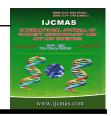
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Original Research Article

Evaluation of macroscopic and microbiological hazards of indigenous pork consumption in south of Benin

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ABSTRACT

Keywords

Pigs, lesions; microorganism; meat; slaughterhouse; Benin This study was conducted in south of Benin and aim to identify macroscopic and microbiological hazards in pork. To identify macroscopic hazard, data were collected on seizures from 77,194 pigs slaughtered at the slaughterhouses of Cotonou-Porto Novo. Assessment of hygienic quality of pork was done on 25 carcasses from the slaughterhouses of Cotonou-Porto-Novo and from the butchers "Bon goût" in Akpakpa and "Chez Clément" in Gbégamey within the administrative district of Cotonou. Among macroscopic hazards recorded, congestion (3.23%), and the lung regurgitation (2.65%) were the most frequent causes of organ seizures (P <0.05). The most seized organs were the heart, liver, intestine, tongue, breasts, lungs, spleen, kidneys and carcass. Among microbiological hazards registered in pork, Total Aerobic Mesophilic Flora (TAMF) loads were higher than the recommended standard with the means of 5.20 logUFC / cm², 8 logUFC / cm² and 11 logUFC / cm², respectively in the carcass of Cotonou-Porto-Novo slaughterhouses, the carcasses collected from butchers of Akpakpa and those collected from Gbégamey. Fecal coliforms loads recorded were unsatisfactory. No contamination by Salmonella spp. was recorded. The loads of Sulfite-Reducing Anaerobic germs Sulfite-Reducing (SRA) were satisfactory for both carcasses collected.

Introduction

The local pigs of Benin or "West Africa dwarf pigs" is found along the coastal countries of West Africa and probably resulted from European pig (Devendra *et al.* 1979; d'Orgeval, 1997). The local pigs of Benin are phylogenetically close to Italian and Turkish boars or to pigs of Iberian, Spanish and Belgian (Ramirez *et*

haplotype E1, which indicates introgression of European strains such as Large White and Asian strains (Ramirez *et al.*, 2006). On the ethnological way, the snout of local pig is long, cylindrical, and conical with tapered extremity. Its profile is slightly concaviligne and their ears are

small, erect and sometimes thrown back. This population of pig of Benin is of small size with a withers height of 40 cm. Indigenous adult pig weight varies from 40 to 50 kg (d'Orgeval, 1997). The dominant phenotype is the black with white belt. It is also met the white phenotype with black or gray ends. These pigs are characterized by a great rusticity and low performance despite several studies carried out to improve their productivity and farming system management (d'Orgeval, 1997; Agbokounou, 2001; Codjo, 2003; Youssao et al., 2008; Youssao et al, 2009a). Other studies had also taken into account the improvement of their carcass composition and meat quality (Youssao et al., 2004a; Youssao et al., 2004b; Youssao et al., 2009b). All those studies carried out on the local pig population of Benin were limited to the technological, organoleptic and nutritional quality of the meat. The hygienic quality of local pig meat had not been taking into account by these authors, and no reference was found in the literature.

Although pork is frequently consumed and well appreciated by their consumers, pork must be up to the hygienic standards to ensure food safety for consumers. According to Dennaï et al., (2001) and Fosse et al., (2007) meat has been traditionally regarded as a vehicle for many foodborne diseases in humans. Its hygienic quality depends the contamination occurring during slaughtering and cutting process and the development and growth of these biocontaminants during cooling, storage and distribution (Dennaï et al., 2001; El Okki et al., 2005). In addition, the slaughterhouse is one of the main critical points of meat hygiene and slaughter is considered to be the stage where the

greatest opportunities of contamination may be occurred (Dennaï et al., 2001, Collebert et al., 2002; Vallaton, 2004; Merle, 2005; Beaubois, 2009). According to Jouve (1990), 80 to 90% of the microflora of meat reaching the consumers resulted from contamination occurring at the slaughterhouse. It is therefore essential to control hazards from meat that may be harmful to consumers. These dangers are even more worrying since the majority of pigs are slaughtered in butchers where slaughtering conditions are unsatisfactory. Unlike microbiological hazards, the safety of meat inspection can identify potential hazards associated with macroscopic meat consumption through seizures slaughterhouses. The present study aims to evaluate the macroscopic and bacterial hazards related to the consumption of local Benin pork.

Materials and Methods

Study areas

Evaluation of bacterial hazards of the consumption of pork was carried out on collected carcasses from slaughterhouses of Cotonou-Porto Novo and from two butchers of Cotonou (bon gout in Akpakpa and "Chez Clément" in Gbegamey) from November 2009 to February 2010. Cotonou is the most important city in Benin, located in the Littoral Department and benefits from subequatorial climate which is characterized by two rainy seasons: the great from April to July and the small from September to November. These two rainy seasons are interspersed with dry seasons. The average rainfall is 1200 mm of rain per year. The average monthly temperatures fluctuate between 27 and 31 ° C and the relative humidity of the air fluctuates between 65% from January to March and 97% from June to July.

Macroscopic hazard assessment

The assessment of macroscopic hazard was done using data collected from seizures recorded on pigs at the slaughterhouses of Cotonou-Porto Novo. These data were collected monthly from April 2007 to March 2010 and include: Causes of seizures; Organs subject to seizure; Number of animals slaughtered.

The data collected were stored in a database taking into account the month and year of slaughter, the monthly number of animals slaughtered, the diseases recorded monthly and the seized organs (heart, liver, intestine, tongue, breasts, lungs, spleen, kidneys and carcass).

Evaluation of bacterial hazards

Sampling

A total of 10 pig carcasses were selected at the slaughterhouses of Cotonou-Porto-Novo, 8 pig carcasses at the butcher "Chez Clément" at Gbégamey and 7 pig carcasses at the butcher "Bon goût" at Akpakpa. Data were collected from 15 January to 26 February 2010 on adult local pigs of 6 months old and with an average live weight varying from 20 to 35kg. Meat sample were collected on the back of the carcass selected, the inside of the throat, the outer side of the thigh, the chest and near to the sternum. These samples were collected before and after the bleeding and dressing, according to the Decision DGAL/SDSSA/N2007-8275 of November 2007 (DGAL, 2007) and the Protocol of Decision 2001-471-EC of 8 June 2001 (JOCE, 2001). Meat samples were obtained by the destructive method

using a punch. The entire sample collected from a carcass was pooled to form one sample. Each pooled sample of each carcass was packed in sterile stomacher bags and weighed and brought immediately to the laboratory in a cooler equipped with dry ice. Transport of samples was done according to the standard ISO 7218: 1996.

Microbiological analysis

Microbiological analysis was performed according to the standard ISO 7218: 1996. Flora counted were Total Aerobic Mesophilic Flora (TAMF), fecal coliforms, Sulphite-Reducing Anaerobic bacteria and Salmonella. Samples were prepared according to the protocol of the decision 2001-471-EC 8 June 2001 (JOCE, 2001). Then, 100 ml of Buffered Peptone Water (BPW, Merk) were introduced into sterile stomacher bag previously containing the 4 samples of the same carcass. The whole sample was crushed and homogenized for minutes. After serial decimal dilution in Peptone Physiological Saline (PPS), 1 ml of the appropriate dilution was pipetted into duplicate plates and the appropriate cell culture medium was distributed according to the pour-plating technique. Total Aerobic Microorganisms Flora (TAMF) were counted in Plate Count Agar (PCA, Oxoid), after incubation at 30°C for 72 h. The colonies obtained were counted and the results expressed in logUFC/cm². The formula for expressing colonies CFU/cm² was:

$$\mathbf{D} = \frac{(\mathbf{N} * \mathbf{V})}{(\mathbf{S} * \mathbf{10}^{-\mathbf{X}})}$$

D = number of CFU / cm²; N = number of Colony Forming Unit at the dilution 10^{-X}: V = volume of the solution; S = total area of the sample.

Total and fecal coliform detection were performed according to standard ISO 4831: 2006 and using the most probable number technique. Then, 1 ml of the appropriate dilution was pipetted in a lactose Brilliant Green Bile broth (BLBVB Oxoid CM 0031). Total coliform were counted after incubation at 37°C for 24 hours and fecal coliform were counted after incubation at 44°C for 24 hours. The test was performed in triplicate and the analyzed with Mac results technique. Positives results were tubes in which there is growth characterized by turbidity and significant production of gas (1/10 of volume of at least a bell).

Detection of Sulphite-Reducing Anaerobic bacteria was performed according to the standard ISO 15213: 2003. One milliliter of the solution was supplemented with 9 ml of peptone water Salt (EPS). The whole was heated at 75 ° C for 20 minutes and supplemented with 30 ml of TSN cell culture medium (TSN Biokar diagnostics BK 001 HA) in cooling condition. The incubation was carried out under anaerobic condition for 48 hours at 37 ° C. Black colonies were counted.

Salmonella was detected as described by the standard ISO 6579:2002 in 25g of product for 4 samples per each carcass. After pre-enrichment with 225 ml of Buffered Peptone Water (BPW Merk), incubation was carried out at 37°C for 24 hours. After incubation, a secondary enrichment in Rappaport-Vassiliadis, green malachite and magnesium chloride broth (Oxoid CM VR 0669) was performed for 24 hours at 37°C. Then, selective flora was done on solid Hektoen cell culture medium (Oxoid CM HEKT

0419) from previously incubated media. This incubation was carried out for 24 hours at 37°C. Differentiation of suspect colonies was performed on Kligler medium. Presumptive Salmonella were purified on Nutrient Agar (Oxoid CM3) and submitted to the oxidase test before biochemical confirmation test using the API 20E.

Statistical Analysis

The Statistical Analysis System (SAS, 9.1.) was used for statistical analysis. The frequencies of organs seized per disease and the frequencies of causes of seizures were calculated using Proc Freq of [25]. Comparisons between the frequencies of seizures were made using the bilateral test of Z. To perform microbiological analysis, the Chi-square test was used to determine significance of the each factor. Comparisons were made in pairs using the bilateral test of Z.

Result and Discussion

Assessment of macroscopic Hazard

The number of pigs slaughtered from April 2007 to March 2010 was 77,194. In total, 15 types of lesions were identified on nine organs seized (Table 1). Among lesions, congestion and regurgitation recorded the highest prevalence (P < 0.05) with respective values of 3.23% and 2.65% of total pigs slaughtered. The other diseases recorded were splenomegaly (1%), nephritis (0.90%), pleurisy (0.78%), emphysema (0.71%), pericarditis (0.65%), abscess (0.63%), hydronephrosis (0.52%), hematoma (0.44%), putrefaction (0.44%), (0.26%), porcine cysticercosis (0.22%), gastroenteritis (0.22%)angioma (0.21%).

The organs frequently seized at the slaughterhouses of Cotonou-Porto Novo were heart, liver, intestine, tongue, breasts, lungs, spleen, kidneys and the entire Among these organs, carcass. frequency of lung seizures was the most This frequency represents important. 0.48% of the total number of pigs slaughtered during this period, and 374 lungs of pigs seized on 77,174 pigs slaughtered. The proportion of lung seized due to regurgitation was the largest (2.65%) and was significantly higher (P <0.05) than lungs seized due to pleurisy (0.78%) and emphysema (0.71%). The other causes of seizure of lungs were congestions, putrefaction and abscesses and their frequency were less than 0.16%. The proportion of spleen seizure was 0.47% of the total number of pigs slaughtered during the study period. The main causes of spleen seizure were congestion and splenomegaly. The number of spleen seizures due to congestion (2.9%) was greater (P < 0.01) than spleen seizures due to splenomegaly (1%). Few spleens were seized because of abscess (0.01%).

Liver (0.12%) and heart (0.07%) are respectively seizures in 3rd and position. The most important diseases observed in the liver was abscess (0.56%) and that lesion was observed more (P <0.05) than seizures due to angioma (0.21%) and cysts (0.26%). Seizures due to congestion and putrefaction were less observed. Pericarditis (0.65%) was the main cause of seizure (P < 0.01) of heart, and that was followed by the putrefaction (0.04%). Nephritis was the most important cause of kidney seizure (0.90%), followed by hydronephrosis and hematoma. No significant difference was observed between the frequency of seizures due to Nephritis, hydronephrosis and hematoma.

Porcine cysticercosis was the main cause of seizure of the entire carcasses (0.01%). The intestine was mostly seized because of enteritis (0.22%), while putrefaction was the second cause of seizure (0.12%). Tongue was primarily condemned because of abscess (0.02%) as well as the breasts (0.01%). Table 1 shows the frequency of organ seizures by lesion.

Hygienic quality of carcasses

Total Aerobic Mesophilic Flora (TAMF) loads were higher than the recommended standard with averages of 5.20 logUFC/cm²; 8 logUFC/cm² and 11 logUFC/cm², respectively for the carcasses collected from the slaughterhouses of Cotonou-Porto-Novo and from butchers at Akpakpa and Gbégamey. Fecal coliform loads in three places slaughtering were ranged from 923 to 1255 CFU / g. Total coliforms load was on average of 1400 cells per gram in carcasses collected from the slaughterhouses and those collected from butchers (Table 2). No case contamination by Salmonella spp was from isolated carcasses. The microbiological loads of Sulfite-Reducing Anaerobic Bacterial (SRA) were well below the recommended standard. The highest microbial load (P <0.05) was observed on the sample provided by the butcher of Akpakpa (9 germs/g) and the lowest load (P < 0.05) was noted on the sample from the carcass provide by the butcher of Gbégamey (1 germ/g). The load of SRA on the carcasses from the slaughterhouses of Cotonou-Porto Novo (4 cell/g) and the both butchers was similar.

Macroscopic hazards identified on the local pig carcasses

The lesions that had been met on the

Table.1 Frequency (%) of organs seized per affection at pork inspection (based on the total number of animals slaughtered)

Lesions	Number	Heart	Liver	Intestine	Tongue	Breasts	Lungs	Spleen	Kidneys	Carcass
Abscess	12501	0.00b	0.56a	0.00b	0.02a	0.01a	0.03d	0.01c	0.00b	0.00b
Angioma	475	0.00b	0.21b	0.00b	0.00a	0.00a	0.00d	0.00c	0.00b	0.00b
Congestion	10239	0.00b	0.13c	0.00b	0.00a	0.00a	0.16c	2.90a	0.04b	0.00b
Porcine cysticercosis	3139	0.00b	0.00c	0.00b	0.00a	0.00a	0.00d	0.00c	0.00b	0.22a
Pulmonary	8833	0.00b	0.00c	0.00b	0.00a	0.00a	0.71b	0.00c	0.00b	0.00b
emphysema										
Enteritis	460	0.00b	0.00c	0.22a	0.00a	0.00a	0.00d	0.00c	0.00b	0.00b
Hematoma	456	0.00b	0.00c	0.00b	0.00a	0.00a	0.00d	0.00c	0.44ab	0.00b
Hydronephrosis	1911	0.00b	0.00c	0.00b	0.00a	0.00a	0.00d	0.00c	0.52ab	0.00b
Cysts	2656	0.00b	0.26b	0.00b	0.00a	0.00a	0.00d	0.00c	0.00b	0.00b
Nephritis	3760	0.00b	0.00c	0.00b	0.00a	0.00a	0.00d	0.00c	0.90a	0.00b
Pericarditis	7721	0.65a	0.00c	0.00b	0.00a	0.00a	0.00d	0.00c	0.00b	0.00b
Pleurisy	7579	0.00b	0.00c	0.00b	0.00a	0.00a	0.78b	0.00c	0.00b	0.00b
Putrefaction	2588	0.04b	0.08c	0.12ab	0.00a	0.00a	0.08c	0.08b	0.00b	0.04b
Regurgitation	8684	0.00b	0.00c	0.00b	0.00a	0.00a	2.65a	0.00c	0.00b	0.00b
Splenomegaly	6192	0.00b	0.00c	0.00b	0.00a	0.00a	0.00d	1.00b	0.00b	0.00b

Frequencies in the same column followed by different letters differ significantly at 5%.

Table. 2 Microbiological characteristics of pig carcasses

Places	TAMF (logUFC/cm ²)	Total Coliforms (germs/g)	fecal Coliforms (germs/g)	Sulphite- Reducing Anaerobic bacteria (germs/g)	Salmonella (germs/25g)
Cotonou-Porto Novo slaughterhouses	5.20	1400 ^a	1255±365 ^a	4±7 ^{abc}	0
Butcher at Akpakpa	8	1400 ^a	1243±416 ^a	9±6.5 ^b	0
Butcher at Gbégamey	11	1231±479 ^a	923±663 ^a	1±2 ^{ac}	0
Standard	m=4, M=5 ¹	-	$1000/g^2$	$100/g^2$	Absent in 25g ²

1= microbiological criteria for foodstuffs RÈGLEMENT (CE) N° 2073/2005, 2=AFNOR 1979.

carcass at the slaughterhouse of Cotonou-Porto Novo were abscesses, angioma, congestion, porcine cysticercosis, emphysema, hematoma, enteritis, hydronephrosis, cysts, nephritis, pericarditis, pleurisy, putrefaction, regrugitation and splenomegaly. Those lesions are the consequences of infections, systemic diseases or infestations. Akpata (2005) reported similar lesions on cattle at the slaughterhouse of Malanville in the country. Among the lesions identified, prevalence of congestion and regrugitation were the most important. Congestion was often encountered in the spleen, liver and lungs. These lesions probably appear with a slaughter in horizontal recumbence and incomplete blood flow during slaughtering of pigs. This method of bleeding leads on an accumulation of blood in organs such as the liver, lungs, spleen and the congestion is often confused observed pathological congestion (Gueye, 1981) due to Bacillus anthracis (Cabre et al., 2005; Fosse et al., 2007). The prevalence of enteritis, putrefaction and abscesses. porcine cysticercosis are low. Abscesses and enteritis are induced respectively by Staphylococcus aureus and Clostridium perfringens (Fosse, 2009). Other studies have identified Clostridium as infectious agent involved in putrefaction (Gueye, microorganisms 1981). Both responsible of food poisoning and can lead on death. The prevalence observed for porcine cysticercosis is low and is on average of 22 infested carcasses out 10000 carcasses inspected. This low prevalence improvements in shows the the management farming systems. Indeed, for Assana et al., (2001) the high prevalence of infection with metacestodes T. solium observed (20.5% ante-mortem and post-mortem 15.7%) in the regions of Cameroon and Chad in 1999 are related to

animal husbandry in freedom, lack of hygiene in the diet of pigs and ignorance by farmers of mode of infestation and pathological aspects of zoonotic parasites. The parasites infest humans following ingestion of undercooked meat and cause serious illness such as nervous symptoms leading generally on death (Cabre, 2005). evaluation of the meat mass corresponding to the input due to porcine cysticercosis was estimated from data on food availability of the FAO (2010) and meat yield of the carcass (DE, 2006) was about 81.5 tons. This mass corresponds to meat consumption of about 130000 people in the town of Cotonou in two years if no entry was made. The prevalence of porcine observed cysticercosis at slaughterhouses of Cotonou-Porto-Novo, suggests strengthening of monitoring by veterinary services upstream of slaughter to improve the level of safety of meat and offal. The other lesions observed (nephritis, pleurisy, emphysema, pericarditis, hydronephrosis, hematoma, cyst, angioma) have very low importance for public health.

Hygienic quality of carcasses

The present study showed that the local carcasses collected pig from slaughterhouses of Cotonou-Porto-Novo and from the butchers of Akpakpa and Gbégamey have an unsatisfactory hygienic quality due to Total Aerobic Mesophilic Flora (TAMF) and fecal coliforms. These very high microbial loads for Total Aerobic Mesophilic Flora (TAMF) and fecal coliforms indicate some very poor hygienic conditions of working in those butchers (Cartier, 1990; Cartier, 1993; Nana, 2000) and the presence recontamination in the slàugntering process. According to Pinochet et al., (1988), the recontamination process can be scalding, depilation, scraping evisceration. The scalding was done at the slaughterhouses of Cotonou-Porto Novo and at the butcher of Gbégamey at a temperature close to 100°C and scalding water was not regularly changed. This temperature is very high compared to that reported by Pinochet et al. (1988) and FAO (1994) for scalding. However, the lack of flushing observed during scalding increases the presence of organic material in important quantity in the water. This increase organic material and the lowering of the temperature of the scald water decrease the bactericidal effect of the process and increase the adhesion of bacteria to the surface of the carcass. Similarly, the use of cold water recycled during the dehairing process ensures recontamination of the carcass (Pinochet et al., 1988; Lo Fo Wong et al., 2002).

Recontamination may also due to contact with the carcass blaze with the ground bacterial cooling. during the The recontamination may also be observed during the scraping process when carcass was in contact with the stone to scrape, or with various manipulations performed with hands or with lastly working tools not disinfected used in the process of evisceration (Guiraud and Galzy, 1980). The loads obtained for Anaerobic Sulphite-Reducing germs allow concluding for a satisfactory hygienic quality. Nevertheless, these charges are significantly different according to the slaughter place. The butcher of Akpakpa presented the highest load and the butcher at Gbégamey presented the lowest load (P <0.05). This difference in microbial loads can be explained by the contact with carcasses with the ground during slaughtering process. This contact with the ground by carcasses is carried out at each slaughtering in the butcher at Akpakpa for

bleeding and cooling processes and at the slaughterhouses of Cotonou-Porto Novo for bleeding, scalding, dehairing and evisceration. The slaughtering performed on the ground is a source of carcasses recontamination by Sulphite-Reducing Anaerobic bacteria especially Clostridium (Guiraud and Galzy, 1980) and May eventually spread to other tools or work tables. No cases of Salmonella spp. was isolated from carcasses of pigs provide by the slaughterhouses of Cotonou-Porto Novo and the butchers. This result can be explained by the sensitivity of Salmonella spp. at a temperature above 50°C (Guiraud and Galzy, 1980; Rozier, 1992) and its competition with fecal coliforms (Van Nierop, 2005). However, other studies (Ahouissi, 2008; Korsak et al., 1998; Daube et al., 2001; Ghafir et al., 2002; Boudry et al., 2002; Daube et al., 2003) carried out on pig carcasses at the slaughterhouse in Belgium found prevalence of Salmonella contamination ranging from 15 to 28%. These studies have used in their method of detection of Salmonella spp., the semi-solid medium Diasalm (Belgian Official Method SP-VG M002) whose composition and principle is based on the mobility of Salmonella spp. This medium achieves a low detection limit of the bacteria. The use of these advanced methods on Salmonella research could confirm the results and confirm the hypothesis that the rules of hygiene in different slaughtering process described in the related Salmonella spp.

The safety inspection allowed us to identify macroscopic potential hazards related to the consumption of pork. Among lesions that cause organ seizure are congestion and regurgitation showed the highest prevalence, followed by the spleen, nephritis, pleurisy, emphysema, pericarditis, abscesses, hydronephrosis,

hematoma, putrefaction, cysts, porcine cysticercosis, enteritis and angioma. The presence of these lesions suggests to improve the monitoring by veterinary services upstream of slaughter to improve the quality of pig meat and offal and the safety of the consumers. TAMF load was higher than the recommended standard. The fecal coliform loads recorded from the three slaughter places show no significant difference. The carcass samples examined were free of Salmonella spp, however, the looking worth to be pursued. Taking into account the load of Sulfite-Reducing Anaerobic bacteria, the quality of pigs carcass sampled is satisfactory in the three places of slaughter. Carcass quality is satisfactory for Salmonella spp. loads, however, research with more advanced methods should be conducted to confirm or refute these results. The hygiene of the slaughtering process and safety inspection is necessary for food safety.

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