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# Assessment of levels of bacterial contamination of large wild game meat in Europe

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ABSTRACT

The variations in prevalence and levels of pathogens and fecal contamination indicators in large wild game meat were studied to assess their potential impact on consumers. This analysis was based on hazard analysis, data generation and statistical analysis. A total of 2919 meat samples from three species (red deer, roe deer, wild boar) were collected at French game meat traders' facilities using two sampling protocols. Information was gathered on the types of meat cuts (forequarter or haunch; first sampling protocol) or type of retail-ready meat (stewing meat or roasting meat; second protocol), and also on the meat storage conditions (frozen or chilled), country of origin (eight countries) and shooting season (autumn, winter, spring). The samples were analyzed in both protocols for detection and enumeration of *Escherichia coli*, coagulase + staphylococci and *Clostridium perfringens*. In addition, detection and enumeration of thermotolerant coliforms and *Listeria monocytogenes* were performed for samples collected in the first and second protocols, respectively. The levels of bacterial contamination of the raw meat were determined by performing statistical analysis involving probabilistic techniques and Bayesian inference. *C. perfringens* was found in the highest numbers for the three indicators of microbial quality, hygiene and good handling, and *L. monocytogenes* in the lowest. Differences in contamination levels between game species and between meats distributed as chilled or frozen products were not significant. These results might be included in quantitative exposure assessments.



## 1. Introduction

The management of microbiological hazards associated with the consumption of meat is of major health and economic significance. Thirty-five pathogens have been identified that are possibly transmitted to humans by consumption of pork and beef, ten of which are bacteria (Fosse et al., 2008b,c). The management of pathogens in meat supply chains, including large wild game, requires both hazard typology analysis and the gathering of specific data on the prevalence and levels of contamination (Fosse et al., 2008b; Lammerding and Fazil, 2000). Moreover, undertaking risk assessment is recommended to evaluate scientifically the known and potential adverse health effects resulting from human exposure to foodborne hazards (World Health Organization, 1995). Several risk assessments have been already undertaken in the meat industry, but for only a limited number of hazards and products; e.g. *Campylobacter* in poultry meat (Nauta et al., 2009; Uyttendaele et al., 2006), *Escherichia coli* O157:H7 in ground beef burgers (Cassin et al., 1998; Delignette-Muller and Cornu, 2008), *Clostridium perfringens* in poultry products (Golden et al., 2009), *Listeria monocytogenes* in ready-to-eat meats (Ross et al., 2009) and *Salmonella* in minced pork (Bollaerts et al., 2009) and in dry-cured pork sausages (Alban et al., 2002). However, no risk assessment has to the best of our knowledge been carried out specifically for large wild game meat; nor are there specific microbiological criteria for such meat within European Union legislation. Indeed, the microbiological quality of meat from large wild game is generally considered to be similar to that of livestock (Atanassova et al., 2008; Gill, 2007), red and roe deer being similar to cattle and wild boar to domestic pigs in this respect. However, for some hazards, and particularly for *C. perfringens* and *Staphylococcus aureus*, there is little published information regarding the prevalence or levels of contamination of meat generally (Fosse et al., 2008a; Golden et al., 2009) and of large wild game meat in particular (Atanassova et al., 2008; Deutz et al., 2000). Most studies on *Salmonella enterica* have demonstrated the rarity of the pathogen in wild mammals such as red deer and wild

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boar (Atanassova et al., 2008; Gill, 2007; Paulsen and Winkelmayer, 2004; Wahlstrom et al., 2003). Nevertheless, enteric pathogens transferred from animals to meat are mainly carried by animals without clinical symptoms and consequently are not detected by macroscopic examination of carcases (Fosse et al., 2008b). In fact, bacterial contamination of meat is mainly associated with hazard transferred from the digestive tract (in particular for *Campylobacter*, *C. perfringens*, *S. enterica*, Shiga-toxin producing *E. coli*, *Yersinia enterocolitica*) or the skin (*S. aureus*, *L. monocytogenes*) to the carcass during slaughter (Beach et al., 2002; Fosse et al., 2008a). Identification and enumeration of indicator organisms for fecal contamination such as *E. coli* have therefore been recommended in addition to pathogens (Commission Regulation, 2007).

Although still low, the consumption of meat from large game animals is increasing in Europe (Bertolini et al., 2005). Annual per capita consumption of game meat varies in European countries from 0.06 kg to 2.6 kg (Reinken, 1998). In France, the annual per capita consumption is estimated to be 0.6 kg (FNC, 2010), and five companies involved in the game meat industry sponsored a scientific study to assess the levels of microbiological contamination of large wild game meat available in France, from both native and imported animals.

In our study, in agreement with the companies, it was decided to obtain information on *C. perfringens*, *S. aureus* (i.e. coagulase + -staphylococci) and *L. monocytogenes* because *C. perfringens* and coagulase + staphylococci are representative of contaminants originating from the digestive track and skin, respectively, and *L. monocytogenes* is representative of contaminants originating from the meat processing environment. In addition to these pathogens, meat was analyzed for *E. coli* and thermotolerant coliforms, for which game meat is routinely tested in France as indicators of fecal contamination.

The aims of this study were to assess the bacterial contamination of wild boar, roe deer and red deer meat in the form of primal cuts (forequarter and haunch) and consumer sale units (roasting and stewing meat); and to collect and analyze data with a view to their subsequent use for risk assessment.

## 2. Materials and methods

### 2.1. Data generation: sampling protocols

A total of 2919 samples was collected at game meat trading facilities in France from 2005 to 2007 in accordance with two sampling protocols. The sampling protocols were devised to provide an overview of what the consumer would be likely to purchase with due consideration for the information labels, i.e. game species, storage conditions and type of cooking to be used. The sampling protocols were not balanced with respect to other recorded factors such as country of origin or hunting season.

In the first sampling protocol, carried out during 2005–2006, 1370 primal meat cuts were collected from chilled and frozen forequarters and haunches (Table 1), and a sample block (5 by 5 cm × 0.5 cm thick) was cut from the surface of each piece; the subsequent microbial analysis unit was log CFU/cm$^2$.

In the second protocol, carried out in 2006–2007, 1549 consumer sale units were collected from roasting or stewing meat (Table 1). Several cubes were cut from each piece for analysis without cauterizing the surface. The subsequent microbial unit was log CFU/g.

### 2.2. Microbiological analysis of meat samples

All samples were analyzed for detection and enumeration of *E. coli*, coagulase + staphylococci and *C. perfringens*. In addition, thermotolerant coliforms and *L. monocytogenes* were detected and enumerated for the samples obtained in the first and second protocols, respectively (Table 2).

The microbiological analyses were carried out on the basis of the standard ISO methods by a COFRAC accredited laboratory, the media being obtained from BIOKAR®. Except for research on *L. monocytogenes*, samples were diluted in 100 mL peptone water, homogenized with a Stomacher before being diluted in tryptone salt and enumerated according to the standard ISO methods. The experimental protocol is detailed in the following text for each pathogen and indicator, the detection range is provided in Table 2.

#### 2.2.1. C. perfringens spores

The ISO 7937 method was followed (Organisation Internationale de Normalisation, 2004a) and TSC medium (AES Chemunex, Bruz, France – AEB152892) plus D cycloseine (AES Chemunex, Bruz, France – AEB184002) was used. Each suspected colony was then picked out and transferred into thioglycolate with resazurine broth (AES Chemunex, Bruz, France – AEB141402) for 8–24 h at 37 °C, then five drops were transferred into lactose-sulfite broth (Biokar Diagnostic, Beauvais, France – BK140HA) in a Durham bell chamber.

#### 2.2.2. Coagulase-positive Staphylococcus

The NF V08-057-2 method (Association Française de Normalisation, 2004) was followed for the detection and enumeration of coagulase-positive staphylococci: 1 mL of parent bacterial suspension was placed on the button of the plate, covered with Baird Baker agar medium plus RPF (rabbit plasma fibrinogen) (AES Chemunex, Bruz, France AEB620314 and AEB184106) and incubated for 24 h at 37 °C.

#### 2.2.3. L. monocytogenes

Identification and enumeration protocols followed the ISO 11290-2/A1 method (Organisation Internationale de Normalisation, 2004b). Twenty-five grams of the sample were pummelled with 225 ml of sterile peptone-buffered water (AES Chemunex, Bruz, France AEB140303) for 60 s in a stomacher bag with a filter. The parent bacterial suspension obtained was incubated for 1 h at 20 °C, then 1 mL was streaked on 140 mm ALOA® plate (AES Chemunex, Bruz, France AEB120082), incubated at 37 °C and examined daily for 2 days. Each suspected colony was identified by culture on ALOA Confirmation® medium (AES Chemunex, Bruz, France AEB120100) and by hemolysis and glucide testing.

**Table 1**
Numbers of samples from meat cuts, species and storage conditions obtained in the two sampling protocols.

| | Meat cut | Red deer | | Roe deer | | Wild boar | | Total | |
|---|---|---|---|---|---|---|---|---|---|
| Sampling protocols | | Frozen | Chilled | Frozen | Chilled | Frozen | Chilled | Frozen | Chilled |
| (1) 1370 samples | Forequarter | 150 | 172 | 95 | 155 | 100 | 150 | 345 | 477 |
| | Haunch | 131 | 149 | 151 | 117 | 0 | 0 | 282 | 266 |
| (2) 1549 samples | Stewing meat | 319 | 167 | 150 | 0 | 296 | 157 | 765 | 324 |
| | Roasting meat | 179 | 155 | 0 | 0 | 0 | 126 | 179 | 281 |

**Table 2**
List of pathogens and indicators investigated in the two sampling protocols and methods used for detection and enumeration.

| Bacterial hazards | Method | Detection range | |
|---|---|---|---|
| | | Sampling protocol 1 (CFU/cm$^2$) | Sampling protocol 2 (CFU/g) |
| *Escherichia coli* | NF ISO 16649-2 | $4 < D < 60\,000$ | $10 < D < 15\,000$ |
| Coagulase + staphylococci | NF V08-057-2 | $4 < D < 60\,000$ | $10 < D < 15\,000$ |
| Thermotolerant coliforms by colony-count technique at 44 °C | NF 08-060 | $4 < D < 60\,000$ | Nd |
| *Clostridium perfringens* | ISO 7937 | $4 < D < 60\,000$ | $10 < D < 15\,000$ |
| *Listeria monocytogenes* | ISO 11290-2/A1 | Nd | $10 < D < 15\,000$ |

Nd: Not done.

#### 2.2.4. E. coli

Detection and enumeration protocols followed the NF ISO 16649-2 (Organisation Internationale de Normalisation, 2001). One mL of the parent bacterial suspension and $10^{-1}$ dilution were streaked on a TBX plate (Biokar, Beauvais France BK146 HA), and incubated for 18–24 h at 44 °C. Then β-glucuronidase-positive *E. coli* were enumerated.

#### 2.2.5. Thermotolerant coliforms

Detection and enumeration protocols followed the NF V 08-060 (Association Française de Normalisation, 1996). One mL of parent bacterial suspension and dilutions were placed on the button of the plate, covered with VRBL agar medium (Biokar, Beauvais France BK152 HA) and incubated for 24 h at 44 °C. Suspected colonies were then enumerated.

### 2.3. Statistical analysis

Two statistical analyses were performed. The response was the microbiological contamination level expressed in log count per cm$^2$ (2005–2206) or per g (2006–2007). In both analyses, a hierarchical model was constructed on the basis of an ANOVA, and the 11 676 microbial counts (2919 samples from the two protocols, four bacteria enumerated per sample) were analyzed in one single model.

The first analysis included the three factors, 'species', 'frozen/chilled storage' and 'cut' (Eq. (1)). As not enough data were collected from meat of roe deer species in the second sampling protocol (Table 1), data for meat from roe deer and red deer were merged.

$$Y_{i,j,k,l,m,n} \sim N\left(\widehat{Y}_{i,j,k,l,m}, \sigma^{w}_{i,j}\right) \quad (1)$$

where $Y_{i,j,k,l,m,n}$ is the microbiological contamination level (log CFU per cm$^2$ or per g) observed for each bacterium ($i$), sampling protocol ($j$), game species ($k$), storage conditions ($l$) and cut ($m$); $n$ is the index encoding the repetition number (number of samples within a specific combination of the factor levels); $\widehat{Y}_{i,j,k,l,m}$ is the mean contamination level including the five factor effects, subsequently analyzed as indicated in Eq. (2), and $\sigma^{w}_{i,j}$ is the standard deviation of the residual error, corresponding to the *intra* variability (within the combination of factor levels).

$$\widehat{Y}_{i,j,k,l,m} \sim N\left(\theta_{i,j,k}, \sigma^{b}_{i,j}\right) \quad (2)$$

where $\theta_{i,j,k}$ is the *supra* mean contamination level, defined for each bacterium ($i$), sampling protocol ($j$) and game species ($k$); $\sigma^{b}_{i,j}$ is the standard deviation of the model error, corresponding to the *inter* variability (variability between the levels of the game species factor), defined for each bacterium ($i$) and sampling protocol ($j$).

The model corresponding to the second statistical analysis with the five explanatory factors, 'species', 'storage condition', 'cut', 'country of origin' and 'hunting season', is expressed in Eq. (3).

As not enough data were available to analyze each country separately, countries were divided into four groups. Great Britain, Hungary, Germany, Czech Republic, Austria and Australia, where the hunting forest management and the hunting practice were assumed to be relatively similar, were merged into one group; Spain and France were kept separate in the analysis as there were many samples from these two countries, Poland was merged with undetermined countries for which the only information available was 'from the European Union'. Moreover, data collected on roe deer were merged with data collected on red deer.

$$Y_{i,j,k,l,m,n,o,q} \sim N\left(\widehat{Y}_{i,j,k,l,m,n,o}, \sigma^{w}_{i,j,k}\right) \quad (3)$$

where $Y_{i,j,k,l,m,n,o,p}$ is the microbiological contamination level (log CFU per cm$^2$ or per g) observed for each bacterium ($i$), sampling protocol ($j$), country ($k$), game species ($l$), storage conditions ($m$), hunting seasons ($n$) and cut ($o$); $q$ is the index encoding the repetition number (number of samples within a specific combination of the factor levels); $\widehat{Y}_{i,j,k,l,m,n,o}$ is the mean contamination level including the seven factor effects, subsequently analyzed as indicated in Eq. (4); $\sigma^{w}_{i,j,k}$ is the standard deviation of the residual error, corresponding to the *intra* variability (within the combination of factor levels). The effect of the country of origin on standard deviation of the residual error was based on preliminary data analysis (not shown) and also on information from the industrial partners associated with the study: variability of contamination level might depend on hunting forest management and on hunting practices which vary between countries.

$$\widehat{Y}_{i,j,k,l,m,n,o} \sim N\left(\theta_{i,j,k,l}, \sigma^{b}_{i,j}\right) \quad (4)$$

where $\theta_{i,j,k,l}$ is the *supra* mean contamination level, defined for each bacterium ($i$), sampling protocol ($j$), country ($k$) and game species ($l$); $\sigma^{b}_{i,j}$ is the standard deviation of the model error, corresponding to the *inter* variability (variability between the country and game species factors), defined for each bacterium ($i$) and sampling protocol ($j$).

### 2.4. Bayesian inference

The findings reported as "below the detection limit" (i.e. less than 1 log CFU/g) were analyzed in the hierarchical model as censored data to construct an overall probability distribution of the contamination level using a Bayesian inference technique. This technique allows the uncertainty due to censored data to be expressed in terms of probability and then avoids making arbitrary choices (e.g. discarding the "not detected" data, or fixing them at 1 log CFU/g). The Bayesian technique was also chosen to introduce

prior information in the estimation process. This technique allows the current state of knowledge, expressed as a prior probability distribution, to be formally combined with new data to reach an updated information state, known as the posterior probability distribution (Eq. (5)).

$$P(\text{parameters}/\text{data}) \equiv P(\text{parameters}) \times P(\text{data}/\text{parameters}) \quad (5)$$

Thus the more informative the data set or the less informative probability distribution, the lower the impact of the prior distribution on the posterior distribution. In contrast, when the prior distribution is informative gathering (what is known before performing the analysis and often obtained by expert knowledge), the impact of the prior on the posterior distribution is greater. One of the values of Bayesian inference lies in this latter property, the lack of knowledge provided by the findings might be compensated for by the information from previous knowledge of the process. In our study, the prior probability distributions were constructed using knowledge from food microbiologists.

The *supra* means of contamination level were assumed to follow a Normal distribution with a standard deviation of 3, this value being considered a realistic informative value to describe the variability of the mean microbial contamination (expressed in log) among several groups of bacteria (Eqs. (6) and (7)).

$$\theta_{i,j,k} \sim N(0,3) \quad (6)$$

$$\theta_{i,j,k,l} \sim N(0,3) \quad (7)$$

The standard deviations of the residual error, $\sigma^{w}_{i,j}$ and $\sigma^{w}_{i,j,k}$, corresponding to the *intra* variability were computed from the precision, $P$ (the precision being the inverse of the variance), while the precision was assumed to follow a Gamma distribution (Eqs. (8) and (9)).

$$P^{w}_{i,j} \sim \text{Gamma}(\nu 1, \nu 2) \quad (8)$$

$$P^{w}_{i,j,k} \sim \text{Gamma}(\nu 1, \nu 2) \quad (9)$$

In Eqs. (8) and (9), $\nu 1$ and $\nu 2$ are described by Gamma distributions (Eqs. (10) and (11)). The prior information associated with the *intra* variability precision was assumed to be compatible with the log count of bacterial contamination level.

$$\nu 1 \sim \text{Gamma}(1,1) \quad (10)$$

$$\nu 2 \sim \text{Gamma}(1,1) \quad (11)$$

Similarly, the standard deviation of the model error, $\sigma^{b}_{i,j}$, corresponding to the *inter* variability, was derived from the precision as written in Eq. (12).

$$P^{b}_{i,j} \sim \text{Gamma}(\nu 3, \nu 4) \quad (12)$$

In Eq. (12), $\nu 3 \sim$ Gamma(1,1) and $\nu 4 \sim$ Gamma(1,1).

### 2.5. Estimation process and software

Computing Eq. (5) is often analytically impossible. A variety of methods have been developed to undertake a modeling approach based on Bayesian inference. One of the most popular methods is the Markov Chain Monte Carlo technique (Dakins et al., 1996; Patwardhan and Small, 1992) in which a Markov Chain is used to sample the posterior probability distribution. This technique was carried out with the Winbugs package (version 1.4.3, Medical Research Council, UK). Three independent chains of 20 000 iterations were run; the first 10 000 iterations were eliminated (burning period). Convergence was checked by visual inspection of the three chains, and no convergence problems were detected.

## 3. Results

### 3.1. Levels of contamination of game meat, first statistical analysis

On a total of 11 676 microbial analyses, 5604 were reported as numerical values in a log count format, 5812 were 'below the detection limit' and then analyzed subsequently as censored data with an upper limit (for example $<1$ log CFU/cm$^2$), and 260 as censored with a lower limit ($>$4.8 log CFU/cm$^2$ or $>$4.2 log CFU/g).

**Table 3**
Standard deviation ($\sigma^{w}$) and mean contamination levels ($\theta$) according to wild game species, results presented for each bacterium and for both sampling protocols. First statistical analysis (Eqs. (1) and (2)).

| Bacterium | Sampling protocol | Standard deviation of contamination levels (5% and 95% credible intervals) | Wild game species | Mean contamination levels (5% and 95% credible intervals) |
|---|---|---|---|---|
| *E. coli* | 2005–2006 | 1.38 (1.32, 1.43) | Red deer | 1.62 (1.01, 2.21) |
| | | | Roe deer | 2.03 (1.43, 2.62) |
| | | | Wild boar | 1.05 (0.20, 1.87) |
| | 2006–2007 | 1.04 (1.01, 1.08) | Red and roe deer | 2.78 (1.90, 3.61) |
| | | | Wild boar | 2.66 (1.66, 3.61) |
| Th. coliforms | 2005–2006 | 1.34 (1.29, 1.39) | Red deer | 1.98 (1.35, 2.58) |
| | | | Roe deer | 2.37 (1.75, 2.97) |
| | | | Wild boar | 1.68 (0.81, 2.53) |
| C + staphylococci | 2005–2006 | 0.93 (0.87, 0.99) | Red deer | −0.07 (−0.60, 0.46) |
| | | | Roe deer | 0.08 (−0.48, 0.62) |
| | | | Wild boar | 0.00 (−0.78, 0.77) |
| | 2006–2007 | 0.75 (0.71, 0.8) | Red and roe deer | 0.38 (−0.41, 1.15) |
| | | | Wild boar | 0.43 (−0.45, 1.30) |
| *C. perfringens* | 2005–2006 | 1.64 (1.57, 1.72) | Red deer | 0.81 (0.06, 1.56) |
| | | | Roe deer | 1.52 (0.78, 2.26) |
| | | | Wild boar | 0.68 (−0.37, 1.73) |
| | 2006–2007 | 1.37 (1.3, 1.44) | Red and roe deer | 1.11 (0.06, 2.11) |
| | | | Wild boar | 1.50 (0.32, 2.64) |
| *L. monocytogenes* | 2006–2007 | 2.03 (1.72, 2.39) | Red and roe deer | −3.57 (−5.07, −2.12) |
| | | | Wild boar | −2.64 (−4.29, −1.10) |

The mean contamination levels ($\theta$) of meat are reported for the various bacteria and wild game species in Table 3. Explicative factors had no significant effect due to high *intra* variability (reported as the standard deviation, $\sigma^w$, in Table 3). Thermotolerant coliforms were the most numerous contaminants overall while *L. monocytogenes* was the least numerous. In the case of *L. monocytogenes*, the predicted log values were negative for the contamination of red and roe deer meat. These negative values result from the choice of analyzing the whole data set, including the 'below the detection limit' results (less than 1 log CFU/g) as probability distributions of contamination levels.

In terms of microbial quality, hygiene and good handling indicators, *C. perfringens* was found in the highest quantities. The means of contamination were estimated to be 0.81–1.52 log CFU/cm$^2$, depending on the game species, for samples collected during the 2005–2006 protocol and 1.11–1.50 log CFU/g for samples from the 2006–2007 protocol (Table 3).

Predicted contamination levels obtained for coagulase + - staphylococci and *C. perfringens* are presented in Figs. 1 and 2 where the predicted contamination level ($Y$ in Eq. (1)) is plotted for the three animal species, and the two types of meat pieces (Fig. 1) or two types of retail-ready meats (Fig. 2), and two storage conditions. Although there was no significant effect of any of the three factors studied in the first statistical analysis, there were some differences between the treatments. For instance, in relation to the storage conditions, the level of contamination when the meat was frozen was slightly lower than when the meat was chilled. This difference was observed for coagulase + staphylococci on forequarters of wild boar and red deer (Fig. 1a), and also with coagulase + staphylococci on wild boar stewing meat (Fig. 2a) and with *C. perfringens* on wild boar stewing meat (Fig. 2b). There was also a slight difference in contamination levels according to the game species. On the basis of the 2005–2006 protocol, where the red and roe deer species were kept separate, it might be concluded that the roe deer meat was slightly more highly contaminated than the red deer and wild boar meat (Fig. 1a and b and also Table 3).

*E. coli* and thermotolerant coliforms were enumerated in the 2005–2006 protocol. Consequently, comparison between these two microbiological contaminant indicators was possible. Not surprisingly, the level of contamination due to thermotolerant coliforms was higher than the level of *E. coli*, but more importantly the levels of contamination were linearly correlated ($\rho = 0.95$). This is the reason why analyses were performed only on *E. coli* in the 2006–2007 protocol.

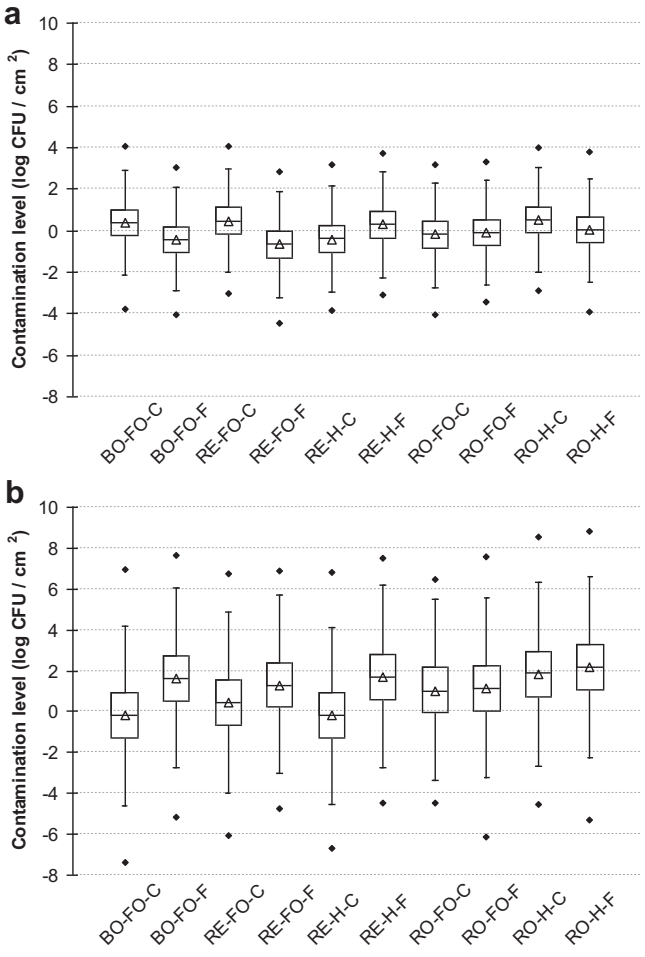


**Fig. 1.** Boxplots representing the predicted contamination levels of coagulase + - staphylococci (a) and *C. perfringens* (b) in wild game meat from the 2005–2006 sampling protocol, derived from the first statistical analysis. BO = wild boar, RE = red deer, RO = roe deer, FO = forequarter, H = haunch, C = chilled, F = frozen. In a boxplot representation, the bottom and top of the box are the 25th and 75th percentiles ($Q_1$ and $Q_3$ respectively), the band in the box is the median and the triangular symbol the mean. The ends of the whiskers represent the lowest figure still within 1.5 IQR (Inter Quartile Range, IQR = $Q_3 - Q_1$) of the lower quartile, and the highest figure still within 1.5 IQR of the upper quartile. The dots correspond to the min. and max. of all data.

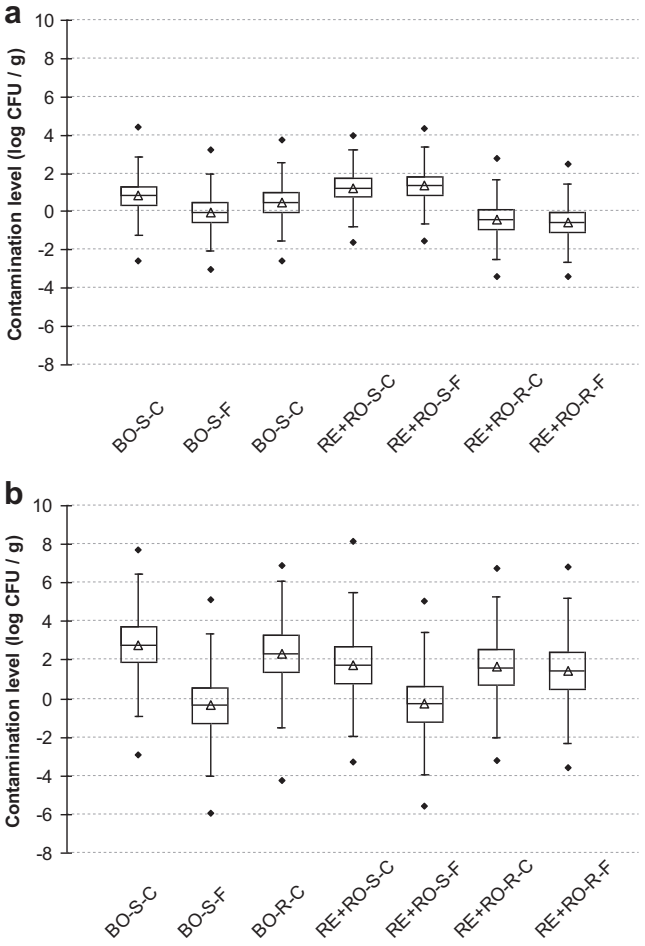


**Fig. 2.** Boxplots representing the predicted contamination levels of coagulase + - staphylococci (a) and *C. perfringens* (b) in wild game meat from the 2006–2007 sampling protocol, derived from the first statistical analysis. BO = wild boar, RE + RO = red and roe deer, S = stewing meat, R = roasting meat, C = chilled, F = frozen. Explanations on boxplot representation are provided in Fig. 1.

### 3.2. Effects of country of origin and hunting season, second statistical analysis

The analysis of the effects of 'game species', 'type of piece or portion' and 'storage' factors showed relatively high variability in contamination levels. It was therefore decided to investigate further this source of variability by performing a second analysis (Eqs. (3) and (4)). Two extra factors (country of origin and hunting season) were added at this stage. The countries were split into four groups and the hunting season into three seasons. The main results of the second analysis are presented in Tables 4 and 5. In this second statistical analysis, the influence of the country of origin on the standard deviation of the residual error was assumed. This hypothesis was based upon preliminary data analysis (not shown) and also on information from the industrial partners associated with the study, who suggested that variability of contamination levels might depend on hunting forest management and on hunting practice, both varying between the countries. This hypothesis was confirmed as the standard deviation of the residual error ($\sigma^W$) differed significantly according to the country; for instance $\sigma^W$ was estimated to be 1.16 for *E. coli* in the 2005–2006 sampling protocol (Table 4) (with 5 and 95% credible interval limits of 1.08 and 1.23) in countries in group 1 and 1.33 (1.24 and 1.44) in France. Similarly $\sigma^W$ varied from 1.28 (1.20–1.37) in group 1 countries to 1.60 (1.45–1.76) in France for *C. perfringens*. No country or group of countries had significantly higher (or lower) contamination levels.

Finally, due to the relatively high *intra* variability, itself due to the uncertainty generated by an insufficient quantity of data for each combination of factors, it was not possible to establish whether there was a hunting season effect.

## 4. Discussion

The contamination rates for raw game meat in this study were in agreement with values reported in the literature. The levels of *Enterobacteriaceae* found in game carcasses in Austria were of the same order of magnitude as the levels of thermotolerant coliforms obtained in our study (Paulsen and Winkelmayer, 2004). Also, Austrian carcasses tested negative for *L. monocytogenes*, which is in agreement with our low estimated of numbers of *L. monocytogenes*. In an exploratory survey carried out in France in 2002–2004, the levels of contamination with *C. perfingens*, *S. aureus* and *E. coli* in wild boar and red/roe deer (Direction Générale de l'Alimentation, 2004) were similar to ours.

Although not statistically significant, storage temperature seems to have an impact on meat hygiene quality, and this might be interpreted as an effect of freezing conditions on the contamination level. However, it is important to emphasize that in the case of staphylococci there is no evidence of sub-lethal damage due to the freezing conditions. On the other hand, according to freezing practices, it is meat of high hygienic quality which is frozen and this might explain why a difference (although not significant) in contamination level was observed according to storage conditions. More generally, the temperature, and the speed at which meat is cooled is an important factor (Paulsen and Winkelmayer, 2004).

From our results in large game species we might conclude that the levels of contamination in roe deer were slightly higher than in the other two species, whereas Paulsen and Winkelmayer reported the opposite (Paulsen and Winkelmayer, 2004). The second statistical analysis revealed a country (or group of countries) effect on the standard deviation of the residual error. High variations in

**Table 4**
Standard deviation ($\sigma^W$) and mean contamination levels ($\theta$) of samples according to country and wild game species, results presented for each bacterium and for the first sampling protocol. Second statistical analysis (Eqs. (3) and (4)).

| Group of countries | Bacterium | Standard deviation of contamination levels: mean and 95% credible intervals | Wild game species | Mean contamination levels: mean and 95% credible intervals |
|---|---|---|---|---|
| Country group 1: Great Britain, Hungary, Germany, Czech Republic, Austria and Australia; 497 data sets | *E. coli* | 1.16 (1.08, 1.23) | Red and roe deer | 2.20 (1.65, 2.76) |
| | | | Boar | 1.31 (0.48, 2.15) |
| | Th. coliforms | 1.07 (1.00, 1.13) | Red and roe deer | 2.56 (2.00, 3.10) |
| | | | Boar | 1.80 (0.95, 2.63) |
| | C + staphylococci | 0.66 (0.59, 0.73) | Red and roe deer | 0.21 (−0.23, 0.64) |
| | | | Boar | −0.35 (−1.09, 0.34) |
| | *C. perfringens* | 1.28 (1.20, 1.37) | Red and roe deer | 1.32 (0.57, 2.06) |
| | | | Boar | 0.72 (−0.37, 1.80) |
| Country group 2: Spain; 286 data sets | *E. coli* | 1.32 (1.22, 1.44) | Red and roe deer | 2.36 (1.75, 2.97) |
| | | | Boar | 2.07 (0.72, 3.42) |
| | Th. coliforms | 1.31 (1.21, 1.42) | Red and roe deer | 2.83 (2.20, 3.48) |
| | | | Boar | 2.77 (1.45, 4.09) |
| | C + staphylococci | 0.96 (0.83, 1.12) | Red and roe deer | 0.11 (−0.41, 0.61) |
| | | | Boar | 0.20 (−0.95, 1.29) |
| | *C. perfringens* | 1.94 (1.75, 2.14) | Red and roe deer | 1.37 (0.50, 2.22) |
| | | | Boar | 1.40 (−0.35, 3.21) |
| Country group 3: France; 421 data sets | *E. coli* | 1.33 (1.24, 1.44) | Red and roe deer | 1.64 (0.95, 2.34) |
| | | | Boar | 1.69 (0.61, 2.78) |
| | Th. coliforms | 1.28 (1.20, 1.38) | Red and roe deer | 1.94 (1.22, 2.63) |
| | | | Boar | 2.25 (1.17, 3.31) |
| | C + staphylococci | 1.07 (0.96, 1.20) | Red and roe deer | −0.13 (−0.70, 0.43) |
| | | | Boar | 0.35 (−0.53, 1.20) |
| | *C. perfringens* | 1.60 (1.45, 1.76) | Red and roe deer | 0.58 (−0.38, 1.52) |
| | | | Boar | 1.01 (−0.38, 2.40) |
| Country group 4: other countries, including undetermined EU countries; 166 data sets | *E. coli* | 0.90 (0.80, 1.01) | Red and roe deer | 1.46 (0.62, 2.28) |
| | | | Boar | 1.02 (−0.13, 2.19) |
| | Th. coliforms | 0.82 (0.73, 0.91) | Red and roe deer | 1.73 (0.92, 2.56) |
| | | | Boar | 1.44 (0.27, 2.58) |
| | C + staphylococci | 0.87 (0.70, 1.09) | Red and roe deer | −0.24 (−0.94, 0.44) |
| | | | Boar | −1.13 (−2.39, 0.00) |
| | *C. perfringens* | 1.01 (0.89, 1.14) | Red and roe deer | 1.12 (0.00, 2.18) |
| | | | Boar | 1.28 (−0.22, 2.74) |

**Table 5**
Standard deviation ($\sigma^w$) and mean contamination levels ($\theta$) of samples according to country and wild game species, results presented for each bacterium and for the second sampling protocol. Second statistical analysis (Eqs. (3) and (4)).

| Group of countries | Bacterium | Standard deviation of contamination levels (5% and 95% credible intervals) | Wild game species | Mean contamination levels (5% and 95% credible intervals) |
|---|---|---|---|---|
| Country group 1: Great Britain, Hungary, Germany, Czech Republic, Austria and Australia; 445 data sets | *E. coli* | 0.59 (0.55, 0.62) | Red and roe deer | 2.61 (1.45, 3.75) |
| | | | Boar | 1.17 (−0.69, 3.00) |
| | C + staphylococci | 0.60 (0.56, 0.65) | Red and roe deer | 0.95 (−0.36, 2.02) |
| | | | Boar | 0.02 (−1.65, 1.67) |
| | *C. perfringens* | 1.17 (1.03, 1.34) | Red and roe deer | 0.41 (−0.83, 1.65) |
| | | | Boar | 0.00 (−2.03, 2.04) |
| | *L. monocytogenes* | 1.18 (0.83, 1.60) | Red and roe deer | −1.64 (−3.39, −0.07) |
| | | | Boar | −3.24 (−6.71, −0.13) |
| Country group 2: Spain; 226 data sets | *E. coli* | 0.80 (0.73, 0.88) | Red and roe deer | 3.06 (2.17, 3.94) |
| | | | Boar | 3.48 (2.02, 4.84) |
| | C + staphylococci | 0.75 (0.48, 1.14) | Red and roe deer | −0.69 (−1.99, 0.43) |
| | | | Boar | −2.39 (−6.46, 0.51) |
| | *C. perfringens* | 0.73 (0.66, 0.81) | Red and roe deer | 2.03 (1.04, 2.99) |
| | | | Boar | 3.04 (1.52, 4.50) |
| | *L. monocytogenes* | 0.82 (0.50, 1.25) | Red and roe deer | −4.00 (−6.97, −1.23) |
| | | | Boar | −2.55 (−5.83, 0.15) |
| Country group 3: France; 381 data sets | *E. coli* | 0.90 (0.84, 0.97) | Red and roe deer | 2.98 (1.60, 4.35) |
| | | | Boar | 3.21 (2.38, 4.05) |
| | C + staphylococci | 0.64 (0.52, 0.79) | Red and roe deer | 0.14 (−1.15, 1.39) |
| | | | Boar | 0.47 (−0.43, 1.24) |
| | *C. perfringens* | 1.08 (1.00, 1.16) | Red and roe deer | 2.18 (0.69, 3.68) |
| | | | Boar | 1.86 (0.94, 2.78) |
| | *L. monocytogenes* | 2.00 (1.66, 2.43) | Red and roe deer | −3.74 (−6.23, −1.44) |
| | | | Boar | −2.37 (−4.03, −1.04) |
| Country group 4: other countries, including undetermined EU countries; 497 data sets | *E. coli* | 1.28 (1.20, 1.37) | Red and roe deer | 2.88 (1.71, 4.03) |
| | | | Boar | 2.68 (1.23, 4.15) |
| | C + staphylococci | 0.96 (0.86, 1.07) | Red and roe deer | −0.06 (−1.29, 1.04) |
| | | | Boar | −0.47 (−2.01, 0.97) |
| | *C. perfringens* | 0.63 (0.56, 0.70) | Red and roe deer | 1.62 (0.37, 2.86) |
| | | | Boar | 1.40 (−0.10, 2.91) |
| | *L. monocytogenes* | 0.98 (0.71, 1.39) | Red and roe deer | −1.19 (−2.92, 0.45) |
| | | | Boar | −1.47 (−3.60, 0.52) |

the contamination levels of wild game meat have already been reported (Direction Générale de l'Alimentation, 2004; Paulsen and Winkelmayer, 2004). Such variations may correspond to variations in forest management between countries but they are more likely to be due to variations in shooting practices. Indeed, in some countries large animal shooting is reserved for specialists, while in others non-shooting experts are allowed to shoot game; this has consequences for microbiological contamination of the meat, with possible cross-contamination from the gastro-intestinal tract to muscles (Gill, 2007). In fact, it has been reported that 'a non-expertly shot animal showed a shot in the abdomen with severe damage and faecal spoiling' (Atanassova et al., 2008).

In the statistical analysis of the game meat contamination rate, the data from the two successive sampling protocols were considered as continuous variables (levels of contamination) and not as discrete variables (prevalence rate and/or class of contamination). In this approach, the numerical data (expressed either in log CFU/cm$^2$ or in log CFU/g) were combined with the 'less than detection limit' data (analyzed as censored data) to generate a single probability distribution of the contamination levels of game meat. This made it possible to incorporate the results in an exposure assessment model in order to calculate the level of contamination in the ready-to-eat portion once the meat is cooked by the consumer. There are several methods to deal with such censored data, non-detectable measurements can be considered equal to either the detection limit or some value below the detection limit. Alternatively, non-detectable microbial population measurements may be completely ignored. Such approaches yield biased estimates of the population parameters (Shorten et al., 2006). Busschaert et al. (2011) recently analyzed microbiological contamination data by comparing a Bayesian analysis with a maximal likelihood estimation procedure. They concluded that the two methods lead to very similar results, are convenient to implement, and finally should be encouraged when censored data sets are analyzed. More importantly, they emphasized that for further use of contamination levels in risk assessment, contamination level data must be analyzed in such a way that probability distributions are generated. The probability distributions obtained in our analysis, for example with *L. monocytogenes*, were rather wide. This was the consequence of the uncertainty generated in the data set analysis, although the data set was fairly large (1549 samples). The lowest limit of the credible interval (−5.07, Table 3) might seem unrealistic, but it is important to keep in mind that the results of this contamination level analysis might subsequently be incorporated into an exposure assessment model to assess whether there is a public health issue. When doing so, it is not the lowest limit of the credible interval which has to be scrutinized but the highest (−2.12 log CFU/g or 1 CFU per 132 g).

More generally, Bayesian techniques have been already used in microbiological risk assessment, for example to construct a hierarchical model (Crépet et al., 2009) or to incorporate prior information in the estimation process (Delignette-Muller et al., 2006). They have been acknowledged as valuable methods to articulate probability distributions (e.g. contamination levels), uncertainty (e.g. due to censored data) and variability (e.g. due to biological materials) in a transparent manner, and consequently to achieve results from complex data set analysis to use in an exposure assessment model.

In conclusion, the bacteriological contamination of large wild game meat distributed in France was determined from an analysis

of 2919 samples, the indicators of microbial quality, hygiene and good handling showing that *C. perfringens* was found in the highest quantities and, *L. monocytogenes* in the lowest. It can be suggested that improving hunting practices across European countries and encouraging good hygiene practices would maintain the microbiological quality of large wild game meat. The results could also be incorporated in an exposure assessment to determine whether there are public health issues.

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