. Reinforced sampling (n=30) on directly surrounding scrums (m-1 and m+1)

shows that the probability of detection in these melee is 66%. These results obtained by process modeling are similar to the data collected from professionals.

The sampling of surrounding scrums is therefore important to maintain for risk control.
Sampling of these surrounding scrums can only be ruled out if the supervising scrums do not share the same HPV batches and a validated cleaning-disinfection procedure guarantees the absence of cross-contamination between the scrums.

• Effectiveness of a prevention strategy including controls on raw materials

The new knowledge acquired since 2014 is not such as to modify ANSES's opinion of 6 May 2014, which concluded that a microbiological criterion applied to mixed foods rather than to HPVs was more effective. To achieve levels of risk reduction comparable to those estimated using a "Mixed" criterion, HPV analysis should involve a very large number of samples, comparable to what is recommended in the United States (60 samples of 6 .25g collected per batch of HPV) (FSIS 2014).

In any case, an integrated approach to prevention and control of EHEC throughout the food chain (including hygiene measures and control of raw materials) should contribute to reducing the risk of HUS. Finally, a method of cooking minced steaks more suitable for young children would significantly reduce the risk (ANSES 2015).

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The National Agency for Food, Environmental and Occupational Health Safety endorses the conclusions of the CES BIORISK.

Dr Roger Genet

KEY WORDS

Enterohaemorrhagic *Escherichia coli* (EHEC); Shiga toxin-producing *E. coli* (STEC); sampling plan; minced beef; Quantitative risk assessment.

Enterohemorrhagic E. coli (EHEC); shigatoxin-producing E. coli (STEC); Sampling plan; ground bovine meat; Quantitative risk assessment.

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APPENDIX 1: PRESENTATION OF SPEAKERS

PREAMBLE : Experts who are members of specialized expert committees, working groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae,* and do not represent their organization to which they belong.

REPORTERS

Mr Frederic AUVRAY - ANSES, Food Safety Laboratory. Molecular characterization and detection of pathogenic *E. coli*

Mr. Olivier CERF – Emeritus Professor. National Veterinary School of Alfort. Microbiological risk assessment, food microbiology

Mr Laurent GUILLIER – ANSES, Food Safety Laboratory. Modelling, quantitative risk assessment, food microbiology

Ms Patricia MARIANI - Laboratory associated with the CNR *E. coli, Shigella* and *Salmonella,* CHU Robert Debré. Clinical infectiology, *E. coli*

Mr Eric OSWALD – Toulouse University Hospital. Clinical infectiology, microbial ecology, *E. coli*

SPECIALIZED EXPERT COMMITTEE

ÿ **CES "Assessment of the biological risks of food" (BIORISK)**

President

Ms Isabelle VILLENA – CHU Reims. Parasitology, infectiology

Members

Mr Jean-Christophe AUGUSTIN – National Veterinary School of Alfort. Modelling, quantitative risk assessment, food microbiology

Ms Anne BRISABOIS – ANSES, Food Safety Laboratory. Food microbiology, microbial ecology, analytical methods

Mr. Olivier CERF – Emeritus Professor. National Veterinary School of Alfort. Microbiological risk assessment, food microbiology

Mr. Pierre COLIN – Emeritus Professor. University of Western Brittany. Food hygiene and microbiology (meat and meat products – poultry)

Mr. Philippe DANTIGNY – AgroSup Dijon. Mycology, decontamination processes, microbial ecology

Ms. Florence DUBOIS-BRISSONNET – AgroParisTech. Food microbiology, stress adaptation mechanisms, biofilms, surface and process hygiene

Mr. Michel FEDERIGHI-ONIRIS, Nantes - Food hygiene and microbiology (meat and meat products), decontamination processes

Mr. Benoit FOLIGNE – Faculty of Pharmacy of Lille. Intestinal microbiota, food ecosystem/microbiota interaction

Ms Florence FORGET-RICHARD – INRA. Mycotoxins, filamentous fungi, biochemistry, cereal sectors

Mr. Philippe FRAVALO – University of Montreal. Food hygiene and microbiology (meat and meat products)

Mr. Pascal GARRY – Ifremer, Nantes. Food hygiene and microbiology (meat and meat products, shellfish)

Mr. Michel GAUTIER – West Agrocampus. Food microbiology, molecular biology, genetic engineering

Mr Laurent GUILLIER – ANSES, Food Safety Laboratory. Modelling, quantitative risk assessment, food microbiology

Ms Nathalie JOURDAN-DA SILVA – Public Health France. Epidemiology of enteric diseases and zoonoses

Mr. Alexandre LECLERCQ – Pasteur Institute. Food microbiology *(Listeria monocytogenes,* enteropathogenic *Yersinia),* phenotypic and molecular methods

Mr. Simon LE HELLO – Pasteur Institute. *Salmonella,* epidemiology, phenotypic and molecular methods

Mr Eric OSWALD – Toulouse University Hospital. Clinical infectiology, Microbial ecology, *E. coli*

Ms Nicole PAVIO – ANSES, Maisons-Alfort Animal Health Laboratory. Virology Ms Sabine

SCHORR-GALINDO – Montpellier 2 University. Mycology, microbial ecology

Ms. Muriel THOMAS – INRA. Gut microbiota, probiotics

ANSES PARTICIPATION

Scientific coordination

Ms Pauline KOOH – Scientific and Technical Project Manager – Food Risk Assessment Unit (UERALIM) – Risk Assessment Department

Ms Nathalie ARNICH - Deputy Head of Unit - UERALIM - Risk Assessment Department

Scientific contribution

Ms Frédérique AAUDIAT-PERRIN – Scientific and technical project manager – UERALIM – Risk Assessment Directorate

Mr. Moez SANAA - Head of Unit - UERALIM – Risk Assessment Directorate

Administrative secretariat

Ms Angélique LAURENT – ANSES – Risk Assessment Department

HEARING OF EXTERNAL PERSONALITIES

Mrs Clémence Bièche-Terrier - Institute of Livestock

Ms Estelle LOUKIADIS - National Reference Laboratory (NRL) *E. coli* including *E. coli* shigatoxin producers

Mrs. Fabienne NIGER - National Federation of Industry and Wholesale Trade of Meats

Mrs. Nathalie VEAUCLIN - Culture Meats

APPENDIX 2: RESULTS OF **EHEC CONTAMINATION MONITORING PLANS IN FOOD IN FRANCE**

- **Prevalence of contamination by top 5 EHEC strains of beef analyzed in France during monitoring plans from 2006 to 2015 (Loukiadis** *et al.* 2012, Loukiadis and Mazuy-Cruchaudet 2013, 2014, Loukiadis, Mazuy-Cruchaudet , *et al.* 2017)

Year	2006	2007	2008	2009	2010	2011	2012	2013	2013	2015
Type of products	VHS	VHR	Minerals	VHR	VHR	VHS	VHS	Ore VHS		ORV
Food chain stage	P	P	P	D	D	P	P	P	P	D
Number positive/number tested	0/796	11/3605	10/992	2/1557	5/2476	9/1878	7/1923	6/495	2/496	1/296
Prevalence and 95% confidence interval	0 [0-0.4]	0.3 [0.2-0.6]	1 [0.5-1.9]	0.1 [0-0.5]	0.2 [0.1-0.5]	0.5 [0.2-0.9]	0.4 [0.1-0.7]	1.2 [0.45 - 2.62]	0.4 [0.05-1.45]	0.3 [0.01-1.9]

P: output; D: Distribution

- **EHEC serotypes isolated from beef as part of surveillance plans for 2006 to 2015**

Year	2006	2007	2008	2009	2010	2011	2012	2013	2013	2015	Total
Type of products	VHS	VHR	Minerals	VHR	VHR	VHS		VHS	Ore VHS	VHR	
Food chain stage	P	P	P	D	D	P	P	P	P	D	
Total number of top 5 EHEC strains of which	0	11	10	2	6	9	7	9	2	1	57
O157:H7		5	2	1	1	3	3	7	1		23
O26:H11		2	5			5	2		1		16
O103:H2		3	3	1	4	1		11		1	14
O145:H28		0	0		1						1
O111:H8		1	0								1

P: Production D: Distribution

- **Prevalence of contamination by EHEC strains of the top 5, raw milk cheeses analyzed in France during monitoring plans between 2005 and 2014**

    (Loukiadis *et al.* 2012, Sergentet, Mazuy-Cruchaudet, and Ruez 2015)

Year	2005	2007	2009	2014
Type of products	Fresh goat's milk cheese believed	Raw cow, sheep and goat milk cheeses	Raw cow, sheep and goat milk cheeses	Raw milk cheeses
Number positive/number tested	0/87	0/392	17/1911	2/1045
Prevalence and 95% confidence interval	0 [0-0.4]	0 [0-0.9]	0.9 0.6-1.4]	0.19 [0.02-0.6]

- **EHEC serotypes isolated from raw milk cheeses as part of monitoring plans between 2005 and 2014**

Year	2005	2007	2009	2014
Type of products investigated	Fresh goat cheese made from raw milk	Raw cow, sheep and goat milk cheeses	Raw cow, sheep and goat milk cheeses	Milk cheeses believed
Total number of strains	0	0	17	2
EHEC top 5 isolated including				
O157:H7			1	
O26:H11			11	2
O103:H2			4	
O145:H28			1	
O111:H8			0	

APPENDIX 3: MODEL PARAMETERS

Settings	Nature	Symbol	Unit	Anses model value 2014	ANSES 2017 model value	Reference
Prevalence of faecal contamination	F	*pf*	%	3.9	*Pflow=1.2* *Pfhigh=3.9*	Bibal *et al.* (2015)
Transfer ratio from faeces to hides	V	*RTfleather*	/	~log-Normal (3.193; 0.106)		Barkocy-Gallagher *et al.* (2003)
EHEC concentration in faeces	V	*See*	log10 CFU/g	~Normal (1.82; 2.43)	*Cflow* ~ Normal (1.59; 1.45) *Cfhigh*~ Normal (2.44;1.98)	Stephens, McAllister, and Stanford (2009)
Transfer fraction of bacteria to the leather	V	*FTf leather*	/	~Log-logistics (-3.77; 2.29; 10.3)		Smith, Fazil, and Lammerding (2013)
Hides to carcass surface transfer ratio	V	*RTcuirC*	/	~Normal (0.237; 0.009)		Barkocy-Gallagher *et al.* (2003)
Bacteria transfer fraction from leather to carcass surface	V	*FTcuirC*	/	~Logistics (-1.97; 0.457)		Smith, Fazil, and Lammerding (2013)
Contaminated surface on the carcass	V	*To*	cm²	~Triangular (log10(30) ; log10(300); log10 (3000))	Equation 1	Cummins *et al.* (2008) Smith, Fazil, and Lammerding (2013)
Proportion of "spot" stains originating from the leather	F	*PSpot*	%	/	80	Cartier (2009)
Proportion of "hand" stains coming from the leather	F	*Main*	%	/	20	Cartier (2009)
Average number of stains per carcass	I	*m_defilement*			1 to 20 (10 by default)	
Area of a spot	F	*Spot*	cm²		7	Cartier (2009)
Area of extensive contamination	F	*Smain*	cm²	/ / /	150	Cartier (2009)
Spot detection/elimination probability	I	*Deposit*	%	/	10 to 90 (50 by default)	
Probability of detection/elimination of a hand stain	I	*tomorrow*	%	/	10 to 90 (80 by default)	
Corrected contaminated surface on the carcass after detection and treatment	V	*OK*	cm²	/	Equation 2	
Viscera Rupture Probability	V	*Ex*	%	Variable between 0.1% and 1%		Cummins *et al.* (2008)
Amount of viscera content soiling the carcass	V	*q*	g	~Uniform (1; 50)	~Uniform (1; 20)	Smith, Fazil, and Lammerding (2013)

Settings	Nature	Symbol	Unit	ANSES 2014 model value	ANSES 2017 model value	Reference
Total carcass area	F	*ASD*	cm²	32,000		Smith, Fazil, and Lammerding (2013)
Number of carcasses per unit of ore	F	*nbc*	/	Scenario 1:5; Scenario 2: 60; Scenario 3: 120	60	
Mass of an ore/melee unit	F	*T*	kg	Scenario 1: 50; Scenario 2: 500; Scenario 3: 1,000	700	
Mass of a piece *j* of a carcass *i*	V	*Mj*	g	~Triangular (50, 500, 1000)		Smith, Fazil, and Lammerding (2013)
Fraction of bacteria found in the pieces intended for the manufacture of minced meat	V	Fvhi		~Uniform (0.75; 0.90)		Smith, Fazil, and Lammerding (2013)
Melee unit heterogeneity coefficient	I	*b*		Homogeneous: 1000; Moderately homogeneous: 1; Heterogeneous: 0.1	0.5-2	Loukiadis, Bièche-Terrier, *et al.* (2017)
Mass of one HPV unit	F/V	*T/MVPH*	kg	Scenario 1: 50 Scenario 2: 500 Scenario 3: 1000	1,000	
Mass of a melee	F/V	*T/MM*	kg		1,000	
Mass of a minced steak	F	*Steak*	g	125g	100g	
Proportion of burgers consumed by children under 15	F	*Pe*		0.16		ANSES (2015)
Probability of development of a HUS knowing the infection	F	*PSHU|Inf*		0.10		Strachan *et al.* (2005)
Parameter of the exponential dose/response relationship	F	*r*		1.13×10^{-3}	equation 12	Perrin *et al.* (2015)
Number of decimal reductions at the end of cooking	V	*DR*		See table 1 review Anses (2014)	See Table 9	Bergis *et al.* (2009)

APPENDIX 4: INFLUENCE OF UNCERTAIN PARAMETERS ON HUS RISK ESTIMATION

Updating the model led, on the one hand, to the estimation of the range of uncertainty surrounding the value of parameter *b* and, on the other hand, to the introduction of three new parameters related to carcass contamination *(mdefilements, Sespot, Week).* At the present time, there is no study making it possible to statistically estimate the values of these three parameters, these have therefore been proposed on the basis of professional data and the opinions of experts. In order to test the influence of these model input parameters *(b, msouillures, Sespot, Semain)* on the model outputs (risk of HUS, probability of detection of contaminated scrums), simulations were carried out for a corresponding scenario at the period of high excretion *(Pfhigh, Cfhigh).* For each uncertain parameter, several values are drawn at random within its range of uncertainty. For each of its values, 100,000 iterations of the model are performed by setting the other uncertain parameters to their default value (Table 14). For each scenario, the average risk of HUS per 100 g serving is estimated for two situations. In the first situation, the reference situation, the minced meat scrums are not analysed. The model therefore considers that all the hamburgers produced are consumed. The estimated risk in this situation is called the reference risk (R0). In the second situation, the minced meat scrums are systematically analyzed (100% of the scrums analyzed) according to the sampling plan n=1, absence in 25 g. The model considers that the minced steaks from the mixes detected positive for the presence of EHEC following the analysis are not consumed (blocked batches). The estimated risk is the residual risk (Rr).

Table 14: Values of uncertain parameters chosen at random and default value

Uncertain parameter	Values	Default value
b	0.01; 0.1; 0.2; 0.5; 1; 2; 10	1
Mstaints	1 to 20 in steps of 1	10
Sespot	10% to 90% in 10% steps	50%
Week	10% to 90% in 10% steps	80%

For each uncertain parameter, the reference risk and the residual risk are estimated and represented according to the value of the uncertain parameter (Figures 16, 18, 20 and 22).

The impact of uncertain parameters on the effectiveness of the sampling plan in terms of probability of detection and on the associated risk reduction are represented in figures 17, 19, 21 and 23.

The value of parameter *b* has little influence on the reference risk level when *b* varies between 0.01 (distribution of heterogeneous contamination) and 10 (distribution of homogeneous contamination). On the other hand, when the frays are analysed, the heterogeneity of the contamination has a significant impact on the efficiency of the sampling and the associated level of risk. Indeed, for b=0.01, the average risk of HUS after analyzes is estimated at 1.0×10^{-5} , i.e. 1 case per 100,000 100 g minced steaks. This risk is lowered to 1.0×10^{-6} for b=0.5, i.e. one case per million servings and to 5.3×10^{-7} , i.e. 1 case for 2 million portions, when b=1 (moderately homogeneous contamination). For values of *b* greater than 1, the influence of the parameter on the average risk level is weaker, the latter being lowered to 3.3×10^{-7} when b=10 (approximately 1 case for 3 million servings) (Figure 16).

The impact of parameter *b* on the risk of HUS after scrum sampling is related to the effectiveness of the plan. Indeed, the more the contamination is evenly distributed within the fray (b>1) the more the probability of detection with the defined sampling plan (n=1, absence in 25g) increases: 0.5% for b =0.1 and about 3% for *b* greater than 1 (Figure 17). Contaminated scrums therefore have a higher probability of being detected when the distribution of contamination is homogeneous, which leads to a reduction in the risk of HUS of more than 95% when b>1 (Figure 17) .
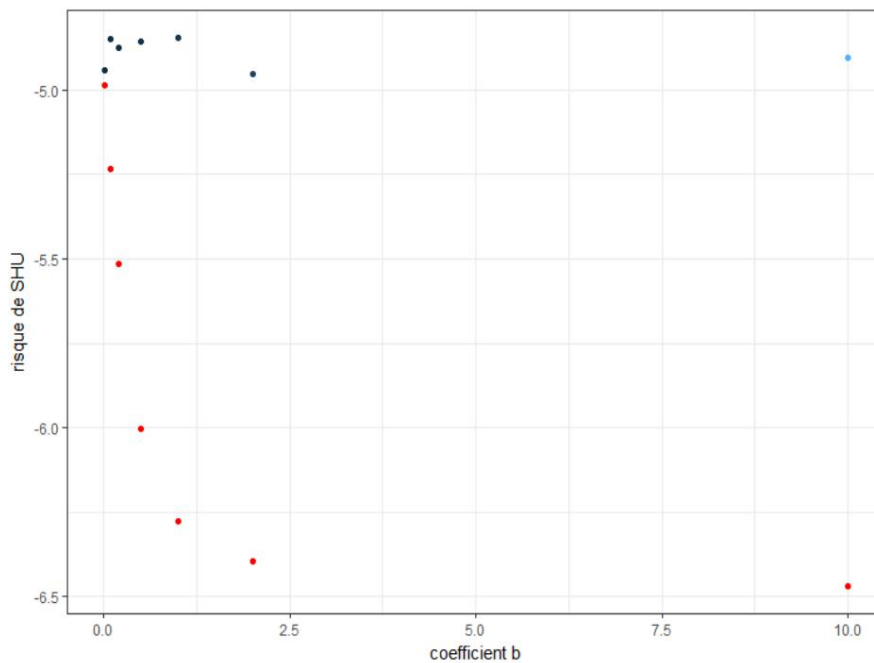
Figure 16: Reference risk (blue dots) and residual risk (red dots) at the log10 scale as a function of the values of the heterogeneity coefficient of the contamination of the frays b.
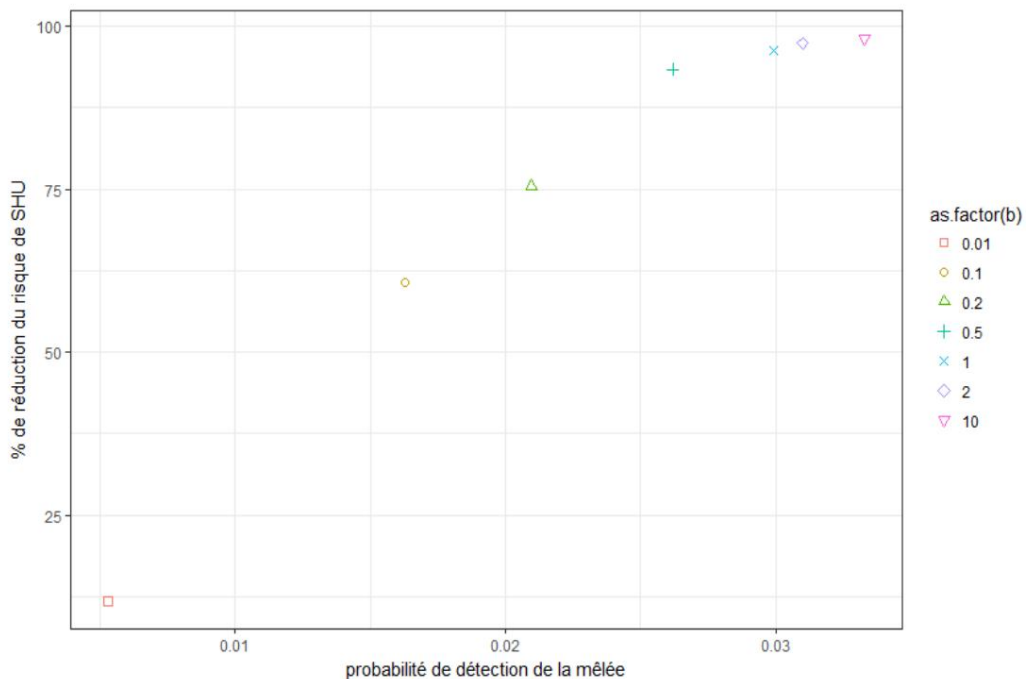

Figure 17: Percentage of risk reduction according to the probability of detection of scrums for the different values of *b*

The average number of stains per carcass has a significant impact on the reference risk and the residual risk, multiplying them by a factor of 5 when the number of stains is increased from 1 to 20 (Figure 18).
For an average number of 10 stains per carcass, the log10 of the reference risk and that of the residual risk are estimated at respectively -4.96 (approximately 1 case per 100,000 servings) and -6.28 (approximately 1 case per 1.9 million servings). The number of stains also slightly influences the efficiency of the sampling: for *mstains=1,* the probability of detecting scrums is 1%; for *mstains=20,* the

probability of detection is 4%. For an average number of stains per carcass of 10, the probability of detection is 3% (Figure 19). The associated risk reduction (between 95% and 98%) is however not directly linked to the number of stains since different reductions are estimated for similar numbers of stains and a similar probability of detection (Figure 19).
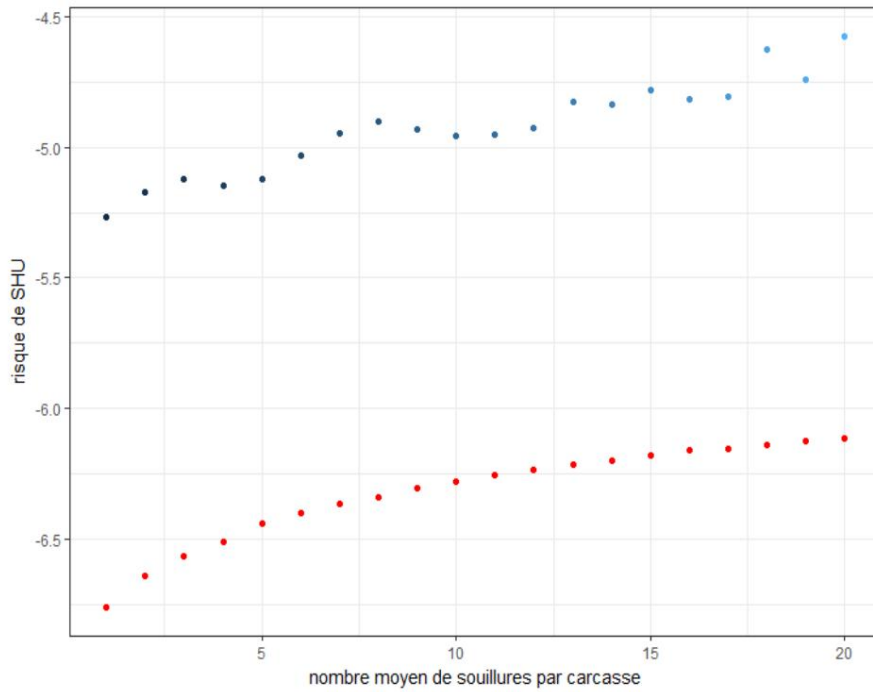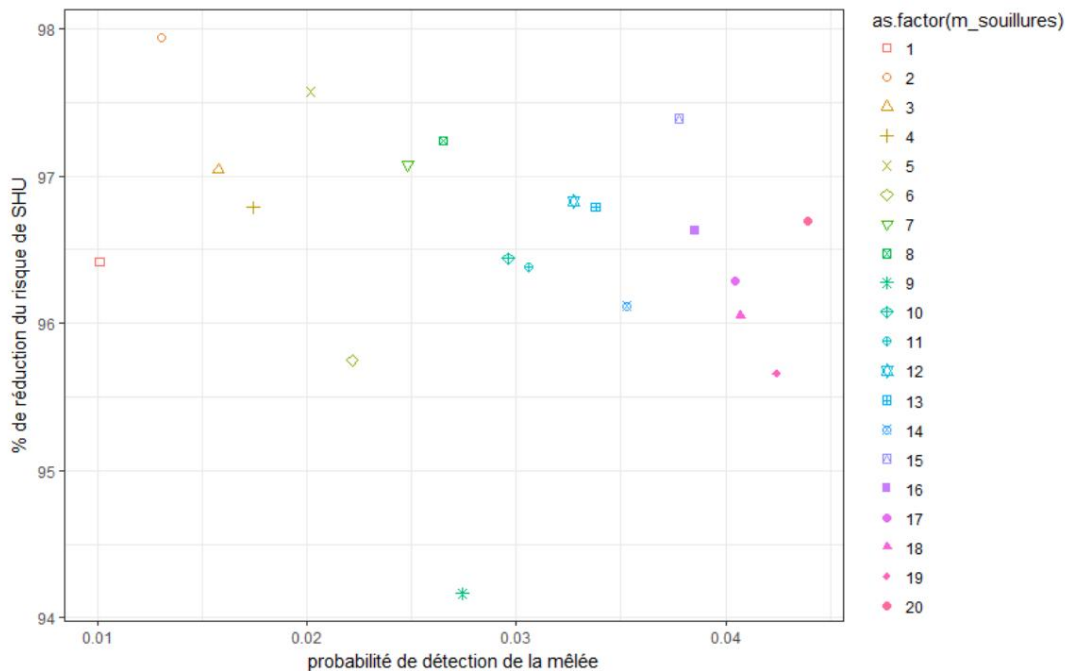

Figure 18: Baseline risk (blue dots) and residual risk (red dots) on a log10 scale as a function of the average number of soilings per carcass.

Figure 19: Percentage of risk reduction according to the probability of detection of scrums for the different values of *mstaining*

The model is not very sensitive to the probability of detection of "spot" stains. Indeed, whatever this probability, between 10% and 90%, the log10 of the reference risk is estimated at -5 and the residual risk, lower, varies between -6.2 and -6.4 (Figure 20) . When the probability of spot detection increases, the sampling efficiency decreases slightly resulting in a smaller reduction in the risk associated with it (Figure 21). For a 50% probability of detection of "spot" soiling, the log10 of the reference risk is estimated at -4.88, i.e. approximately 1 case for 80,000 servings and that of the residual risk at -6.28 (approximately 1 case for 1.8 x 106 servings) i.e. a reduction in the risk associated with sampling of 96%.
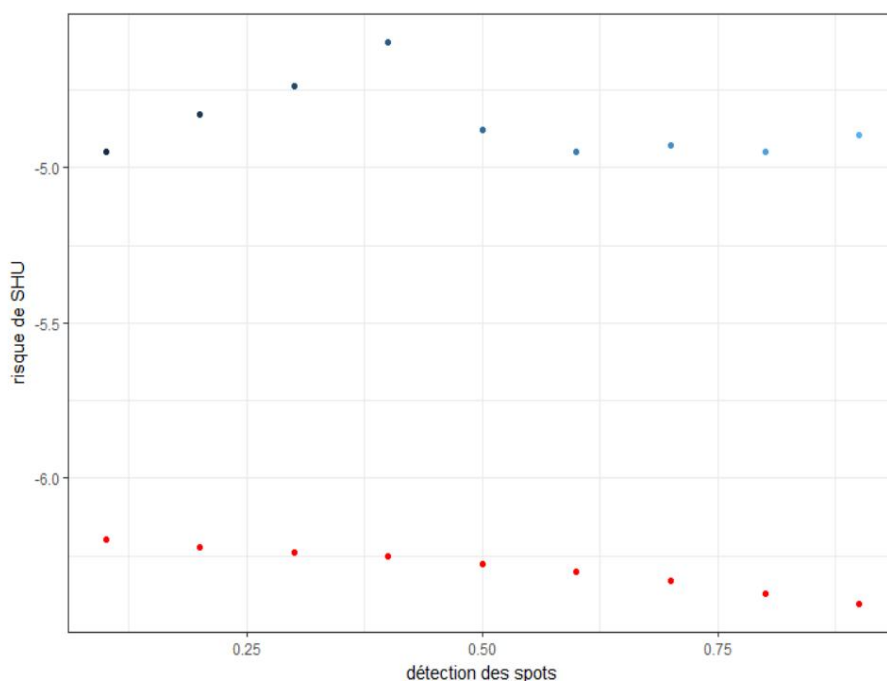
Figure 20: Baseline risk (blue dots) and residual risk (red dots) on the log10 scale according to the probability of detection of "spot" type soiling on the carcasses.
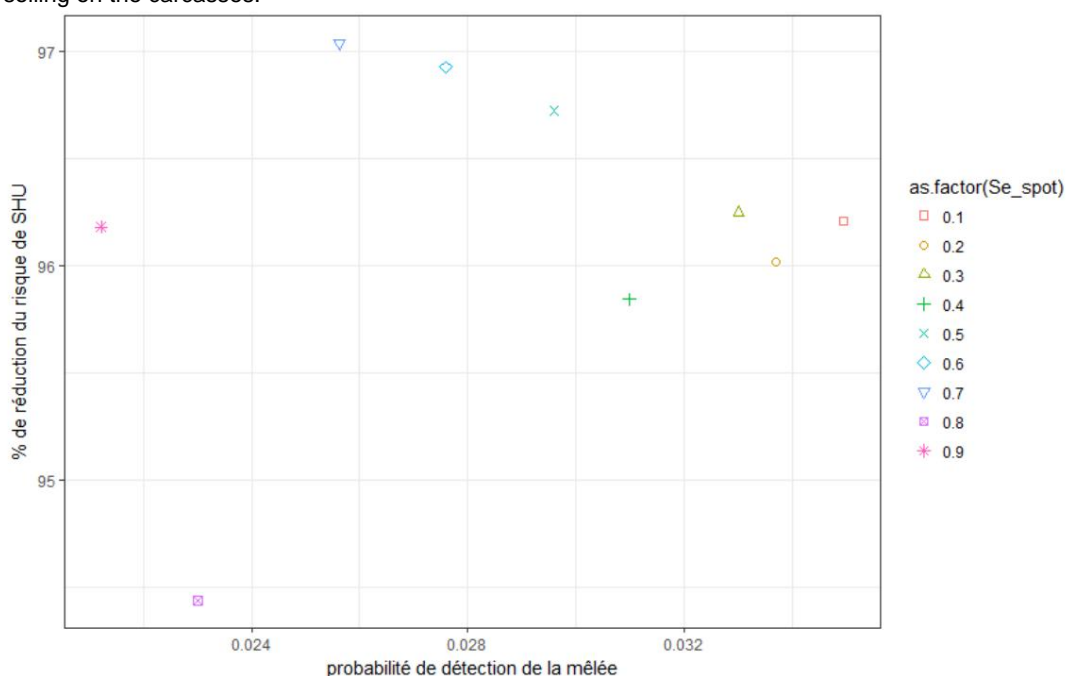
Figure 21: Percentage of risk reduction according to the probability of detection of melee for the different values of probability of detection of "spot" type soiling on carcasses (Sespot)

On the other hand, the probability of detection of "hand" type stains, larger in size but less frequent than spots, influences the outputs of the model. Indeed, the log10 of the reference and residual risks are reduced by approximately 0.5 when the probability of detection increases from 10% to 90%, ie a reduction by a factor of 3 (Figure 22). In the same way as for the spots, the greater the probability of detecting "hand" type stains on the carcasses, the less effective the sampling plan tested is, which leads to a reduction in the risk reduction, which remains understood between 95% and 97% (Figure 23). For a probability of detection of "hand" soiling of 80%, the log10 of the reference risk R0 is estimated at -5, i.e. 1 case for 100,000 servings and that of the residual risk Rr is estimated at -6.3 (approximately 1 case for 2 million servings) or a 95% risk reduction.
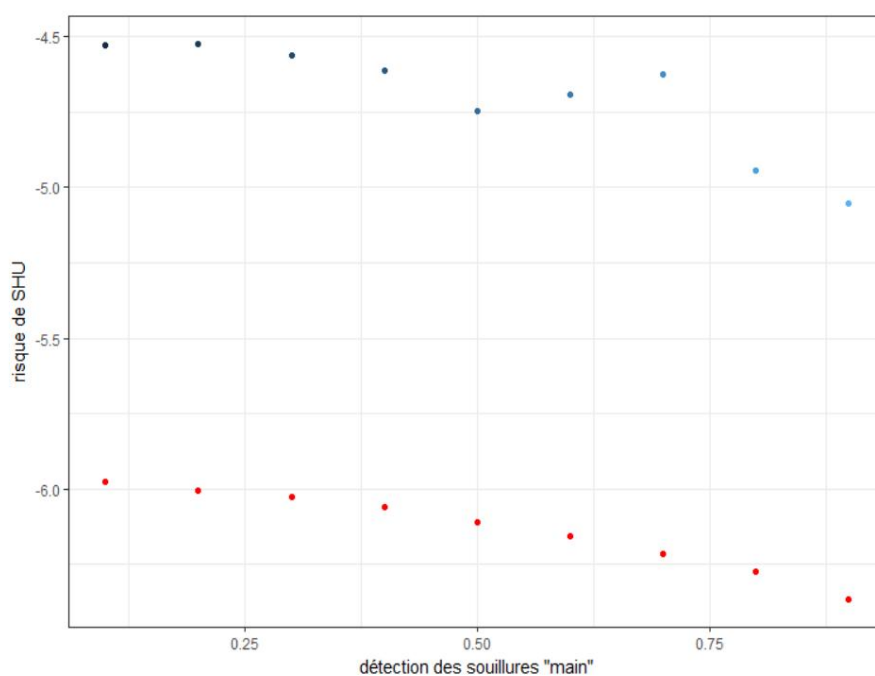

Figure 22: Reference risk (blue dots) and residual risk (red dots) at the log10 scale according to the probability of detection of "hand" type soiling on the carcasses.
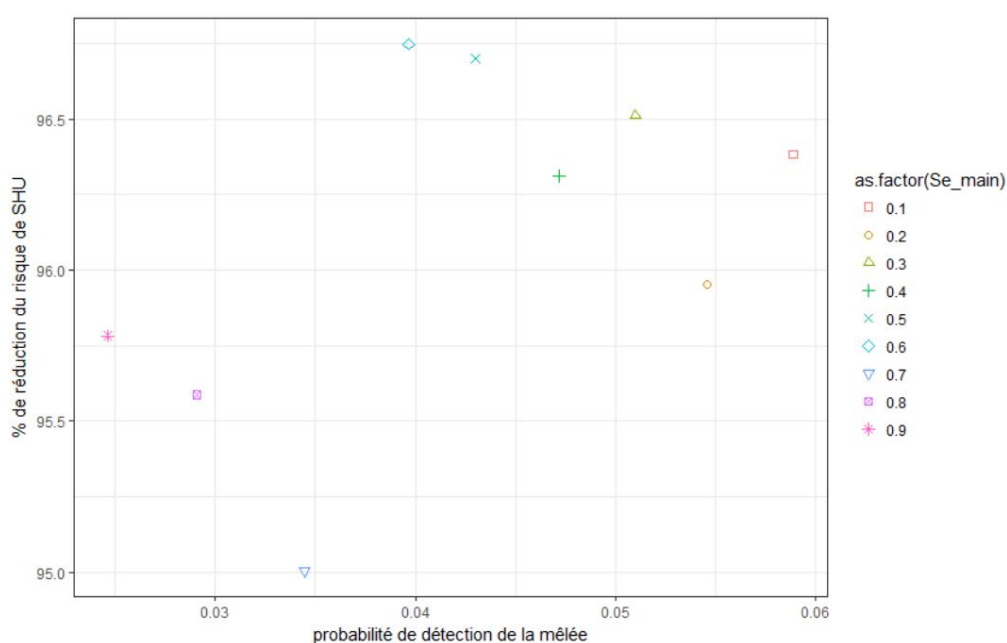

Figure 23: Percentage of risk reduction as a function of the probability of detection of melee for the different values of probability of detection of "hand" type soiling on the carcasses (Week) .