ANSES Opinion
Request No 2015-SA-0114
Related Request Nos 2007-SA-0149, 2007-SA-0160, 2009-SA-0055

The Director General

Maisons-Alfort, 18 December 2015

OPINION[1]
of the French Agency for Food, Environmental and Occupational Health & Safety

on a draft decree pursuant to Article L. 214-1 of the French Consumer Code and concerning the labelling of raw milk intended to be provided for direct consumption by the final consumer

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

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

Its opinions are made public.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 18 December 2015 shall prevail.

On 2 June 2015, ANSES received a formal request from the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) to undertake the following expert appraisal: Request for an opinion on a draft decree pursuant to Article L. 214-1 of the French Consumer Code and concerning the labelling of raw milk intended to be provided for direct consumption by the final consumer.

1. BACKGROUND AND PURPOSE OF THE REQUEST

1.1. Regulatory background

Regulation (EC) No 853/2004 allows an EU Member State to establish national rules limiting the marketing on its territory of raw milk intended for direct human consumption.

The Ministerial Order of 13 July 2012 lays down the conditions for production and placing on the market of raw milk from cattle, small ruminants and domestic solipeds provided for direct consumption by the final consumer. The draft order had been the subject of an AFSSA[2] Opinion issued on 19 June 2009 (Request 2009-SA-0055). The draft decree that is the subject of this current request relates to this order in that it stipulates the mandatory labelling statements for raw milk provided for direct consumption by the final consumer.

1.2. Recommendations of the AFSSA Opinion of 19 June 2009 on labelling statements for raw milk

The AFSSA Opinion of 19 June 2009 stated that the following information should be communicated to the consumer:

"1. *date of milking;*

[1] This Opinion cancels and replaces the Opinion of 5 August 2015 which was sent to the author of the request but not published on the Agency's website.
[2] Became ANSES on 1 July 2010

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2. "use clean containers, made of a food-safe material";

3. "store at a maximum of +4°C and consume before …";

4. " boil before consumption for children under fifteen years of age, pregnant women and people whose immune system is weakened";

5. "cook thoroughly, to at least +70°C, any cakes and pastries prepared with raw milk; heat raw milk to at least +70°C prior to making yoghurt or cheese". "

Lastly, AFSSA recommended establishing a maximum regulatory shelf life for raw milk of three days after milking, applicable to raw milk that is packaged, in bulk, or provided via vending machines.

1.3. The main provisions of the draft decree

The mandatory labelling statements for raw milk contained in Article 4 of the draft decree are as follows:

"The following information is brought to the attention of the final consumer via the label of each individually packaged item, or on an easily-read poster prominently displayed to the final consumer for bulk sales:

a. "the words "store at +4°C maximum";

b. the use-by date in [the conditions laid down by] Article 24 of Regulation (EU) No. 1169/2011 referred to above;

c. the words "boil before consumption for children under five years of age, pregnant women and people whose immune system is weakened";

d. the identification number of the establishment, issued by the prefect;

e. the name and address of the operator;

f. the words "use a clean container", when the milk is provided directly to the final consumer via a liquids vending machine." .

Article 5 states that the use-by date should be no more than three days after the date of the earliest milking operation.

1.4. Recent epidemiological data

The BIOHAZ panel[3] of EFSA[4] issued an Opinion on 13 January 2015 on the public health risks related to the consumption of raw milk. According to this opinion, the main microbiological hazards associated with the consumption of raw milk, within the European Union, are: *Brucella melitensis*, *Campylobacte*r spp., *Mycobacterium bovis*, *Salmonella* spp., Shiga toxin-producing *Escherichia coli* (STEC) and tickborne encephalitis virus (TBEV). According to the epidemiological data, *Campylobacte*r, *Salmonella* and STEC are the most widespread at European level. *Campylobacter* is the primary agent responsible for outbreaks associated with the consumption of raw milk in Europe (responsible for 21 of the 27 outbreaks reported between 2007 and 2013). Because of the lack of data, however, the BIOHAZ Panel was unable to quantify the public health risks associated with the consumption of raw milk in the EU (EFSA BIOHAZ Panel, 2015).

Another study published by the CDC[5] reports 81 outbreaks related to the consumption of unpasteurised milk, between 2007 and 2012, in the United States, of which 77% were caused by *Campylobacter* (Mungai *et al.,* 2015).

1.5. Issues investigated

The draft decree only partially takes into account the AFSSA opinion of 2009, and, in particular, modifies the age below which the boiling of raw milk is recommended (15 years replaced by 5 years). Furthermore,

[3] Panel on Biological Hazards
[4] European Food Safety Authority (EFSA)
[5] Centers for Disease Control and Prevention, U.S. Department of Health & Human Services

particularly in light of the recent data on the risk associated with *Campylobacter,* questions should be asked about the need to extend the recommendation to boil raw milk to all consumers, in order to take this risk into account.

The questions examined are as follows:

- How does sensitivity vary with regard to the main pathogens that can be transmitted by raw milk according to age and the population category?
- In light of the available data, should the recommendation to boil raw milk be directed at one or more vulnerable sub-population(s), or should it be generalised to the whole population?

2. ORGANISATION OF THE EXPERT APPRAISAL

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

The collective expert appraisal was carried out by the Expert Committee (CES) on Assessment of the biological risks in foods (BIORISK) which met on 6 November 2015.

The rapporteurs' initial expert appraisal mainly drew on:

- The French regulations concerning the production and placing on the market of raw milk in France;
- The information available on 23 October 2015 concerning the conditions for production and placing on the market in other countries of the EU (Opinion of EFSA's BIOHAZ panel, communication by the members of EFSA's "Microbiological risk assessment" network in 2013 and 2014[6]);
- French epidemiological data and the dose/response relationships relating to the main pathogens that can be transmitted by raw milk;
- Scientific publications.

ANSES analyses the links of interest declared by the experts prior to their appointment and throughout the work, in order to avoid potential conflicts of interest with regard to the matters dealt with as part of the expert appraisal. The experts’ declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE CES

3.1. Review of the regulatory requirements concerning the production and placing on the market of raw milk in France

Regulation (EC) No 853/2004 of 29 April 2004 lays down specific hygiene rules for food of animal origin, including raw milk, in its Annex III, Section IX, Chapter I "raw milk-primary production". The definition of raw milk is "milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or undergone any treatment that has an equivalent effect". In Chapter I of its Section IX, this text clarifies the health requirements to be met for operators producing or collecting raw milk as to the health of the animals, the organisation and running of the production establishment, hygiene during milking, collection and cooling, and also has provisions concerning staff hygiene.

Compliance with these hygiene and health conditions, when producing raw milk, must lead to satisfaction of the sole microbiological criterion proposed by the European text. The raw milk produced must be analysed at least twice a month in the establishment. This involves comparing a rolling geometric average of bacterial counts at 30°C obtained over a period of two months (at least n=4) with a limit: 10^5 CFU/mL for cow's milk, or $1.5x10^6$ CFU/mL for milk from other species. It is stated that the samples must be taken by random samplingand that their number should be representative of production, but this number is not specified by the text of the Regulation.

The temperature provisions associated with collection and distribution conditions for raw milk are specified in the text and are as follows: the milk must be cooled immediately to a temperature of not more than 8°C in the case of daily collection or, if collection takes place over several days, the maximum temperature is then 6°C.

[6] Network meeting of 19-20 November 2013 and replies to the questionnaire sent by EFSA on 18 April 2014

Regulation (EC) No 853/2004 allows an EU Member State to establish national rules limiting the marketing on its territory of raw milk and raw cream intended for human consumption. In France, the conditions for the collection and distribution of raw milk are minimally those of the Regulation, with supplementary provisions specified in the Ministerial Order of 13 July 2012 relating to the "conditions for production and placing on the market of raw milk from cattle, small ruminants and domestic solipeds provided for direct consumption by the final consumer". The distribution of raw milk is subject to authorisation, which is granted subject to compliance with additional provisions concerning the conditions for collection and distribution.

Raw milk collected over a period of more than 24 h may not be distributed. The maximum holding temperature, before and during distribution, is between 0 and 4°C and not 8°C as indicated in Regulation (EC) No 853/2004.

In addition, the microbiological criteria imposed by the Order go beyond simply counting the cultivable flora at 30°C. They include safety criteria relating to *Salmonella (*absence in 5 samples of 25 mL) and *Listeria monocytogenes* (<100 CFU/mL). The frequency of analysis is not imposed and remains under the operator's responsibility.

An additional hygiene criterion is imposed. This is the enumeration of *E. coli* applicable both for raw cow's milk and for the milk of small ruminants or solidungulates. The analysis of the results of *E. coli* enumeration follows a three-class plan: m=10; M=100; n=5 and c=2.

The limit of the criterion relating to the total plate count at 30°C is lowered to $5x10^4$ CFU/mL, tested under the conditions of the Regulation (twice a month) for raw cow's milk, except if the holding does not have its milk collected; in this case, the frequency is determined under the operator's responsibility. For species other than cows, the limit of the criterion is $5x10^5$ CFU/mL.

Direct sale or sale by vending machine of raw cow's milk is authorised in the great majority of European countries (exceptions: Greece, Spain, Norway, Scotland). There are significant differences between the reported levels of contamination of milk by cultivable flora at 30°C (without specifying the destination, direct sale or not) among representatives of the Member States, with average levels of between 3000 and more than 10^5 CFU/mL. The average is around 4.5 log CFU/mL. Few countries (Denmark, Sweden and France) give the distribution by class of concentration. The vast majority of milk analysed (90% of milk and more), contains counts below 5.10^4 CFU/mL. In France, in 2012 more than 92% of tested milk had counts below 5.10^4 CFU/mL (communication by the members of EFSA's "Microbiological risk assessment" network, 2014).

In conclusion, in France, authorisation to collect and distribute raw milk for direct human consumption is subject to the satisfaction of additional conditions to those of Regulation (EC) No 853/2004. These relate to compliance with microbiological criteria relating to *Listeria monocytogenes* and *Salmonella* (pathogens), as well as *E. coli* (hygiene indicator). In addition, the French Order is more stringent than the European Regulation with regard to the microbiological limit (m) for the l the total plate count at 30°C.

3.2. Available information concerning foodborne outbreaks (FBOs) associated with the consumption of raw milk

There is little feedback on episodes of FBOs associated with the consumption of raw milk in Europe, between 2007 and 2014. The countries reporting episodes related to raw milk only describe them rarely (less than once a year).

In France, seven FBOs related to the consumption of raw milk, including one caused by *Campylobacte*r, were identified by the InVS in the period 2007-2014 (InVS communication, 2 July 2015). The FBO caused by *Campylobacter* occurred in 2010, following the consumption of raw milk by a group of children during a visit to a farm (11 sick children out of 24, presence of *Campylobacter* confirmed in stool cultures). This FBO is not mentioned in the opinion of EFSA's BIOHAZ panel because France considered the level of evidence to be low; indeed, *Campylobacter* was identified only on human samples, and not in the food, so the hypothesis of another source of contamination (contact with farm animals, for example) cannot therefore be ruled out.

Since 2012, Finland has reported three FBOs associated with the consumption of raw milk: 1) an episode of eight cases of haemolytic uremic syndrome (HUS) in children who consumed raw milk purchased at a farm, 2) an episode of 18 cases of campylobacteriosis among schoolchildren following the distribution in class of raw milk brought by a classmate, 3) an outbreak of yersiniosis (55 cases) occurring in 2014 and associated with the consumption of raw milk contaminated by *Yersinia pseudotuberculosis* O:1 (Parn *et al*., 2015).

Two countries that prohibited the sale of raw milk in or before 2010 have reported episodes of FBOs since this implementation. The first of these two episodes dates from 2013 and involved *Campylobacter* (Norway). In the second, dating from 2012, *S.* Enteritidis was mentioned, but without any real evidence of a causal link with the consumption of raw milk (Spain).

Episodes of FBOs associated with raw milk consumed directly appear to be relatively limited in Europe. Several outbreaks have concerned children.

3.3. Sensitivity by age group and category of adult population to the main pathogens that can be transmitted by raw milk

3.3.1. Approach

The hazards taken into consideration in light of the conclusions of the Opinion of EFSA's BIOHAZ panel and the regulatory criteria of the Ministerial Order of 13 July 2012 were: S*almonella*, *Campylobacter*, enterohaemorrhagic *Escherichia coli* (EHEC) and *Listeria monocytogenes.* One possible approach to defining the different population categories to which a recommendation about boiling milk should be addressed could be based on the estimate of the number of patients for each of the potential categories (children under 5 years of age, under 10 years of age, people over the age of 65, etc.). However, the analysis of epidemiological data ("top-down" approach), like the use of the quantitative risk assessment ("bottom-up" approach) is unable to calculate this estimate in the current state of knowledge (due to a lack of data on the fraction of diseases concerned that are attributable to raw milk, the level of consumption, consumption patterns by age group, etc.).

It is however possible to estimate the relative sensitivity of the different population categories to the various hazards and to deduce the attributable risk. One of the ways to measure this attributable risk involves the calculation of the Gini coefficient (Lee, 1997), which quantifies the dispersion of the levels of risk in a given population. This coefficient is calculated from the Lorenz curve, which represents the difference between the percentage of the number of patients associated with a sub-population and the percentage of this population in the general population (Figure 1).

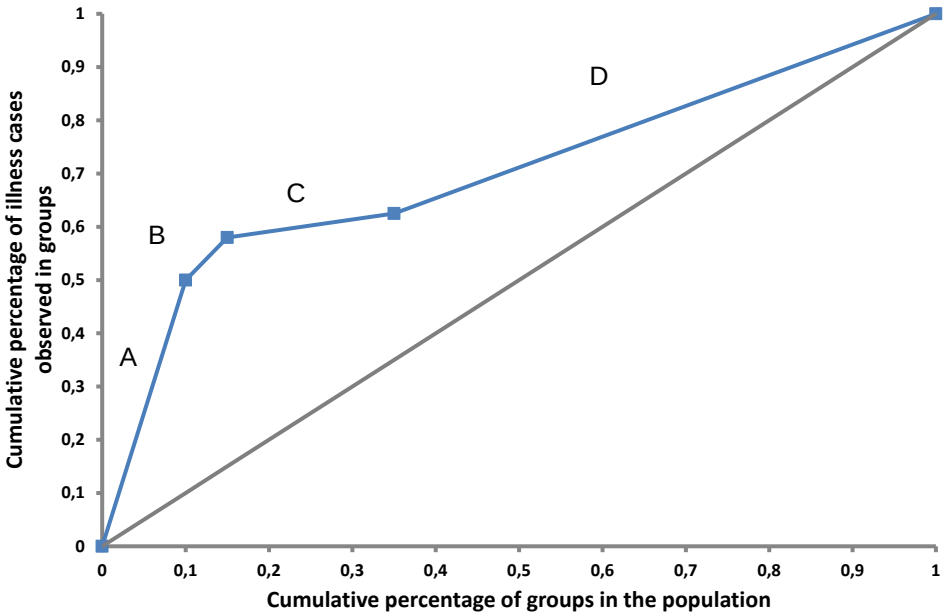


Figure 1: Lorenz curve (blue) for four sub-populations A, B, C and D, each representing respectively 10%, 5%, 20% and 65% of the population and 50%, 8%, 5% and 37% of the number of patients. The grey curve represents a situation in which each individual, each group contributes equally to the number of cases. The Gini coefficient represents the area between the two curves

In the example shown in Figure 1, populations A and B only represent 15% of the population but 58% of cases of disease. This dispersal of risk levels may be associated with either a difference in exposure or a difference in sensitivity of the different population categories.

In order to assess the relative sensitivity of the different age groups, it is assumed that exposure to the microbiological hazards in question (all routes combined) is identical between the different age groups. In other words, any differences in incidence, for the different population categories, of diseases associated with the pathogens under study would mainly be explained by a difference in sensitivity, rather than a difference in exposure.

The relative sensitivities of the different population categories were assessed using incidence data observed in the French population and also from the available dose-response relationships. The different approaches were compared in order to propose the relative sensitivity values ultimately used to guide the choice of the target populations for the recommendations.

3.3.2. Incidence of disease by age group and by population type

Salmonella infections

The *Salmonella* National Reference Centre (NRC) receives strains from private and hospital laboratories. In 2009, the exhaustiveness was estimated at 48%, i.e. 48% of the strains of *Salmonella* isolated in France were received at the NRC.

The breakdown of strains by age group is relatively stable from one year to the next: over the last 5 years (2010-14), between 5.8% and 7.4% of the strains received at the NRC were for children under 1 year of age, 24% to 26% for children aged 1-5 years, 14% for children aged 6-14 years, from 36.5% to 39.2% for the 15-64 age group, and between 14.9% and 17.1% for those aged 65 years and older. The data on annual incidence by age group in 2014 are shown in Table 1.

Table 1: Annual incidence of salmonellosis* by age group in France, 2014 (*Salmonella* CNR, 2014)

Age group (years)	Number of cases*	Population size (Insee data[7] 2015)	Annual incidence (per 100,000)
0-5	2718	4,851,509	56
6-10	910	4,156,534	22
11-14	357	3,335,942	11
15-64	3552	41,788,916	8
>=65	1522	12,185,093	12

*strains of patients received at the NRC

Campylobacter infections

Surveillance of infections caused by *Campylobacte*r relies on a network of bio-medical analysis laboratories and hospital laboratories. The voluntary participating laboratories systematically screen for *Campylobacter* in any stool culture and send the strains they isolate to the NRC with a corresponding data sheet. In 2014, the NRC received 5080 strains. The median age of people infected by *Campylobacte*r (and related bacteria) was 24 years old (extremes: 0 months-102 years). The average age was 31 years and this was significantly higher in the case of *C. coli* than with *C. jejuni* (36 years vs 29 years, $p<10^{-3}$). The average incidence (annual number of cases reported per 100,000 inhabitants) was 10 cases per 100,000. The incidence was highest among young children (23 cases per 100,000 for children 5 years and under, 16 cases per 100,000 for children aged 6 to 10 years and 10 cases per 100,000 for children aged 11 to 14 years), lowest in adults (incidence of 7 cases per 100,000 for 15-64 years) and was 8 cases per 100,000 for those 65 years and older.

[7] National Institute for Statistics and Economic Studies

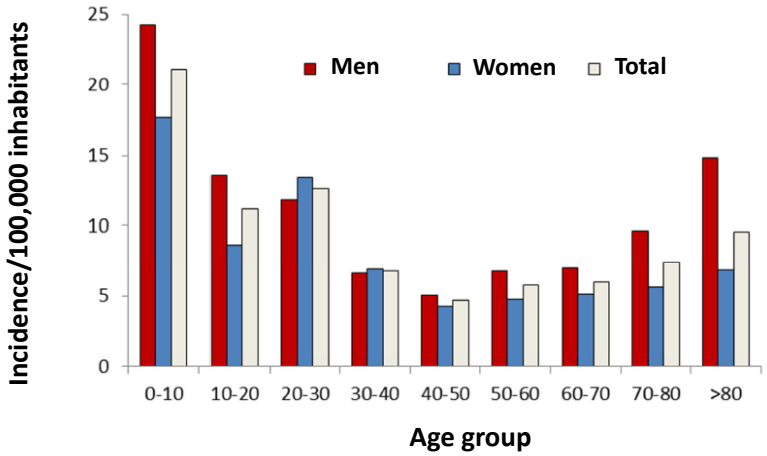


Figure 2: Annual number of cases (strains received at the NRC) of *Campylobacter* and related bacteria per 100,000 inhabitants by age and sex, France 2014 (*Campylobacter* CNR, 2014)

***Listeria monocytogenes* infections**

Listeriosis has been a notifiable disease since 1998. Surveillance of human listeriosis is conducted jointly in France: by the InVS by means of mandatory reporting, which is used to collect the clinical characteristics of patients with the help of a specific reporting sheet, and by the *Listeria* CNR which carries out microbiological surveillance of the strains it receives. In addition to this transmission of clinical and microbiological data, a dietary survey is systematically performed for any case of listeriosis, using a specific questionnaire. From 250 to 380 cases of listeriosis are diagnosed each year in France, an annual incidence of 4 to 6 cases per million inhabitants.

Goulet *et al*. (2012) examined the cases of listeriosis reported in France from 2001 to 2008 (on the basis of 1959 reported cases). The number of cases and deaths were calculated according to age and other underlying conditions. The impact of these specific underlying conditions on the onset of the listeriosis was calculated.

Table 2: Number of cases of listeriosis reported in France from 2001 to 2008 by population categories (Goulet *et al.*, 2012)

Population categories	Population		Cases of listeriosis	
	Size	Percentage	Number	Average annual incidence (per 100,000)
Adult population in good health under the age of 65	48,909,403	76.7%	189	0.05
Over the age of 65 without other underlying conditions	7,038,068	11.0%	377	0.67
Pregnant women	774,000	1.2%	347	5.60
Non-haematological cancers	2,065,000	3.2%	437	2.65
Haematological cancers	160,000	0.3%	231	18.05
Kidney or liver failure	284,000	0.4%	164	7.22
Organ transplant	25,300	0.0%	16	7.91
Inflammatory disease (Crohn's disease, rheumatoid arthritis, etc.)	300,674	0.5%	68	2.83
HIV	120,000	0.2%	22	2.29
Diabetes (type I or II)	2,681,000	4.2%	79	0.37
Heart disease	1,400,000	2.2%	29	0.26
Total	63,757,445	100%	1959	0.38

Table 3: Incidence of listeriosis by age group, 2012-2014 (unpublished data, M. Tourdjman, InVS, 21/10/2015)

Age group (years)	2012		2013		2014	
	Number of cases	Annual incidence (per 100,000)	Number of cases	Annual incidence (per 100,000)	Number of cases	Annual incidence (per 100,000)
0-5	0	0	3	0.06	1	0.02
6-10	0	0	1	0.02	2	0.05
11-14	1	0.03	0	0	0	0
15-64	132	0.31	132	0.32	141	0.34
>=65	213	1.90	233	2.02	230	1.94

In 2014, the incidence of listeriosis in France was 5.7 cases per million inhabitants. This rate was stable compared to 2013 but represents a moderate increase compared with the annual incidences observed during the period 1999-2012, which varied between 2.9 and 5.3 cases per million inhabitants. This increase mainly concerns elderly individuals with co-morbidities, and follows an increase already noted in other European countries since 2006. Children under 15 years of age are relatively unaffected by listeriosis (Table 3). For this hazard, therefore, they were considered as belonging to the general population.

In 2014, 374 cases of listeriosis were reported in France (of which 364 were in metropolitan France): 49 maternal-neonatal forms and 325 non-maternal-neonatal forms. 51 deaths occurred among the 325 non-maternal-neonatal forms, a mortality rate of 16%. Over the past 5 years, the mortality rate has varied between 16% and 20%.

Enterohaemorrhagic *E. coli* (EHEC) infections

In France, as screening for EHEC in stools is not carried out routinely in medical analysis laboratories, surveillance of EHEC infections since 1996 has been based on the monitoring of cases of HUS in children under 15 years of age. HUS particularly affects young children and represents the main cause of acute renal failure in children under 3 years of age. In the literature, the mortality rate of HUS varies from 3 to 5%, 1% according to French surveillance data, and more than a third of patients suffer long-term kidney damage.

In 2014, 117 cases of indigenous HUS were reported by 37 hospitals, including 12 cases by 10 hospitals outside the network of paediatric nephrology services. The annual incidence of HUS was 0.99 cases/100,000 children under the age of 15. Since 1996, the annual incidence of HUS has varied between 0.6 and 1.3 cases/100,000 children under the age of 15. In 2014, 66% of the children affected were aged 3 years and under (median: 29 months; extremes: 2 months-14 years). Since surveillance began in 1996, the highest incidence has been observed in children under 3 years of age. In 2014, it was 2.9/100,000 (Table 4).

Table 4: Incidence of HUS per 100,000 children under 15 years of age by age group. France, 2014 (InVS, 2015)

Age group (years)	Annual incidence (per 100,000 children)
0-5	1.95
6-10	0.6
11-14	0.1

3.3.3. Hospitalisation data

Annual hospitalisation rates in metropolitan France per 100,000 inhabitants over the period 2008-2013 were calculated by dividing the number of stays with a corresponding ICD-10[8] code in the PMSI[9] (for enteritis caused by *Campylobacter*, code A045, and for *Salmonella* codes A020, A021, A022, A028 and A029) by the Insee population data (2010) by age group (0-5 years, 6-10 years, 11-14 years, 15-64 years, and 65 years and older), corrected for the sensitivity of the culture for *Campylobacter* and *Salmonella*, respectively 0.6 and 0.95.

[8] International Classification of Diseases - Version 10

[9] French Programme for the Medicalisation of Information Systems

- ***Campylobacter* infections**

For *Campylobacter* infections, the average annual hospitalisation rate over the period was 8 hospitalisations per 100,000. The hospitalisation rate was higher among children 0-5 years of age (21 hospitalisations/100,000). This rate was similar among individuals aged 6-10 years, 11-14 years and over 65 years (respectively 12, 9 and 12 per 100,000). Finally, the hospitalisation rate was lowest in the 15-64 years age group (5 hospitalisations per 100,000).

- ***Salmonella* infections**

For *Salmonella* infections, the average annual hospitalisation rate over the period was 7 hospitalisations per 100,000. The hospitalisation rate was highest among children 0-5 years of age (33 hospitalisations/100,000). The rate was 12 hospitalisations per 100,000 among children aged 6-10 years. The hospitalisation rate was similar among individuals aged 11-14 years (6 hospitalisations per 100,000) and 65 years and older (9 hospitalisations per 100,000). Finally, the hospitalisation rate was lowest in the 15-64 years age group (3 per 100,000).

Table 5: Hospitalisation rates related to infections by *Campylobacter* and *Salmonella* (Van Cauteren *et al.,* 2015)

Age group (years)	Population size (in millions) (Insee 2010 data)	*Campylobacter 2008-2013*			*Salmonella 2008-2013*		
		PMSI	0.60 sensitivity	Hospitalisations /100,000	PMSI	0.95 sensitivity	Hospitalisations /100,000
0-5	4.62	586	976	21	1448	1525	33
6-10	3.89	285	474	12	452	476	12
11-14	3.03	167	278	9	193	203	6
15-64	40.7	1316	2193	5	1189	1252	3
>65	10.5	737	1228	12	909	957	9
Total	62.7	3089	5149	8	4192	4413	7

- ***Listeria monocytogenes* infections**

For *Listeria monocytogenes,* cases are usually hospitalised and surveillance by mandatory reporting is exhaustive. Surveillance data for *Listeria monocytogenes* therefore reflect the actual incidence of listeriosis in France.

- **Haemolytic uraemic syndrome**

Paediatric cases of HUS are usually all hospitalised. The PMSI analysis for paediatric cases of HUS was not therefore carried out.

3.3.4. Review of the dose-response relationships

A **dose-response relationship** is used to establish a link between the level of exposure to a hazard (total amount of microorganisms ingested expressed as a colony forming unit – CFU) and the probability that an effect will occur. Several dose-response relationships have been published for the four hazards discussed. The objective here, for each of the hazards, is to use the dose-response relationships defined for the general population and the vulnerable population(s) and to calculate the relative sensitivity between these populations using the probabilities of diseases for the doses of hazard found in raw milk.

- **For *Salmonella***

Teunis *et al.* (2012) defined two types of populations with regard to the dose-response relationship for *Salmonella*: one for the vulnerable population (defined by the authors as children under 12 years of age, people over the age of 65 and hospital patients) and one for the general population.

A dose-response relationship was established for each of these populations. The two dose-response relationships serve to estimate the relative sensitivity of each of these populations for different doses (*d*). Figure 3a shows the probability of salmonellosis for different doses for the two populations. The concentration of *Salmonella* in raw milk was not characterised but in principle the ingested doses are low (Soboleva, 2013). For the low levels of ingested dose (selected value of 10 CFU), the likelihood of developing salmonellosis is 1.65 times higher in the vulnerable population compared to the general population.

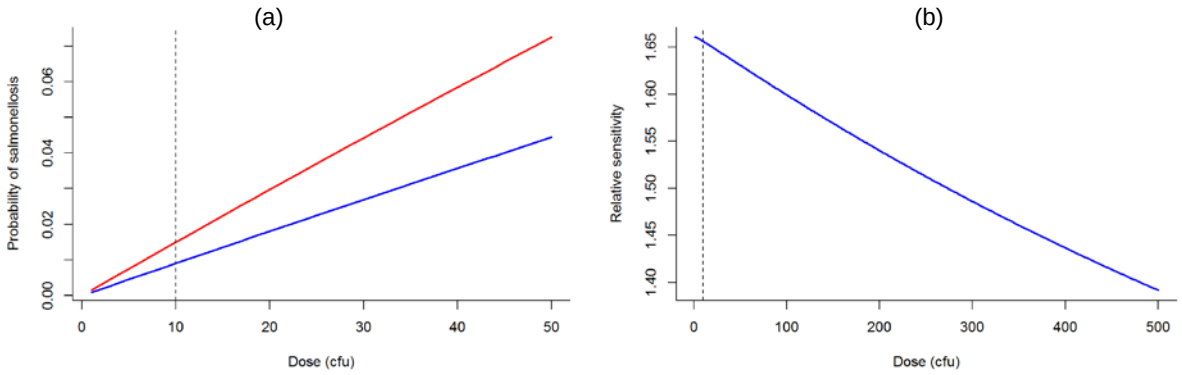


Figure 3. (a) Dose-response relationship for *Salmonella* (from Teunis et al., 2010) for the vulnerable (red) and general (blue) populations and (b) relative sensitivity as a function of the dose

- **For *Campylobacter***

The reference dose-response relationship for *Campylobacter* also considers two categories of population; the general population and the vulnerable population (Teunis *et al*., 2005; Nauta *et al.,* 2009). According to these authors, the vulnerable population consists of children. The dose-response relationship for this category was established using data from two FBOs related to raw milk, in the United Kingdom and Denmark, which affected children aged between 4 and 13 years. In our situation, the dose-response relationship is extended to all children under the age of 15. Figure 4a shows the probability of campylobacteriosis for different doses for the two populations. The concentrations of *Campylobacter* in raw milk can be of the order of 100 CFU/L (Soboleva, 2013). The raw milk responsible for these two FBOs contained between 10 and 100 CFU/L (Teunis *et al*., 2005). For these levels (100 CFU ingested) the probability of campylobacteriosis would be 2.5 times higher in children than in the general population. Nauta *et al.* (2009) noted that the vulnerable population could include other categories than children, but without giving any details.

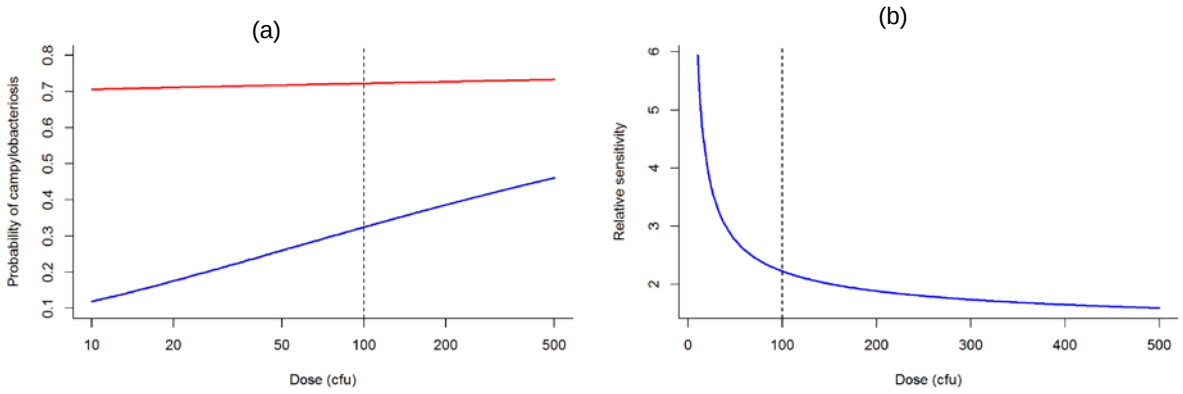


Figure 4. (a) Dose-response relationship for *Campylobacter* (from Teunis et al., 2005) for the vulnerable (red) and general (blue) populations and (b) relative sensitivity as a function of the dose

- **For EHEC**

For *E. coli* O157:H7, Delignette-Muller *et al*. (2010) and Perrin *et al*. (2015) proposed a dose-response relationship for the risk of developing HUS as a function of the ingested dose and age (for age categories less than 15 years old). This relationship was established using data collected during an outbreak associated with the consumption of minced meat. For most dose-response relationships, it is considered that this relationship is valid for all food matrices. It is also assumed that this relationship is valid for all serotypes of EHEC.

From this relationship, it is possible to calculate the probability of HUS for the different age groups presented in Table 6. For individuals over 15 years of age, the assumption is that individuals have the same dose-response relationship as children aged 15 years of age (Perrin *et al*., 2015).

As there are few data on contamination of raw milk (Perrin *et al.*, 2015), the relative sensitivity was calculated from the probabilities of HUS for a dose of 1 CFU.

Table 6: Relative sensitivity of different population categories with regard to EHEC according to the dose-response relationships proposed by Delignette-Muller *et al.* (2010) and Perrin *et al.* (2015)

Age groups (years)	Population		Dose-response relationship	
	Size (Insee, 2015)	Relative share	Probability of HUS for 1 cell	Relative sensitivity compared to the general population
[0,5]	4,851,509	7.2%	2.5E-3	110.0
[6,10]	4,156,534	6.2%	3.7E-4	16.4
[11,14]	3,335,942	5.0%	6.5E-5	2.8
General population (≥15 years)	53,974,009	81.6%	2.3E-5	-

- **For *Listeria monocytogenes***

Recently, a new dose-response relationship incorporating the variability in the virulence of strains of *L. monocytogenes* and the variability in the sensitivity of the host, established for 11 sub-groups of the population (with identical underlying co-morbidities), was published (Pouillot *et al*., 2015, Table 7). Relative sensitivities were estimated for a dose of 1 CFU, which corresponds to the median dose of *L. monocytogenes* ingested by consumers of raw milk (Giacometti *et al.,* 2015).

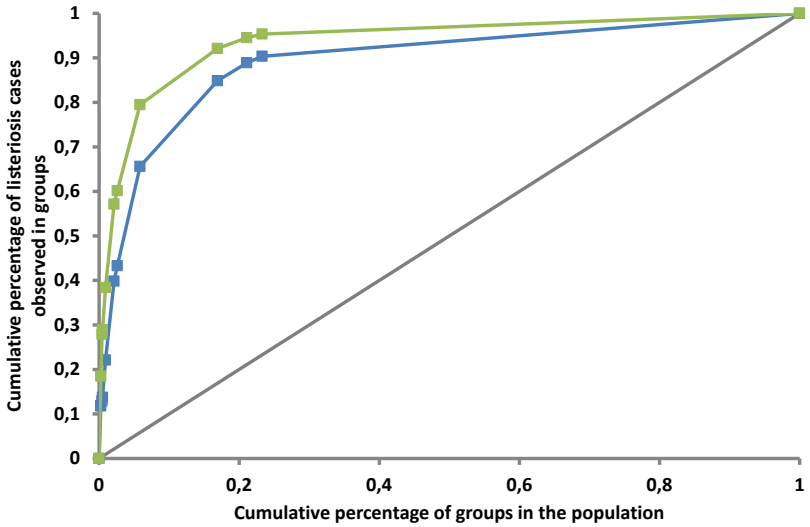
Table 7: Relative sensitivity of different population categories to *L. monocytogenes* according to the dose-response relationships proposed by Pouillot *et al.* (2015)

Population categories	Population		Dose-response relationship		
	Size	Percentage	Probability of listeriosis for 1 cell	Relative sensitivity compared to the general population	Classification by decreasing relative sensitivity
Adult population in good health under the age of 65	48,909,403	76.7%	7.9E-12	-	-
Haematological cancers	160,000	0.3%	9.6E-09	1215	1
HIV	120,000	0.2%	6.5E-09	823	2
Organ transplant	25,300	0.0%	3.14E-09	397	3
Kidney or liver failure	284,000	0.4%	2.79E-09	353	4
Pregnant women	774,000	1.2%	2.01E-09	254	5
Inflammatory disease (Crohn's disease, rheumatoid arthritis, etc.)	300,674	0.5%	8.43E-10	107	6
Non-haematological cancers	2,065,000	3.2%	7.76E-10	98	7
Over the age of 65 without other underlying conditions	7,038,068	11.0%	1.49E-10	19	8
Diabetes (type I or II)	2,681,000	4.2%	7.47E-11	9	9
Heart disease	1,400,000	2.2%	5.01E-11	6	10
Total		100%			

3.3.5. Comparison of the results obtained from the dose-response relationships and the epidemiological data

The Lorenz curves for *Listeria monocytogenes* (Figure 5 top) and EHEC (Figure 4 bottom) show very high Gini coefficients, indicating a significant disparity in the levels of risk within the population. The dose-response data, like the incidence data in the sub-populations, reveal similar relative sensitivities.

For EHEC, the difference is explained in part by the epidemiological data available (they only concern children under 15 years old) and by the assumption that the adult population contributes to the risk with identical sensitivity to that of children aged 15 years old. For *L. monocytogenes*, the relative sensitivity estimated from the dose-response relationships was assessed at low doses. The calculation of relative sensitivity established from the epidemiological data is probably similar to the relative sensitivity for high doses (Pouillot *et al.,* 2015).



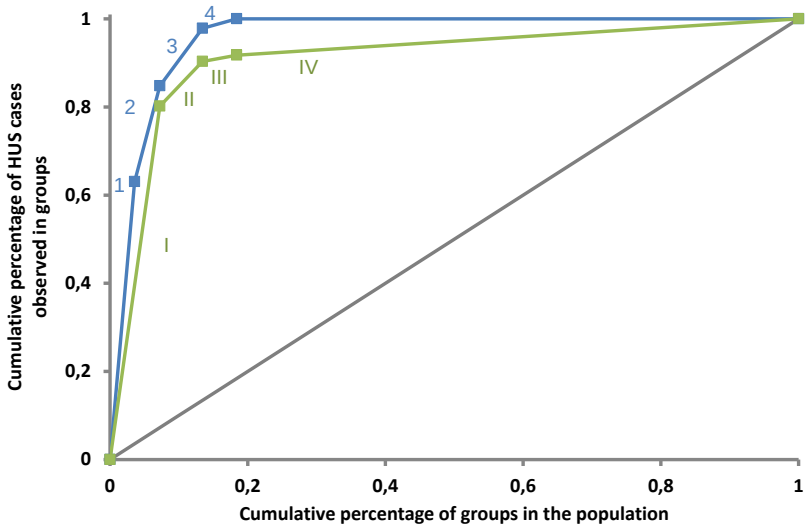
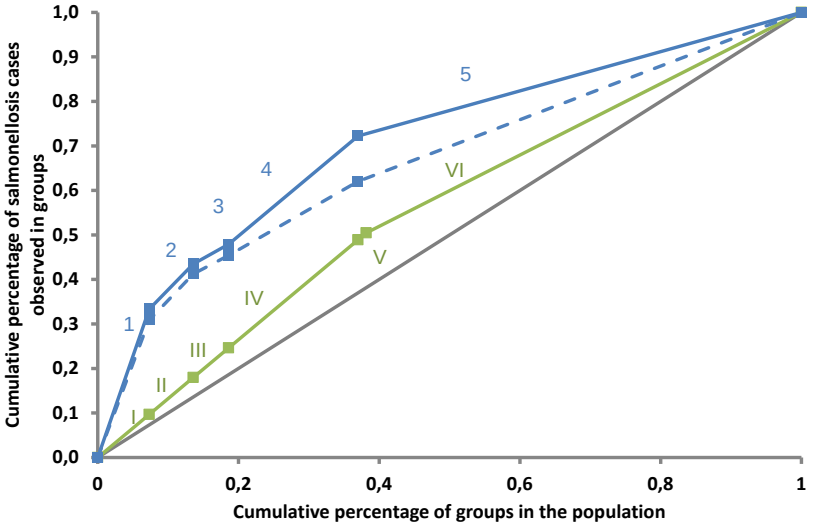


Figure 5. Lorenz curves obtained from the epidemiological data (blue) or the dose-response relationships (green) for (top) *L. monocytogenes* (see Table 7 for the reference to sub-populations, classified in the same order as the figure) and (bottom) EHEC (1, 2, 3) for sub-populations ([0,5], [6,10], [11,14]). (I, II, III, IV) for sub-populations ([0,5], [6,10], [11,14]) and the general population

The Lorenz curves for *Salmonella* (Figure 6 top) and *Campylobacter* (Figure 6 bottom) show less disparity in the levels of risk within the population than that for *L. monocytogenes* and EHEC. The dose-response relationships tend to indicate lower relative differences in sensitivity than those reported by the epidemiological data.



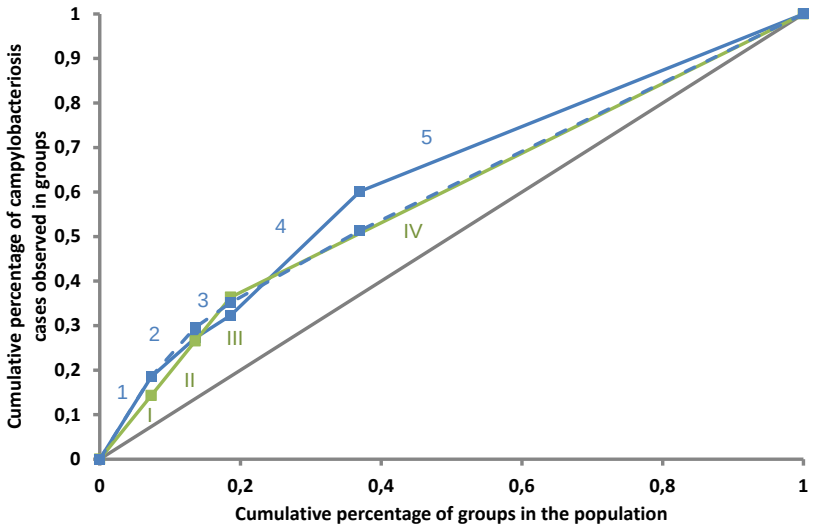


Figure 6. Lorenz curves obtained from the epidemiological data (solid blue = CNR cases, dashed blue = from the data relating to hospitalised cases) or the dose-response relationships (green) for (top) *Salmonella* (1, 2, 3, 4, 5) for the sub-populations ([0,5], [6,10], [11,14], >65 years and general population). (I, II, III, IV, V, VI) for the sub-populations ([0,5], [6,10], [11,14], >65 years, pregnant women and general population, and (bottom) *Campylobacter*. (1, 2, 3, 4, 5) for the following sub-populations ([0,5], [6,10], [11,14], >65 years and general population). (I, II, III, IV) for the sub-populations ([0,5], [6,10], [11,14]) and the general population

The relative sensitivities of the different population categories are summarised in Table 8:

- Children aged 0 to 5 years may have sensitivity between 10 and 110 times higher than the general population for HUS, between 1.6 and 10.3 times for salmonellosis and between 2.5 and 4 times higher for campylobacteriosis.
- Children aged 6 to 10 years may have sensitivity between 6 and 16.4 times higher than the general population for HUS, between 1.6 and 3.7 times for salmonellosis and from 2.2 to 2.5 times higher for campylobacteriosis.
- Children aged 11 to 14 years may have sensitivity 2.8 times higher than the general population for HUS, between 1.4 and 2 times for salmonellosis and between 1.4 and 2.5 times higher for campylobacteriosis.
- Elderly people over the age of 65 may have sensitivity between 14 and 19 times higher than the general population for listeriosis and between 1.6 and 3 times higher for salmonellosis.
- Pregnant women may have sensitivity between 116 and 254 times higher for listeriosis and 1.6 times for salmonellosis.
- Other vulnerable populations may have sensitivity 1.6 times higher for salmonellosis, and a sensitivity for listeriosis that varies between 5 and 1215 times according to the underlying disease (see Table 8).

Table 8: Summary of the sensitivities relative to the general population calculated using different methods (epidemiological data and dose-response relationship) of the different categories of vulnerable population selected for the hazards considered

Method	Hazard	Children				Pregnant women	Other vulnerable populations								People over the age of 65
		[0,5]		[6,10]	[11,14]		Other cancers	Haematological cancer	Liver or kidney failure	Organ transplant	Inflammatory diseases	HIV	Diabetes	Heart disease	
		[0-2]	[3-5]												
Dose-response relationship	*Salmonella*	1.6				1.6	1.6								1.6
	Campylobacter	2.5		2.5	2.5	-	-								-
	Listeria monocytogenes	-[a]		-	-	254	98	1215	353	397	107	823	9	6	19
	EHEC	110		16.4	2.8	-	-								-
Incidence of infections	*Salmonella*	7.0		2.8	1.4	-	-								1.5
	Campylobacter	3.3		2.3	1.4	-	-								1.1
	Listeria monocytogenes	-		-	-	116	54	373	149	163	58	47	7	5	14
	EHEC	29	10	6	1[b]	-	-								-
Hospitalisation data	*Salmonella*	10.3		3.7	2.0	-	-								3
	Campylobacter	4.0		2.2	1.6	-	-								2.4

[a] Group attached to the general population for the hazard considered
[b] The reference group corresponds to children aged between 11-14 years

From the Lorenz curves, we can also determine the part of the potential risk represented by the populations targeted by the labelling statements (Table 9). The intervals shown are bounded by the extreme values from the three methods of estimating the relative risk, for each category.

- The recommendation "*boil before consumption for children under five years of age, pregnant women and people whose immune system is weakened*" is addressed to the categories of populations that may account for 34 to 57% of potential cases of salmonellosis, 80 to 85% of potential cases of HUS, 27 to 46% of potential cases of campylobacteriosis, and 90 to 95% of potential cases of listeriosis associated with raw milk consumed directly.
- The recommendation "*boil before consumption for children under ten years of age, pregnant women and people whose immune system is weakened*" is addressed to the categories of populations that may account for 42 to 67% of potential cases of salmonellosis, 90 to 98% of potential cases of HUS, 40 to 55% of potential cases of campylobacteriosis, and 90 to 95% of potential cases of listeriosis associated with raw milk consumed directly.
- The recommendation "*boil before consumption for children under fifteen years of age, pregnant women and people whose immune system is weakened*" is addressed to the categories of populations that may account for 49 to 72% of potential cases of salmonellosis, 92 to 100% of potential cases of HUS, 49 to 60% of potential cases of campylobacteriosis, and 90 to 95% of the risk of listeriosis associated with raw milk consumed directly.

Table 9: Part of the potential risk represented by the different categories of population for the four pathogens considered and according to the methods of estimation available (*DR*: dose-response relationship, *inf*: epidemiological data on cases of infection, *hosp:* epidemiological data on hospitalised cases)

Recommendations	*Salmonella*			EHEC		*Campylobacter*			*L. monocytogenes*	
	DR	*inf*	*hosp*	*DR*	*inf*	*DR*	*inf*	*hosp*	*DR*	*inf*
Age group (years)										
[0,5]	10%	31%	33%	80%	85%	14%	19%	18%		
[0,10]	18%	41%	43%	90%	98%	27%	30%	27%		
[0,14]	25%	45%	48%	92%	100%	36%	35%	32%		
>65 years	24%	17%	24%			13%	16%	28%	19%	13%
Pregnant women									18%	19%
Other vulnerable individuals									53%	64%
Sum 1 ([0,5] + >65 years + pregnant women + vulnerable individuals)	34%	48%	57%	80%	85%	27%	35%	46%	90%	95%
Sum 2 ([0,10] + >65 years + pregnant women + vulnerable individuals)	42%	58%	67%	90%	98%	40%	46%	55%	90%	95%
Sum 3 ([0,14] + >65 years + pregnant women + vulnerable individuals)	49%	63%	72%	92%	100%	49%	51%	60%	90%	95%

CONCLUSIONS OF THE "BIORISK" EXPERT COMMITTEE (CES)

The burden of infectious diseases associated with raw milk consumed directly, in France, remains difficult to estimate. Nevertheless, taking into account:

- (i) specific requirements relating to the conditions for the production and distribution of raw milk and its low consumption in France,
- (ii) the low number of FBOs detected by the French surveillance system,
- (iii) risk assessments carried out in other countries,

the contribution of raw milk consumed directly to infectious diseases such as salmonellosis, listeriosis, campylobacteriosis and HUS can be regarded as low compared to other known contributors (meat and meat products, eggs, ready-to-eat processed products, etc.).

Nevertheless, the severity of the adverse effects associated with certain microbiological hazards potentially found in raw milk may justify consumer information measures. The draft decree proposes the following labelling statement: *"boil before consumption for children under five years of age, pregnant women and people whose immune system is weakened".*

The proposed approach for guiding the choice of populations targeted by the prevention message is to determine the relative sensitivity of the different population categories to the hazards in question. It is based on the dose-response relationships published in the literature as well as incidence and hospitalisation data as indicators of the sensitivity of the monitored populations.

The information presented in this opinion and summarised in Tables 8 and 9 can be used as a decision-support tool for choosing the population categories to appear on the labelling statement (vulnerable sub-population(s) or general population). In the absence of data on the effectiveness of labelling on changes in consumer behaviour, the impact on risk reduction of the different labelling statements cannot be assessed. The estimates proposed should be regarded as a maximum percentage of risk reduction. Indeed, other work conducted by ANSES has shown that the recommendations given on the labelling only affect consumer behaviour to a very limited degree (ANSES, 2015).

For the choice of categories of vulnerable populations, a limit to the increase in risk relative to the general population should first be established. For example:

- if the limit is set as an increase by a factor of 10 in the relative risk of different diseases (shown here in parentheses), the populations targeted by the message would be: children under 10 years of age (HUS), pregnant women, elderly people over the age of 65 and the other vulnerable populations (listeriosis). These population categories may account for 42 to 67% of potential cases of salmonellosis, 90 to 98% of potential cases of HUS, 40 to 55% of potential cases of campylobacteriosis, and 90 to 95% of potential cases of listeriosis associated with raw milk consumed directly.
- if it is set as an increase by a factor of 2 in the relative risk of different diseases (shown here in parentheses), the populations targeted by the message would be: children under 15 years of age (HUS, campylobacteriosis), pregnant women, elderly people over the age of 65 and the other vulnerable populations (listeriosis). These population categories may account for 49 to 72% of potential cases of salmonellosis, 92 to 100% of potential cases of HUS, 49 to 60% of potential cases of campylobacteriosis, and 90 to 95% of the risk of listeriosis associated with raw milk consumed directly.

Generalising the recommendation to the entire population would prevent the remainder of the potential cases: 28% to 51% of potential cases of salmonellosis, 8% of potential cases of HUS, 40 to 51% of potential cases of campylobacteriosis and 5 to 10% of potential cases of listeriosis associated with raw milk consumed directly.

Lastly, the CES BIORISK recommends collecting data for estimating the current level of risk associated with raw milk marketed in France (prevalence and concentration of the main hazards, level of consumption and methods of storage and use) in particular for the different categories of vulnerable individuals.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

The French Agency for Food, Environmental and Occupational Health & Safety endorses the CES BIORISK's conclusions.

Implementing good husbandry and hygiene practices at the farm, as well as compliance with the cold chain, are essential for limiting contamination and the growth of pathogenic microorganisms in raw milk. Nevertheless, in the absence of measures ensuring the elimination of these hazards in the production of raw milk intended to be provided for direct consumption by the final consumer, any deviation from good practices may result in an increase in the level of risk, as evidenced by the outbreaks identified to date. The boiling of raw milk at the time of consumption can significantly reduce the risk.

The draft decree concerning the labelling of raw milk intended to be provided for direct consumption by the final consumer only partially takes into account the AFSSA opinion of 2009 and, in particular, modifies the age below which the boiling of raw milk is recommended (15 years replaced by 5 years). The Agency's Opinion provides information on the potential impacts of such a recommendation for different population categories according to their sensitivity with regard to the main pathogens that can be transmitted by raw milk.

Therefore, the Agency considers it necessary to inform consumers about the need to boil raw milk, especially for vulnerable populations.

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KEYWORDS

Raw milk; labelling; Decree; microbiological risks

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