B A
S E

Biotechnol. Agron. Soc. Environ. 2013 **17**(3), 441-449

Bacteriological assessment of smoked game meat in Lubumbashi, D.R.C.

Rosette Kabwang a Mpalang [1, 2], Mireille Kakubu a Mpalang [3, 4], Clarence Mukeng Kaut [3], Raphaël Boreux [1], Pierrette Melin [1], Faustin Khang'mate Akir Ni Bitiang [2], Georges Daube [5], Patrick De Mol [1]

[1] Univ. Liege. Laboratory of Medical Microbiology. CHU Sart-Tilman B23. B-4000 Liege (Belgium). E-mail: rosettekabwang@yahoo.fr
[2] University of Lubumbashi. Faculty of Veterinary Medicine. Laboratory of Expertise, Hygiene and Technology of Foods from Animal Origin. P.O. Box 1825. Lubumbashi (Democratic Republic of Congo).
[3] University of Lubumbashi. Faculty of Medicine. Department of Basic Sciences. P.O. Box 1825. Lubumbashi (Democratic Republic of Congo).
[4] Ministry of Health and Social Services Namibia. P.O.Box 70762. Windhoek (Namibia).
[5] Univ. Liege. Faculty of Veterinary Medicine. Food Science Department-Microbiology. Sart-Tilman B43 bis. B-4000 Liege (Belgium).

Received on December 21, 2012; accepted on May 8, 2013.

The bacteriological quality of smoked game meat in Lubumbashi has not been studied much to date. The present study focused on the analysis of 182 samples of smoked game meat from three species, *Syncerus caffer* (n = 63), *Phacochoerus aethiopicus* (n = 60) and *Sylvicapra grimmia* (n = 59), sold at retail outlets in Lubumbashi. The isolation of *Escherichia coli* from 81.3% of samples (mean 4.87 ± 0.6 $\log_{10}$ CFU·g^{-1} of sample) confirms significant faecal contamination of smoked game meat. The study has determined by culture prevalences of 0.0%, 4.3% [$CI_{95\%}$ 1.4-7.4], 3.8% [$CI_{95\%}$ 1.1-6.6] and 14.2% [$CI_{95\%}$ 9.2-19.4] respectively for Shiga toxigenic *Escherichia coli* (STEC), *Salmonella* spp., *Campylobacter jejuni* and *Campylobacter coli*. Using Polymerase Chain Reaction, these prevalences were of 2.2% [$IC_{95\%}$ 0.1-4.3], 6.0% [$IC_{95\%}$ 2.6-9.5], 3.8% [$IC_{95\%}$ 1.1-6.6] and 15.9% [$IC_{95\%}$ 10.6-21.3] respectively for STEC, *Salmonella* spp., *C. jejuni* and *C. coli*. *Syncerus caffer* was established as a potential vehicle of STEC carrying *stx1* gene (3.2%), *stx2* gene (1.6%) and the combination of *stx2* and *eae* genes (1.6%). On the basis of these data, we suggested the need for developing monitoring plans of the production, preparation, handling and distribution of smoked game meat in Lubumbashi.
Keywords. Smoked meat, game meat, bacteriological analysis, PCR, epidemiology, D.R.C.

Qualité bactériologique de la viande boucanée de gibier à Lubumbashi, R.D.C. La qualité bactériologique de la viande boucanée de gibier n'était pas bien connue à Lubumbashi jusqu'à présent. Pour cette étude, 182 échantillons de viande boucanée provenant de trois espèces de gibier, à savoir *Syncerus caffer* (n = 63), *Phacochoerus aethiopicus* (n = 60) et *Sylvicapra grimmia* (n = 59) ont été achetés au détail dans les marchés de Lubumbashi et analysés. Le dénombrement de *Escherichia coli* (en moyenne 4,87 ± 0,6 $\log_{10}$ CFU·g^{-1} d'échantillon) dans 81,3 % d'échantillons montre une contamination fécale significative de viandes boucanées de gibier. Pour l'ensemble de l'échantillonnage, nous avons déterminé par culture des prévalences de 0,0 %, 4,3 % [$IC_{95\%}$ 1,4-7,4], 3,8 % [$IC_{95\%}$ 1,1-6,6] et 14,2 % [$IC_{95\%}$ 9,2-19,4] respectivement pour *Escherichia coli* producteur de Shiga toxine (STEC), *Salmonella* spp., *Campylobacter jejuni* and *Campylobacter coli*. Par contre, à l'aide de la PCR (*Polymerase Chain Reaction)*, ces prévalences ont été de 2,2 % [$IC_{95\%}$ 0,1-4,3], 6,0 % [$IC_{95\%}$ 2,6-9,5], 3,8 % [$IC_{95\%}$ 1,1-6,6] et 15,9 % [$IC_{95\%}$ 10,6-21,3] respectivement pour STEC, *Salmonella* spp., *C. jejuni* et *C. coli*. *Syncerus caffer* a été établi comme vecteur potentiel de STEC portant les gènes *stx1* (3,2 %), *stx2* (1,6 %) et la combinaison des gènes *stx2* et *eae* (1,6 %). Sur base de ces données, nous avons suggéré la nécessité de développer des plans de surveillance pour la production, la préparation et la distribution des viandes boucanées de gibier à Lubumbashi.
Mots-clés. Viande fumée, viande de gibier, analyse bactériologique, PCR, épidémiologie, R.D.C.

1. INTRODUCTION

Wild mammals contribute widely to the diet of African populations (Bachand et al., 2012). Their meat is considered to be a good alternative to beef meat (Onyango et al., 1998). Chomel et al. (2007) have reported that in the Congo basin, trade and consumption of game meat could reach up to 4.5 million tons annually. In Katanga, the southern province of Democratic Republic of Congo (D.R.C.), smoked game meat is generally provided by rural populations living around woodlands. In Lubumbashi, the main city of Katanga, as well as in others countries of Central Africa, smoked game meat is highly appreciated by consumers because of its organoleptic characteristics, such as taste, smell and color. Smoked game meat is of great value for traditional ceremonies or when welcoming an important host. Traditional and fetish practitioners often include it in magical preparations, illustrating its major importance in superstitious beliefs. Smoking is a transformation process for meat aimed at prolonging their shelf life and improving their organoleptic properties. This procedure was developed because of the lack of an appropriate storage system for transporting meat, as hunting is usually done in villages far from urban centers. Thus, game meat could be transported to dealers in urban centers in the form of dried or smoked meat. The process of smoking game meat consists of boiling the game meat, draining it, then placing it on metal sieves above a low heat. Smoke is produced by burning wood or sawdust beneath the sieves.

Numerous factors may be responsible for the spoilage of fresh game meat, such as conditions of evisceration, and exposure to ambient temperature and relative humidity. Macroscopic alterations like the presence of moisture, mould, maggots and a nauseating smell are often observed on the meat at the selling point to consumers. This situation can be explained by illegal practices by salesmen, which involve injection of water into smoked game meat or soaking of smoked game meat in order to add volume to the dried muscle tissue. Consumption of such meat can constitute a threat to public health. However, there is no study conducted to assess the bacteriological quality of this type of meat in Lubumbashi. Although zoonotic transmission of enteric pathogens to human through the consumption of contaminated food such as meat has been reported (Ojo et al., 2010). In developed world, studies focusing on the microbiological quality of game meat as a threat to public health have been conducted previously (Atanassova et al., 2008; Miko et al., 2009). Indeed, wildlife could act as a potential source of human Salmonellosis (Hilbert et al., 2012). In developing world, enteric diseases are a major threat to public health (Bachand et al., 2012) and more than 60% of human infectious diseases are shared with wild or domestic animals (Karesh et al., 2012). Pathogenic *Escherichia coli*, *Campylobacter* spp. and *Salmonella* spp. are the most incriminated in gastroenteritis from zoonotic origin (Mohammed, 2012; Goualié et al., 2012). Immunocompromised people and children under the age of 5 are the most affected and 4.9 deaths per 1,000 among children are reported every year (Fhogartaigh et al., 2009).

The objective of this study is to assess, by means of culture and Polymerase Chain Reaction (PCR), the prevalence of major Enterobacteriaceae such as STEC and *Salmonella*, as well as other major foodborne pathogens such as *Campylobacter jejuni* and *Campylobacter coli* in smoked game meat sold at retail outlet in Lubumbashi.

2. MATERIALS AND METHODS

2.1. Sample collection

In the city of Lubumbashi, game meat is mainly sold in the central market of Lubumbashi municipality and in the central market of Kenya municipality; both sites were used to acquire samples of game meat. A total of 182 samples of smoked game meat were collected between December 2009 and December 2010 in the city of Lubumbashi (D.R.C.). Ninety-six samples were collected from the municipality of Kenya and 86 from the municipality of Lubumbashi. Sampling was performed monthly, 3 to 6 samples of each type of game meat were collected on the day of harvest according to the availability of meats on the market. Stalls selling smoked game meat are located in semi-open sheds, among stalls of other foods, including dried and salted fish, fried or dried and smoked fish. Various game meats like *Potamocherus porcus* (Red River Hog), *Cephalopus* sp. (Duiker), *Lepus saxatilis* (Scrub Hare), *Paraxerus boehmi* (Boehm's Bush Squirrel) and *Cryptomys hottentotus* (African Mole Rat) are consumed in Lubumbashi. But we have included in this study three species that are common and sold in retail cuts. These included *Syncerus caffer* (African buffalo called *Mbo* in Swahili, the local language of Lubumbashi; n = 63), *Phacochoerus aethiopicus* (warthog or *Lupenge* in Swahili; n = 60) and *Sylvicapra grimmia* (common duiker or *Nkasha* in Swahili; n = 59). Smoked meat is sold as specified cuts like thigh, shoulder and ribs, but also as unspecified retail cuts of 50 to 100 g, hand-separated or separated with a sharp instrument such as a knife, axe, machete or hacksaw. We have included in this study only unspecified retail cuts of smoked game meat prior to sale.

2.2. Sample preparation and enrichment procedure

Analyses were performed at the Laboratory of Expertise, Hygiene and Technology of Food from Animal Origin, University of Lubumbashi. All samples were transported to the laboratory in an isothermal box at 4 °C and analyzed within 2 to 4 h after collection.

Isolation, enumeration and presumptive identification of *E. coli*. The isolation of *E. coli* was performed on samples diluted in Buffered Peptone Water (BPW) (dilution 10^{-1}) and further diluted in sterile water up to 10^{-4}. Diluted samples were then spread onto Violet Red Bile Agar with Lactose (VRBL) (Merck, Darmstadt, Germany) and incubated for 24 h at 44 °C. Violet colonies of 5 mm in diameter were counted. For presumptive identification 1 to 4 violet colonies were streaked onto Eosin Methylene Blue (EMB) agar (Merck) and incubated at 37 °C for 24 h (protocol of enumeration of *E. coli* in the Laboratory of Expertise, Hygiene and Technology of Food from Animal Origin, University of Lubumbashi). Green colonies with a metallic sheen were characterized as *E. coli* using the API 20E (bioMérieux, France). Data on the enumeration of *E. coli* were entered into Excel and transformed into $\log_{10}$ Colony-Forming Units per gram ($CFU \cdot g^{-1}$) of food sample.

According to Congolese criteria, an *E. coli* colony count between 2.75 to 3.70 $\log_{10}$ $CFU \cdot g^{-1}$ in a food sample is considered high and above 3.70 $\log_{10}$ $CFU \cdot g^{-1}$ the food is then declared unsuitable for human consumption. For the interpretation of our results, we considered positive only the samples that showed a count above 3.70 $\log_{10}$ $CFU \cdot g^{-1}$.

Isolation and presumptive identification of STEC. A quantity of 25 g of each sample was mixed with 225 ml of BPW supplemented with vancomycin, cefsulodin and cefixime (BPW-VCC) according to the protocol of Chapman (1995), and then homogenized using a Stomacher 400 (Seward, London, UK). A 1 ml volume of the BPW-VCC mixture was then enriched in brain heart broth (Merck) in order to enhance the bacterial level necessary for PCR sensitivity. The enrichment mixture was incubated at 37 °C for 18 to 24 h and each enriched sample was stored at -20 °C for further characterization by PCR in the Laboratory of Medical Microbiology, University of Liege. The interpretation of PCR results was based on the "presence" of "absence" of the target gene in a 25 g sample. Isolation of STEC was carried out on sorbitol MacConkey Agar (SMAC) (Merck). The characterization of *E. coli* strain was carried out with the API 20E (bioMérieux). In addition, agglutination of O157 antigen using dry SpotTM for identification of *E. coli* O157 (Oxoid, Basingstoke, UK) was used for colonies suspected to be STEC of O157 serotype.

Isolation and presumptive identification of *Salmonella* spp. A quantity of 25 g of each sample was mixed with 225 ml of BPW. Then the mixture was homogenized using a Stomacher 400 (Seward). Following the techniques described by Woldemariam et al. (2005), 1 ml of the BPW mixture was enriched in selenite broth (Merck) and incubated at 37 °C for 18 to 24 h. Enriched samples were stored at -20 °C for further characterization by PCR in the Laboratory of Medical Microbiology, University of Liege. Cultures of *Salmonella* were performed on Xylose-Lysine-Deoxycholate (XLD) agar (Merck) and incubated at 37 °C for 24 h. The characterization of *Salmonella* was achieved using the API 20E (bioMérieux).

Isolation and presumptive identification of *Campylobacter* spp. A quantity of 25 g of each sample was mixed with 225 ml of BPW and homogenized using a Stomacher 400 (Seward). A volume of 1 ml of the mixture was further enriched in Bolton broth (Oxoid, Basingstoke, UK) and incubated at 42 °C for 18 to 24 h under microaerophilic conditions. Enriched samples were stored at -20 °C for further characterization by PCR in the Laboratory of Medical Microbiology, University of Liege. *Campylobacter jejuni* and *C. coli* were isolated on Blood Free *Campylobacter* selective agar base (Merck) supplemented with Charcoal Cefoperazone Deoxycholate Agar Supplement (CCDA selective supplement) (Merck) and incubated at 42 °C for 48 h in a microaerophilic atmosphere, using GENbox microaer, atmosphere generators (bioMérieux). The morphology of colonies, the motile characteristics and biochemical tests, such as production of oxidase (Merck) and catalase (Merck) were used to confirm *Campylobacter* spp. Then, the hippurate hydrolysis test (Rosco, Taasturp, Denmark) was used to differentiate between *C. jejuni* and *C. coli*, following the protocols described by Sallam (2007) and Enokimoto et al. (2007).

2.3. DNA extraction

DNA was directly extracted from enriched samples. Thus, 200 μl of each enriched sample were centrifuged and the bacterial pellets were collected. For cell lysis, 100 μl of a buffer containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 0.5% Nonidet P40 (Applied Biosystem, Branchburg, USA. Manufactured by Roche®) and 0.5% Tween 20 (Promega®, Madison, USA) supplemented with 3 μl of proteinase K (20 $mg \cdot ml^{-1}$) (Qiagen®, Hilden, Germany) were added to bacterial pellets and heated for 30 min at 60 °C and then 30 min at 100 °C. Cell debris were removed

by centrifugation at 13,000 rpm for 5 min using a centrifuge 5415R (Eppendorf, Hamburg, Germany). The supernatant was used as the DNA template for PCR assays.

2.4. PCR assays

Simplex PCRs for identification of *stx1* and *stx2* genes encoding type I and type II Shiga toxins and for identification of the *eae* gene encoding intimin responsible for lesions of attachment-effacement caused by STEC were developed. Another simplex PCR was developed to detect the *ompC* gene of *Salmonella* spp. Finally, 2 simplex PCR were developed to identify *C. jejuni* and *C. coli* based on *ceuE* genes present in both species. Target genes, primers, primers sequences and amplicon sizes are listed in **table 1**.

2.5. Reference strains

The PCR experiments were performed on reference strains of *E. coli* O157:H7 ATCC 43890, EH 384 and EH 1340; *Salmonella typhi* LO8493, *Salmonella* spp. 04 and *Salmonella* spp. 4818; *C. jejuni* N204, T276 and L359 and *C. coli* Y58, T287 and A747 provided respectively, from the Reference Laboratory of Enterohemorrhagic *Escherichia coli*, VUB (Belgium); the Laboratory of Medical Microbiology, UHC of Liege (Belgium); and the Reference Laboratory of *Campylobacter*, UHC Saint Pierre (Belgium).

2.6. Statistical analysis

Statistical analysis of results was conducted *via* IBM SPSS Statistics (Version 19.0 Copyright©, SPSS Inc., Chicago, USA), and the methods used were the chi-squared contingency table test and Fisher's exact test to determine whether there were associations between prevalence of researched bacteria with species of game meat analysed. The level of agreement according to detection rate by culture and by PCR was expressed using Coehen's kappa coefficient.

To minimize error related to sampling, Confidence Interval (CI) with 95% confidence was calculated as follows:

$$\hat{p}_0 \pm 1{,}96\sqrt{\frac{\hat{p}_0\hat{q}_0}{n}}$$

where p_0 is the observed percentage, q_0 the complementary percentage, n the number of observations and 1.96 the coefficient for a risk error of 5%.

3. RESULTS

Faecal contamination was found in 81.3% of all sampling. The prevalence of *E. coli* in different groups was of 100%, 78.3% and 64.4% respectively from meat of *Syncerus caffer*, *Phacochoerus aethiopicus* and *Sylvicapra grimmia*. The mean count of *E. coli* was ranging from 4.8 ± 0.8 $\log_{10}$ CFU·g^{-1} in the meat of *Syncerus caffer*; 4.8 ± 0.8 $\log_{10}$ CFU·g^{-1} in the meat of *Phacochoerus aethiopicus*; and 4.9 ± 0.9 $\log_{10}$ CFU·g^{-1} in the meat of *Sylvicapra grimmia*. Other findings by culture determined for the all sampling prevalences of 0%, 4.3% and 18.1% respectively for STEC, *Salmonella* spp. and *Campylobacter* spp. Among *Campylobacter* spp., 3.8% were *C. jejuni* and 14.2% were *C. coli*. Nevertheless, it is important to mention that during the whole sampling process no culture could yield positive

Table 1. Target genes, primers sequences and amplicon sizes — *Gènes cibles, séquences des amorces et taille des amplicons*.

Gene	Primer	Sequence of primer	Size of amplicon	Reference
eae	SK1	CCCGAATTCGGCACAAGCATAAGC	863 bp	Zweifel et al., 2006
	SK2	CCCGGATCCGTCTCGCCAGTATTCG		
stx1	KS7	CCCGGATGAAAAAAACATTATTAATAGC	285 bp	Friedrich et al., 2002
	KS8	CCCGAATTCAGCTATTCTGAGTCAACG		
stx2	VT2e	AATACATTATGGGAAAGTAATA	348 bp	Piérard et al., 1998
	VT2f	TAAACTGCACTTCAGCAAAT		
ompC	S29	CAGTATCAGGGCAAAAACGGC	360 bp	Gilbert et al., 2003
	S30	TTCAAAGTTCTGCGCTTTGTT		
ceuE	Cj1	CTGCTACGGTGAAAGTTTTGC	783 bp	Houng et al., 2001
	Cj2	GATCTTTTTGTTTTGTGC		
ceuE	CC2	GATTTTATTATTTGTAGCAGCG	645 bp	Houng et al., 2001
	CC3	TCCATGCCCTAAGACTTAACG		

results for STEC, even though culture tests were specifically performed to identify STEC of serotype O157: absence of fermentation of sorbitol in < 24 h and colorless on Sorbitol MacConkey agar (SMAC). Biochemical and serological tests performed on suspected colonies were negative.

By PCR, we determined for the all sampling prevalences of 2.2%, 6.0% and 19.7% respectively for STEC, *Salmonella* spp. and *Campylobacter* spp. Among *Campylobacter* spp., 3.8% were characterized as *C. jejuni* and 15.9% as *C. coli*. STEC were especially recovered from the meat of *Syncerus caffer* (6.3%) while cultures were negatives. Among the STEC-positive samples, two were carrying the *stx1* gene (3.2%), one was harboring the *stx2* gene (1.6%) and the last one was carrying the combination of *stx2* and *eae* genes (1.6%). Comparing both methods, agreement between the PCR and the cultural method was observed ($\varkappa = 0.834$, $\varkappa = 1.000$ and $\varkappa = 0.936$ respectively for *Salmonella*, *C. jejuni* and *C. coli*), although the detection rate was slightly higher by PCR than by culture (**Table 2**).

The highest prevalence of *Salmonella* (10%), *C. jejuni* (6.7%) and *C. coli* (20.0%) were found by PCR in meat of *Phacochoerus aethiopicus*, there were statistically no difference of level of contamination compared to other types of smoked game meat (p = 0.44, p = 0.13 and p = 0.39 respectively). A significant difference was observed in prevalence of STEC in meat of *Syncerus caffer* compared to other types of smoked game meat (p = 0.02). In the other hand, the association between the prevalence of *E. coli* and the prevalence of pathogens was not established (p = 0.33; p = 0.10; p = 0.76 and p = 0.24 respectively for STEC, *Salmonella*, *C. jejuni* and *C. coli*).

4. DISCUSSION

Bacteriological analyses by culture and PCR were conducted to assess the contamination of smoked game meat by STEC, *Salmonella*, *C. jejuni* and *C. coli* in Lubumbashi. Our findings showed higher prevalences of these pathogens

Table 2. Prevalence of *Escherichia coli*, STEC, *Salmonella* and *Campylobacter* determined by culture and PCR. Positive numbers/tested numbers(%) — *Prévalence des* Escherichia coli, *STEC*, Salmonella *et* Campylobacter *déterminée par culture et par PCR. Nombres positifs/nombres testés (%).*

Sample type	*E. coli**	STEC		*Salmonella* spp.		*Campylobacter* spp.				Total	
						C. jejuni		*C. coli*			
	Culture	Culture	PCR	Culture	PCR	Culture	PCR	Culture	PCR	Culture	PCR
Syncerus caffer (n = 63)	63/63 (100)	0/63 (0)	4/63 (6.3)**	2/63 (3.1)	2/63(3.1)***	0/63 (0)	0/63 (0)	6/63 (9.5)	7/63 (11.1)***	6/63 (9.5)	7/63 (11.1)
$CI_{95\%}$	-	-	[0.3 - 12.3]	[0 - 7.5]****	[0 - 7.5]****	-	-	[2.3 - 16.8]	[3.4 - 18.9]	-	-
Phacochoerus aethiopicus (n = 60)	47/50 (78.3)	0/60 (0)	0/60 (0)**	3/60 (5.0)	6/60 (10.0)***	4/60 (6.7)	4/60 (6.7)***	12/60 (20.0)	12/60 (20.0)***	16/60 (26.6)	16/60 (26.6)
CI 95%	-	-	-	[0 - 10.5]****	[2.4 - 17.6]	[0.4 - 13.0]	[0.4 - 13.0]	[9.9 - 30.1]	[9.9 - 30.1]	-	-
Sylicapra grimmia (n = 59)	38/59 (64.4)	0/59 (0)	0/59 (0)**	3/59 (5.1)	3/59 (5.1)***	3/59 (5.1)	3/59 (5.1)***	8/59 (13.5)	10/59 (16.9)***	11/59 (18.6)	13/59 (22.0)
$CI_{95\%}$	-	-	-	[0.5 - 10.7]	[0.5 - 10.7]	[0.5 - 10.7]	[0.5 - 10.7]	[4.8 - 22.3]	[7.4 - 26.5]	-	-
Total (n = 182)	148/182 (81.3)	0/182 (0)	4/182 (2.2)	8/182 (4.3)***	11/182 (6.0)***	7/182 (3.8)	7/182 (3.8)	26/182 (14.2)***	29/182 (15.9)***	33/182 (18.1)	36/182 (19.7)
$CI_{95\%}$	[75.7-87.0]	-	[0.1 - 4.3]	[1.4 - 7.4]	[2.6 - 9.5]	[1.1 - 6.6]	[1.1 - 6.6]	[9.2 - 19.4]	[10.6 - 21.3]	-	-

*: count > 3.70 $\log_{10}$ CFU·g^{-1} — *dénombrement* > 3,70 $\log_{10}$ CFU·g^{-1}; **: significant difference p < 0.05 — *différence significative p < 0,05*; ***: no significant difference (p > 0.05) — *différence non significative (p > 0,05)*; ****: negative lower endpoint of CI, represented by 0 — *limite inférieure négative de l'IC représenté par 0.*

by PCR than by cultural method. We can explain these findings by the sensitivity of PCR as a diagnostic tool, which has been demonstrated in many studies (Debruyne et al., 2008; Shelton et al., 2008; Karmali et al., 2010).

Faecal contamination of smoked game meat was assessed by the count of *E. coli* colonies. In the all sampling the mean count of *E. coli* was ranging from 4.8 ± 0.6 $\log_{10}$ CFU·g^{-1}, and the prevalence of *E. coli* was of 81.3%. This illustrates an important faecal contamination of smoked game meat as the acceptable Congolese criteria is < 3.7 $\log_{10}$ CFU·g^{-1}. Such faecal contamination could be explained by the lack of hygiene during the preparation of smoked meat, during storage or during the display of smoked meat for sale. In contrast to our case, lower values were found in a study performed in South Africa, on the microbiological quality of matured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazelle*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equus burchelli*). Indeed, the count of *E. coli* ranged from 1.5 ± 0.5 to 2.0 $\log_{10}$ CFU·g^{-1}. These values were within acceptable limits, as the southern African retail norm for red meat is < 4.7 $\log_{10}$ CFU·g^{-1} for *E. coli* (van Schalkwyk et al., 2011). In Nigeria, the highest count for *E. coli* (7.5 $\log_{10}$ CFU·g^{-1}) was found in beef meat when studying the safety of street-vended meat products (Ologhobo et al., 2010). This is much higher than in Lubumbashi. In the developed world, several studies have demonstrated faecal contamination of fresh game meat, such as red deer, roe deer and wild boar in France (Membré et al., 2011), kangaroo meat in Australia (Holds et al., 2008) and carcasses of bison in Canada (Gill, 2007).

In Lubumbashi, STEC were only found in smoked game meat of *Syncerus caffer*. Buffaloes belong to the Bovidae family, of which domestic cattle is a member as well as the main reservoir of STEC. These animals are generally considered as healthy carriers of STEC and therefore they can constitute a source of contamination for other animals (Ateba et al., 2011). Meat is often contaminated at slaughtering and dressing of carcasses. This would explain the presence of STEC in smoked game meat of *Syncerus caffer*. In our case, it cannot be excluded that contamination of meats due to lack of hygiene occurs during evisceration or when smoking the game meat. Indeed, the tools used for slaughtering and evisceration are often used during smoking. There are few data on contamination of game meat by STEC in Africa. However, in an outbreak of haemorrhagic diarrhoea reported in Ngoïla in Cameroon, STEC was isolated in 50% of samples from contaminated people after consumption of smoked game meat (Germani et al., 1998). In the other hand, this pathogen has been reported in various livestock such as healthy cattle in Uganda (Kaddu-Mulindwa et al., 2001), cattle, sheep, goats and pigs in Nigeria (Ojo et al., 2010), meats like beef meat and meat products in South Africa (Abong'o et al., 2009; Ateba et al., 2011), smoked zebu meat in Central African Republic (Raji et al., 2006) and poultry in Senegal (Nzouankeu et al., 2010).

In the developed world, studies performed by PCR-based detection of STEC in game meat are rare. However, STEC serotype O157:H7 was isolated by culture from stool of patients contaminated following consumption of sausages made from roe deer in Missouri (Ahn et al., 2009); and from the faeces of wild ruminants such as *Cervus elapsus*, *Capreolus capreolus*, *Dama dama*, *Ovis musimon* and wild boar in Spain (Sánchez et al., 2009; Sánchez et al., 2010). To avoid underestimation of the real STEC prevalence in Lubumbashi, great care should be taken in order to optimize the STEC isolation methods such as the combination of immunomagentic separation and culture on SMAC supplemented with Cefixim and Tellurite, which has showed an increased isolation rate of STEC O157 (de Boer, 1998). In addition, serological tests and PCR could allow the characterization of STEC serotypes potentially involved in food contamination in Lubumbashi. The presence of one STEC organism can be a threat to public health, as demonstrated by Paton et al. in 1996. Thus, much attention must be accorded to this pathogen.

The presence of *Salmonella* in game meat has never been reported in D.R.C. In Zimbabwe, southern Africa, Madsen (1996) determined 20% of *Salmonella* respectively from refrigerated and frozen crocodile meat. In other regions of Africa, *Salmonella* was reported in a wide range of sources: various livestock like sheep and goats, poultry dishes, fresh sausages and chicken carcasses (Cardinale et al., 2005; van Nierop et al., 2005; Woldemariam et al., 2005; Mrema et al., 2006). But there is a lack of data focused on the contamination of smoked game meat by *Salmonella*.

In developed countries, contamination of fresh game meat by *Salmonella* has been previously described. Among 385 kangaroo carcasses, 4 (1.0%) were identified as being contaminated by *Salmonella* in Australia (Holds et al., 2008). But this prevalence is much lower than the prevalence determined in game meat from Lubumbashi. According to Congolese standards, the presence of one *Salmonella* spp. per 25 g of analyzed food sample is not acceptable, and food must be considered unsuitable for human consumption. Thus, in addition to our qualitative study, quantitative studies must be conducted to determine the levels of bacteria that can constitute a threat for public health as usually the food is cooked at high temperature, which significantly reduces the bacterial load.

Little is known about contamination of game meat by *Campylobacter* in Africa. However, *Campylobacter*

contamination of food animals like sheep, goats, chickens, meats and meats products, such as chicken dishes, beef meat, goat meat, sheep meat, has been already reported (Cardinale et al., 2005; Kassa et al., 2007; Dadi et al., 2008; Woldemariam et al., 2009). In Lwiro, the eastern region of D.R.C., among patients who attended as outpatients because of diarrhoea and among children admitted in the same hospital because of malnutrition, *C. jejuni* was identified in 13.7% of outpatients and in 24.0% of inpatients. The same study was also conducted on animals handled by the local population and *C. jejuni* was found in goats (13.0%), pigs (38.4%) and chickens (40.0%) (De Mol et al., 1983).

In our study, *C. coli* was more detected (15.9%) than *C. jejuni* (3.8%). Similar findings were reported in developed world, although the prevalence of *Campylobacter* in game meat was very low. Among 1.0% of *Campylobacter* detected in meat samples of wild boar and feral pigs, two were identified as being *C. coli* and one as *C. jejuni* (Atanassova et al., 2008). In contrast to us, Gill (2007) could not detect any *Campylobacter* from fresh game meat sold at retail. Consideration must be taken for *C. jejuni* and *C. coli* as major foodborne pathogens, though the Congolese standard does not stipulate a standard level of *Campylobacter* spp. in food. Further studies should be conducted on characterization of the *Campylobacter* species frequently isolated, as well as determining the antibiotic susceptibility of these pathogens. This, in order to prevent emergence or dissemination of antimicrobial-resistant *Campylobacter*, which has increased the concern that treatment of foodborne campylobacterisis can be compromised in cases of severe symptoms or in immunocompromised patients (Ge et al., 2003; Uaboi-Egbeni et al., 2011).

This is the first assessment of bacteriological quality of smoked game meat conducted in Lubumbashi. However, the study encountered limitations particularly the accessibility to villages to meet hunters and/or producers of smoked game meat, and to convince these people to participate in our survey. The cost of smoked game meat was also a limitation for the sampling process, as all the samples were purchased.

In conclusion, smoked game meat sold at retail outlets in Lubumbashi is contaminated by STEC, *Salmonella*, *C. jejuni* and *C. coli*, with respective detection rates of 2.2%, 6.0%, 3.8% and 15.9% determined by PCR. These results provide reliable epidemiological data on the bacteriological quality of smoked game meat in Lubumbashi. Further studies should be conducted on serotyping of STEC and *Salmonella*, and characterization of the *Campylobacter* species frequently isolated in meat Lubumbashi. Although trade in smoked game meat is allowed, it remains difficult to control the production chain of such meat, because sometimes game meat comes from hunting by poaching in national parks or from occasional hunting which are generally illegal and the markets are often supplied with game meat from unknown origin. With the establishment of supervised hunting within defined hunting periods and a quota of game per season, in addition to the identification of hunters and intermediate sellers of game between hunters, smoked game meat dealers and salesmen in local markets, it would be possible to develop monitoring plans of the production chain and commercialization of smoked game meat in Lubumbashi.

Acknowledgements

The authors would like to thank all individuals who participated to the study, as well as those who took part by identifying sites of smoked game meat preparation and interviewing game meat salesmen. Special thanks are also extended to the people who helped to carry out the sampling and later analyses to the laboratories in Lubumbashi (DRC) and Liege (Belgium). Thanks to the Reference Laboratory of *Campylobacter*/UHC Saint-Pierre (Belgium) and the Reference Laboratory for Enterohemorrhagic *E. coli*/VUB (Belgium) for providing reference strains. The Belgian Technical Cooperation is sincerely thanked for its financial support.

Bibliography

Abong'o B.O. & Momba M.N., 2009. Prevalence and characterization of *Escherichia coli* O157:H7 isolates from meat and meat products sold in Amathole District, Eastern Cape Province of South Africa. *Food Microbiol*., **26**(2), 173-176.

Ahn C.K. et al., 2009. Deer sausage: a newly identified vehicle of transmission of *Escherichia coli* O157:H7. *J. Pediatr*., **155**(4), 587-589.

Atanassova V., Apelt J., Reich F. & Klein G., 2008. Microbiological quality of freshly shot game in Germany. *Meat Sci*., **78**(4), 414-419.

Ateba C.N. & Mbewe M., 2011. Detection of *Escherichia coli* O157:H7 virulence genes in isolates from beef, pork, water, human and animal species in the northwest province, South Africa: public health implications. *Res. Microbiol*., **162**, 240-248.

Bachand N. et al., 2012. Public health significance of zoonotic bacterial pathogens from bushmeat sold in urban markets of Gabon, Central Africa. *J. Wildl. Dis*., **48**(3), 785-789.

Cardinale E. et al., 2005. Risk factors for contamination of ready-to-eat street-vended poultry dishes in Dakar, Senegal. *Int. J. Food Microbiol*., **103**(2), 157-165.

Chapman P.A., 1995. Verocytotoxin-producing *Escherichia coli*: an overview with emphasis on the epidemiology

and prospects for control of *E. coli* O157. *Food Control*, **6**(4), 187-193.

Chomel B.B., Beletto A. & Meslin F.-X., 2007. Wildlife, exotic pets, and emerging zoonoses. *Emerg. Infect. Dis.*, **13**(1), 6-11.

Dadi L. & Asrat D., 2008. Prevalence and antimicrobial susceptibility profiles of thermotolerant *Campylobacter* strains in retail raw meat product in Ethiopia. *Ethiop. J. Health Dev.*, **22**(2), 195-200.

de Boer E., 1998. Update on media for isolation of Enterobacteriaceae from foods. *Int. J. Food Microbiol.*, **45**(1), 43-53.

Debruyne L. et al., 2008. Comparative performance of different PCR assays for the identification of *Campylobacter jejuni* and *Campylobacter coli*. *Res. Microbiol.*, **159**(2), 88-93.

de Mol P. et al., 1983. Enteropathogenic agents in children with diarrhoea in rural Zaire. *Lancet*, **1**(8323), 516-518.

Enokimoto M. et al., 2007. Enumeration and identification of *Campylobacter* species in the liver and bile of slaughtered cattle. *Int. J. Food Microbiol.*, **118**(3), 259-263.

Fhogartaigh C.N. & Edgeworth J.D., 2009. Bacterial gastroenteritis. *Medicine*, **37**(11), 586-593.

Friedrich A.W. et al., 2002. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J. Infect. Dis.*, **185**(1), 74-84.

Ge B. et al., 2003. Antimicrobial-resistant *Campylobacter* species from retail raw meats. *Appl. Environ. Microbiol.*, **69**(5), 3005-3007.

Germani Y. et al., 1998. Enterohaemorrhagic *Escherichia coli* in Ngoila (Cameroon) during an outbreak of bloody diarrhoea. *Lancet*, **352**(9128), 625-626.

Gilbert C., Winters D., O'Leary A. & Slavik M., 2003. Development of a triplex PCR assay for the specific detection of *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli* O157:H7. *Mol. Cell. Probes*, **17**(4), 135-138.

Gill C.O., 2007. Microbiological conditions of meats from large game animals and birds. *Meat Sci.*, **77**(2), 149-160.

Goualié G.B. et al., 2012. Prevalence and antimicrobial resistance of thermophilic *Campylobacter* isolated from chicken in Côte d'Ivoire. *Int. J. Microbiol.,* Article ID 150612, 5 pages.

Hilbert F., Smulders F.J.M., Chopra-Dewasthaly R. & Paulen P., 2012. *Salmonella* in the wildlife-human interface. *Food Res. Int.*, **45**(2), 603-608.

Holds G. et al., 2008. Microbial profiles of carcasses and minced meat from kangaroos processed in South Australia. *Int. J. Food Microbiol.*, **123**(1-2), 88-92.

Houng H.S. et al., 2001. Development of a ceuE-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. *Diagn. Microbiol. Infect. Dis.*, **40**(1-2), 11-19.

Kaddu-Mulindwa D.H. et al., 2001. Occurrence of Shiga toxin-producing *Escherichia coli* in fecal samples from children with diarrhea and from healthy zebu cattle in Uganda. *Int. J. Food Microbiol.*, **66**(1-2), 95-101.

Karesh W.B. et al., 2012. Ecology of zoonoses: natural and unnatural histories. *Lancet*, **380**, 1936-1945.

Karmali M.A., Gannon V. & Sargeant J.M., 2010. Verocytotoxin-producing *Escherichia coli* (VTEC). *Vet. Microbiol.*, **140**(3-4), 360-370.

Kassa T., Gebre-Selassie S. & Asrat D., 2007. Antimicrobial susceptibility patterns of thermotolerant *Campylobacter* strains isolated from food animals in Ethiopia. *Vet. Microbiol.*, **119**(1), 82-87.

Madsen M., 1996. Prevalence and serovar distribution of *Salmonella* in fresh and frozen meat from captive Nile crocodiles (*Crocodylus niloticus*). *Int. J. Food Microbiol.*, **29**(1), 111-118.

Membré J.M., Laroche M. & Magras C., 2011. Assessment of levels of bacterial contamination of large wild game meat in Europe. *Food Microbiol.*, **28**(5), 1072-1079.

Miko A. et al., 2009. Assessment of Shiga toxin-producing *Escherichia coli* isolates from wildlife meat as potential pathogens for humans. *Appl. Environ. Microbiol.*, **75**, 6462-6470.

Mohammed M.A.M., 2012. Molecular characterization of diarrheagenic *Escherichia coli* isolated from meat products sold at Mansoura city, Egypt. *Food Control*, **25**, 159-164.

Mrema N., Mpuchane S. & Gasha B.A., 2006. Prevalence of *Salmonella* in raw minced meat, raw fresh sausages and raw burger patties from retail outlets in Gaborone, Botswana. *Food Control*, **17**, 202-212.

Nzouankeu A. et al., 2010. Multiple contaminations of chickens with *Campylobacter*, *Escherichia coli* and *Salmonella* in Yaounde (Cameroon). *J. Infect. Dev. Ctries.*, **4**(9), 583-686.

Ojo O.E. et al., 2010. Potentially zoonotic shiga toxin-producing *Escherichia coli* serogroups in the faeces and meat of food-producing animals in Ibadan, Nigeria. *Int. J. Food Microbiol.*, **142**(1-2), 214-221.

Ologhobo A.D. et al., 2010. Safety of street vended meat products-chicken and beef suya. *Afr. J. Biotechnol.*, **9**, 4091-4095.

Onyango C.A., Izumimoto M. & Kutima P.M., 1998. Comparison of some physical and chemical properties of selected game meats. *Meat Sci.*, **49**(1), 117-125.

Paton A.W. et al., 1996. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing *Escherichia coli*. *J. Clin. Microbiol.*, **34**(7), 1622-1627.

Piérard D. et al., 1998. Identification of new verocytotoxin type 2 variant B-subunit genes in human and animal *Escherichia coli* isolates. *J. Clin. Microbiol.*, **36**(11), 3317-3322.

Raji M.A., Minga U. & Machangu R., 2006. Current epidemiological status of enterohaemorrhagic *Escherichia coli* O157: H7 in Africa. *Chin. Med. J.*, **119**(3), 217-222.

Sallam K.I., 2007. Prevalence of *Campylobacter* in chicken and chicken by-products retailed in Sapporo area, Hokkaido, Japan. *Food Control*, **18**, 1113-1120.

Sanchez S. et al., 2009. Detection and characterization of Shiga toxin-producing *Escherichia coli* other than *Escherichia coli* O157:H7 in wild ruminants. *Vet. J.*, **180**(3), 384-388.

Sanchez S. et al., 2010. Detection and characterization of O157:H7 and non-O157 Shiga toxin-producing *Escherichia coli* in wild boars. *Vet. Microbiol.*, **143**(2-4), 420-423.

Shelton D.R., Karns J.S. & Park C.H., 2008. A multiple protocol to improve diagnosis and isolation of Shiga toxin-producing *Escherichia coli* from human stool specimens. *Diagn. Microbiol. Infect. Dis.*, **62**(1), 7-10.

Uaboi-Egbeni P.O., Bessong P.O., Samie A. & Obi C.L., 2011. Prevalence and antimicrobial susceptibility profiles of *Campylobacter jejuni* and *coli* isolated from diarrheic and non-diarrheic goat faces in Venda region, South Africa. *Afr. J. Biotechnol.*, **10**(64), 14116-14124.

van Nierop W. et al., 2005. Contamination of chicken carcasses in Gauteng, South Africa, by *Salmonella*, *Listeria monocytogenes* and *Campylobacter*. *Int. J. Food Microbiol.*, **99**(1), 1-6.

van Schalkwyk D.L. et al., 2011. Physico-chemical, microbiological, textural and sensory attributes of matured game salami produced from springbok (*Antidorcas marsupialis*), gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*) and zebra (*Equus burchelli*) harvested in Namibia. *Meat Sci.*, **88**(1), 36-44.

Woldemariam E., Molla B., Alemayethu D. & Muckle A., 2005. Prevalence and distribution of *Salmonella* in apparently healthy slaughtered sheep and goats in abattoir in Debre Zeit area, Ethiopia. *Small Rumin. Res.*, **58**(1), 19-24.

Woldemariam T., Asrat D. & Zwede G., 2009. Prevalence of thermophilic *Campylobacter* species in carcasses from sheep and goats in an abattoir in Debre Zeit area, Ethiopia. *Ethiop. J. Health Dev.*, **23**(3), 229-233.

Zweifel C. et al., 2006. Virulence profiles of Shiga toxin 2e-producing *Escherichia coli* isolated from healthy pig at slaughter. *Vet. Microbiol.*, **117**(2-4), 328-332.

(49 ref.)