IJAMBR 5 (2017) 110-122 ISSN 2053-1818

International Journal of Applied Microbiology and Biotechnology Research

www.bluepenjournals.org/ijambr

Pork consumption and risk factors related to *Yersinia enterocolitica* 4/O:3 in pork production chain in Côte d'Ivoire

Atobla Koua[1]*, Kouadio-Ngbesso Nadège[2], Dadié Adjehi[3], Karou Tago Germain[1] and Niamké Lamine Sebastien[1]

[1]Laboratory of Biotechnologies, Research and Training Unit of Biosciences, Félix Houphouët-Boigny University, Côte d'Ivoire.
[2]Laboratory of Microbiology of Oceanographic Research Center, Côte d'Ivoire.
[3]Laboratory of Biotechnology and Microbiology of Foods, Department of Foods Sciences and Technologies, Nangui Abrogoua University, Côte d'Ivoire.

Article History
Received 02 September, 2017
Received in revised form 06 October, 2017
Accepted 10 October, 2017

Keywords:
Pork,
***Yersinia enterocolitica*,**
Infection risks,
Public health.

Article Type:
Full Length Research Article

ABSTRACT

Pork consumption was evaluated based on transversal and retrospective survey, coupled with direct observations in Abidjan District, Côte d'Ivoire. Methods of collecting data were face-to-face interviews using standardized questionnaires for both consumers or non-consumers. In addition, *Yersinia enterocolitica* strains from pork samples were characterized by using biotyping, and serotyping methods as well as virulence markers. The results of the survey showed that smoked pork was consumed more (51.9%) at home, followed by fresh pork (24.7%) and then fried pork (17.3%). Outside the home, consumers preferred baked pork (51.3%). Observation of pork vendors and consumers revealed that good hygiene practices were not observed. As a result, 39.5% of consumers surveyed evoked some infection cases. The most common digestive disorders evoked were diarrhea (46.0%), abdominal pain (21.3%) and vomiting (5.2%). Thus, among the 400 samples collected, three *Y. enterocolitica* 4/O:3 strains were isolated, including 2 from 200 tongues and 1 strain in 200 carcasses. *Y. enterocolitica* 4/O:3 showed antibiotic resistances and harbored virulence genes, indicating their pathogenicity. All these findings revealed that pork consumption and processing conditions would be a risk factor for *Yersinia* infections. Therefore, good hygiene practices campaign along the pork chain should be conducted in Côte d'Ivoire.

INTRODUCTION

Among foodborne diseases, zoonoses constitute public health problem. These biological hazards present in food products, which can have an adverse effect on health, are most often associated within apparent carriage in animals (Singer et al., 2007) such as pig. Pork is the most widely eaten meat in the world, accounting for over 36% of the world meat intake, followed by poultry and beef, with about 35 and 22%, respectively (FAO, 2014).

In Africa, the accelerated urbanization of the last 20 years has showed an increase in pork production. Indeed in 2005, 800,000 tons of pork were consumed, compared to 500,000 tons in 1990 (CTA, 2007). In Côte d'Ivoire, pork consumption increased because of the high cost of other protein sources such as beef, sheep meat, chicken meat and fish in the market. Pigs have been considered to be the primary reservoir for the pathogenic *Yersinia*

*Corresponding author. E-mail: atobla@yahoo.fr.

enterocolitica that has been isolated in the oral cavity especially from tonsils, tongues or in lymph nodes (Kapperud, 1991; Simonova et al., 2008). They shed enteropathogens in the environment with their stools, which can be a source of contamination for other parts of the carcasses during slaughter procedures (Nesbakken et al., 2003; Van Damme et al., 2015). The European Food Safety Authority (EFSA) considers *Y. enterocolitica* as one of the most relevant biological hazards in the context of meat inspection of swine (EFSA, 2011).

Contaminated raw or undercooked pork meat is particularly important in transmitting *Y. enterocolitica* to humans (Rosner et al., 2012). The incidence rate of human yersiniosis attributed to pork consumption has been estimated to be 2.826 cases per 100,000 inhabitants per year in Europe. Therefore, *Y. enterocolitica* is the most prevalent biological contaminant in pigs after *Salmonella* (3.374/100,000 inhabitants) in Europe (Fosse et al., 2009). The biotype of *Y. enterocolitica* the most frequently responsible for human infections worldwide is biotype 4 (Drummond et al., 2012), which is almost systematically associated with serotype O:3 (4/O:3). Pigs are the main reservoir of bioserotype 4/O:3 strains (Drummond et al., 2012). Infections are the most dangerous in immunocompromised patients with a septicemic mortality rate which can reach 50% (Krauss et al., 2003). Although infections are often mild and self-limiting, more severe clinical presentations such as pseudo-appendicular syndromes mimicking an appendicitis (Perdikogianni et al., 2006) or septicemia in elderly and immunocompromised patients (Savin and Carniel, 2008) can occur. The chromosomally located *ail* gene (attachment and invasion locus) is an essential virulence factor in strains of *Yersinia* spp. (Miller et al., 1989) and it is the most frequently used target to detect human pathogenic *Y. enterocolitica* (Miller et al., 1989; Fredriksson-Ahomaa and Korkeala, 2003). The consumption of pork is increasing, and there are no scientific data related to the exposure risk of the population.

The objectives of this study were to assess the prevalence of *Y. enterocolitica* 4/O:3 isolated from pork destined for consumption and to describe the attitudes and practices surrounding consumption of pork in Côte d'Ivoire.

MATERIALS AND METHODS

Conducting the survey

The survey was carried out from February to November 2015 in Abidjan District. A preliminary survey of one month was performed in order to identify the main sites of pork consumption locations. It helped to settle a survey questionnaire describing the following sections: consumer profiles, eating habits, culinary techniques, storage conditions and risks associated with consumption.

The transversal and retrospective survey coupled with direct observation was conducted in 12 communes of Abidjan District, namely: Yopougon, Cocody, Treichville, Adjamé, Port-Bouët, Abobo, Marcory, Attecoubé, Plateau, Koumassi, Bingerville and Anyama (Figure 1).

Data collection

Methods of collecting data were face-to-face interviews using standardized questionnaires (written surveys) for both consumers or non-consumers. Written surveys allowed the researchers to ask consumers questions. The same questionnaire was used for both consumers and non-consumers. The survey was conducted by a random sampling of 3,200 people.

The survey was performed in households and out-of-home (streets, *maquis* and market). In those chosen locations, the consumers or non-consumers were randomly selected and questioned. In addition, sales and consumption practices were observed. At home, interviews were carried out with the people in charge of cooking the family meal. The questionnaire focused on three themes, namely eating habits of pork, the frequency of consumption and digestive disorders related to pork consumption. Socio-demographic information such as age, gender and education level were also collected. Administration of the questionnaire was followed by direct observation of the participants.

Population size

In this study, 3,200 people were questioned about pork consumption in Abidjan District. They were composed of 834 housewives (group I), 712 pupils and students (group II), 922 officials (group III) and 732 people from other socio-professional categories (group IV). Among the 3,200 people surveyed, 2,400 were consumers including 200 consumers per commune (Table 1).

Bacteriological analysis

Sources of sampling

The samples were collected at the slaughterhouse of SIVAC (Ivorian Society of Slaughter and pork butchery) from November 2015 to April 2015. The SIVAC Company was chosen because of the diverse origin of pigs slaughtered and its supply with large quantities of pig carcasses. Indeed, pig samples collected in this slaughterhouse were from modern and traditional farms of Abidjan District (Abobo, Bingerville, Yopougon,

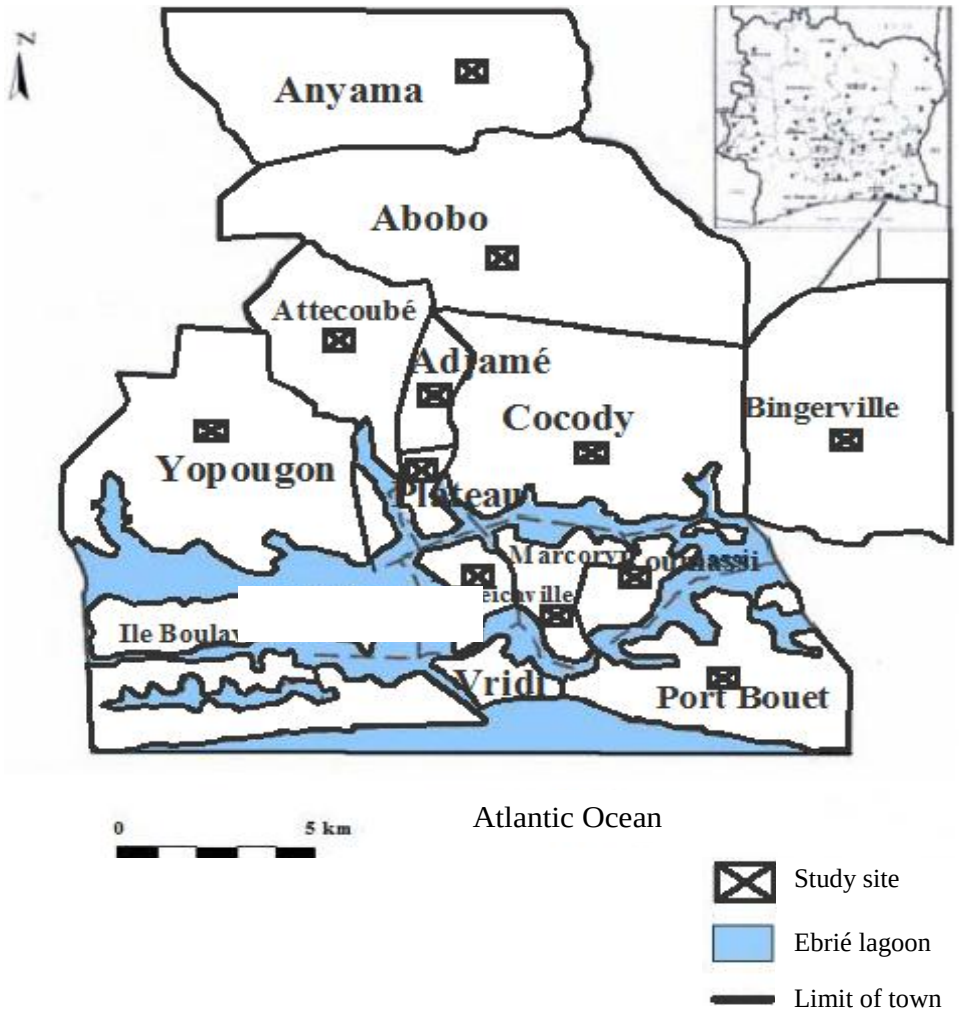

Figure 1. Map of Abidjan district.

Table 1. Distribution of people surveyed by commune.

Residential locations	Socio-professional categories				Total consumers and non-consumers	Consumers
	Housewives (group I)	Pupils and students (group II)	Officials (group III)	Other categories (group IV)		
Abobo	81	64	82	61	288	200
Anyama	82	61	79	67	289	200
Adjamé	79	56	80	59	274	200
Attécoubé	72	66	79	65	282	200
Bingerville	63	53	70	58	244	200
Cocody	64	65	73	59	261	200
Koumassi	74	60	87	66	287	200
Marcory	62	57	66	61	246	200
Plateau	73	57	95	58	283	200
Port-Bouët	60	58	67	60	245	200
Treichville	65	62	75	63	265	200
Yopougon	59	53	69	55	236	200
Total	834	712	922	732	3,200	2,400

Table 2. Pig sources and distribution of analyzed samples.

Communes	Carcasses	Tongues	Total
Abobo	20	20	40
Anyama	20	20	40
Bouaké	20	20	40
Bingerville	20	20	40
Divo	20	20	40
Grand-Bassam	20	20	40
Man	20	20	40
Songon	20	20	40
Yamoussoukro	20	20	40
Yopougon	20	20	40
Total	200	200	400

Anyama, Songon, Divo, Grand-Bassam, Man and Bouaké) (Table 2). A total of 400 samples (200 carcasses and 200 tongues) were collected from apparently healthy pigs randomly selected at SIVAC. The carcasses and tongues samples were swabbed with sterile cotton wool. The cotton swab samples were transferred into tubes containing 9 ml of Trypticase Soya Broth with Novobiocin (MERCK, Darmstadt, Germany). All samples were stored in ice box during transportation at 4°C within 2 h from collection and were taken to the laboratory for immediate *Yersinia* detection.

Isolation and identification of Yersinia strains

Yersinia strains were isolated as described by ISO 10273 (2003) method slightly modified. *Y. enterocolitica* strains were isolated using two stages enrichment procedures including pre-enrichment in trypticase soya broth (TSB) with Novobiocin (MERCK, Darmstadt, Germany) at 28°C for 24 h and selective enrichment using cold method (3 days at 25°C) in PSMB (Phosphate buffered saline supplemented with 1% mannitol, 1% sorbitol, 0.15% bile salts and 0.5% soy peptone). A volume of 0.5 ml TSB was transferred into 4.5 ml of PSMB. In order to reduce the contaminating flora, an alkali treatment of 0.5 ml of the enriched sample in 4.5 ml of 0.25% potassium hydroxide was performed as described by Aulisio et al. (1980). Immediately, 10 µl of the enriched sample was then streaked onto MacConkey agar (Bio-Rad, Marnes-La-Coquette, France) supplemented with 1% sorbitol. The plates were incubated at 30°C for 48 h. After incubation, the plates were examined for presumptive colonies. Oxidase-negative, glucose-positive, H_2S-negative and urease-positive colonies were finally identified with API 20E (BioMérieux, Marcy l'Etoile, France) and then with API 50CH (BioMérieux, Marcy l'Etoile, France).

Antibiotic susceptibility testing

A bacterial suspension of *Y. enterocolitica* in saline was prepared to the density of a McFarland 0.5 turbidity standard approximately corresponding to 1-2×10^8 CFU/mL. Antibiotic susceptibilities testing were determined by the disc diffusion method on Mueller-Hinton agar (Oxoid, France) according to the procedure of Bauer et al. (1966). The results were interpreted according to the CA-SFM/EUCAST (2015). The antimicrobial drugs tested and their concentrations on the discs (Bio-Rad, Marnes-La Coquette, France) were the following: penicillin (10UI), ampicillin (10µg), ticarcillin (75 µg), amoxicillin (25 µg), amoxicillin-clavulanic acid (20 µg/10 µg), Imipenem (10 µg), cefalotin (30 µg), cefoxitin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), gentamicin (15 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), sulphonamide (200 µg), and tetracycline (30 UI).

Primers and PCR conditions

DNA was extracted using the phenol/chloroform methods based on the procedures of Sambrook and Russel (2001). Fresh colonies of bacteria suspensions from 24 h culture on trypticase soy agar (TSA, Oxoid) were resuspended in 500 µl TE bufferpH (8.0) and genomic DNA of the purified isolates were extracted. Primers for the chromosomal genes *ail* (attachment invasion locus) a chromosomally located virulence marker of *Y. enterocolitica* and *ystA* (*Yersinia* heat-stable enterotoxin) of *Y. enterocolitica* were used (Table 3).

PCR reactions were carried out in a volume of 50 µl containing 2.5 µl of 5X Green Flexi buffer (Promega), 2.5 µl of 5X Colorless Flexi buffer, 3 µL of 25 mM $MgCl_2$; 1 µl of 10 µMnucleotides dATP, dTTP, dGTP and dCTP; 1 µl of a 20 µM solution of each primer (Table 3); 0.5 µl of 5

Table 3. Primers for the detection of virulence gene in *Yersinia enterocolitica* 4/O:3.

Gene	Primer	Sequence (5'-3')	Amplicon length	References
ail	ail-F	TAATGTGTACGCTGCGAG	351	Thoerner et al. (2003)
	ail-R	GACGTCTTACTTGCACTG		
ystA	ystA-F	AATGCTGTCTTCATTTGGAGC	145	Ibrahim et al. (1997)
	ystA-R	ATCCCAATCACTACTGACTTC		

ail, attachment invasion locus; *ystA*, *Yersinia* heat-stable toxin gene.

U/µlTaq DNA polymerase (Promega) and 5 µl of DNA. The amplifications were performed in a thermal cycler (GeneAmp 9700, Applied Biosystems, Singapore) with the following conditions: denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 5 min. A quantity of 10 µl of PCR products were subjected to electrophoresis in a 2% agarose gel (Invitrogen) and stained with ethidium bromide (Eurobio).

Statistical analysis

Statistical analysis was performed using SPSS software version 20.0 (IBM Corporation, Somers, NY, USA). The Chi-square test was used to test the relationships between the variables. The difference between the variables was considered significant at $p< 0.05$.

The relative risk (RR) was calculated from SPSS software to determine the risk of sickness related to pork consumption.

RESULTS

Profile and distribution of consumers

Among the 3,200 people surveyed, 2,400 (75%) were pork consumers. Based on the data collected, 1,554 (48.6%) consumed pork at home and 846 (26.4%) people preferred to consume pork out-of-home. For the rest of the results, only people consuming pork were taken into account (n = 2,400).

Socio-demographic characteristics of pork consumers

The survey of 2,400 consumersof pork revealed that 62.3 and 37.7% of respondents were women and men, respectively with a sex ratio of 0.6. The education level for most respondents (30.9%) was secondary school, 24.7% had no level, and 23.9% had a primary school level (Table 4).

Considering the frequency of pork consumption, the survey also showed that 49.7% of respondents consumed pork every day (about 7 times a week), compared to 37% of respondents who consumed pork occasionally (Table 4).

Modes and places of pork consumption

Pork consumption at home

At home, pork was preferentially consumed in three modes. This source of protein was more consumed as smoked meat (51.9%) in sauce, but also as boiled fresh pork (24.7%) in sauce, as fried meat (17.3%) in sauce and other forms (6.1%) of cooking were consumed (Figure 2).

Pork consumption outside the home

Pork vendors were mostly located near the *maquis*, cabarets, bars, etc. Pork was consumed in the *maquis*[1] (38.5%), bars (7.7%), cabarets (9.6%), schools (7.7%), restaurants (13.6%), street vendors (17.3%), markets (3.8%) and offices (1.9%) sometimes (Figure 3). In *maquis*[1], baked pork[2] (51.3%) was the most consumed. Skewers (11.4%) and pork steaks (4.9%) were less consumed (Figure 4).

Risks related to pork consumption

Storage conditions and pre-processing of pork

After purchasing pork, 46.7% of consumers cooked the same day for consumption, compared to 53.3% who stored the pork before cooking. Indeed, 23.8% of the respondents kept their pork in the refrigerator, 15.2% of respondent said that they kept pork at ambient temperature after smoking. Other respondents mentioned that they kept their pork at room temperature after boiling (10.5%) or after frying (5.7%). Only 8.6% of consumers used a freezer to store their pork meat. Some housewives and sellers kept their pork under ice (7.6%) for 24 h (one night) before cooking (Figure 5).

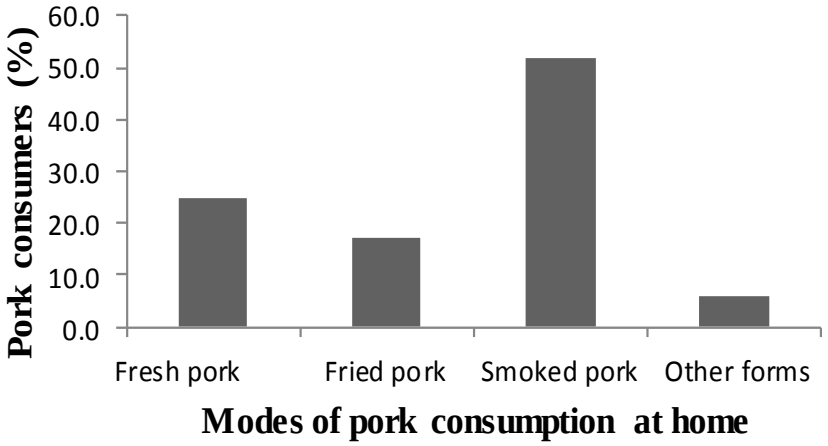

Figure 2. Different modes of pork consumption at home.

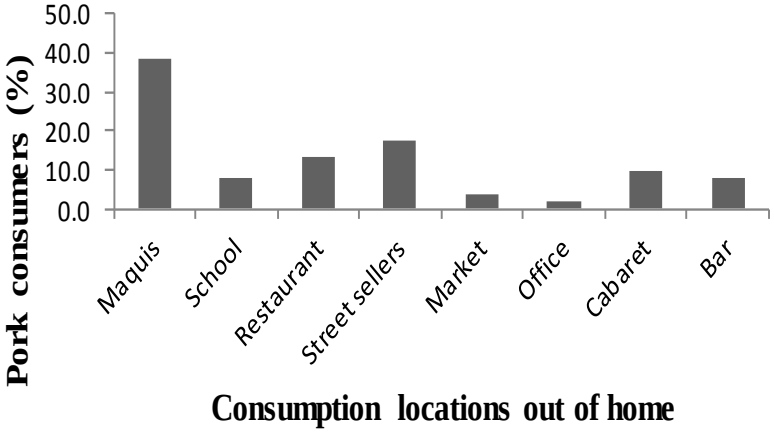

Figure 3. Locations of pork consumption outside the home.
[1]*Maquis*: In Africa particularly in Côte d'Ivoire. Bar, dancing (Larousse). Location of sale of beverages, refreshment bar, dancing and restaurant.

Table 4. Socio-demographic characteristics of pork consumers.

Characteristics	Total	Ratio (%)
Gender		
Female	1,495	62.3
Male	905	37.7
Study level		
None	586	24.7
Primary	569	23.9
Secondary	742	30.9
University	503	21.0
Consumption frequency of pork		
Everyday	1,193	49.7
Occasionally	888	37.0
Rarely	319	13.3

Every day (~ 7 times a week); occasionally (~ once a month); rarely (~ once a year).

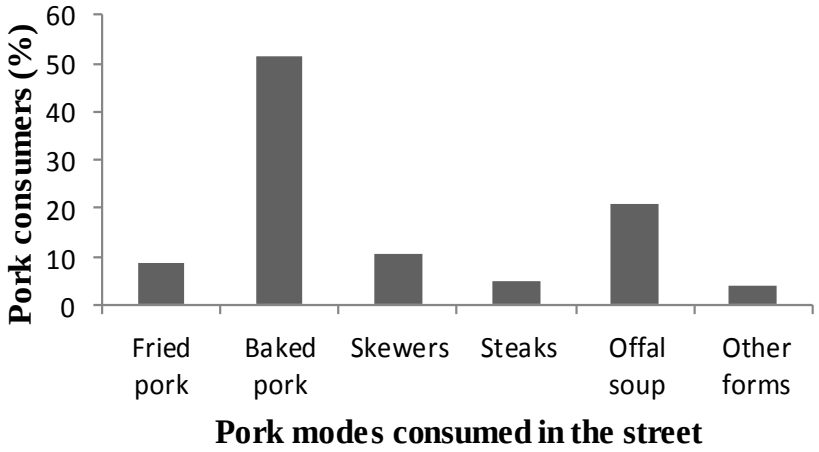

Figure 4. Distribution of pork consumption consumed in the street.
[2]Baked pork: pork cooked in an oven by wood.

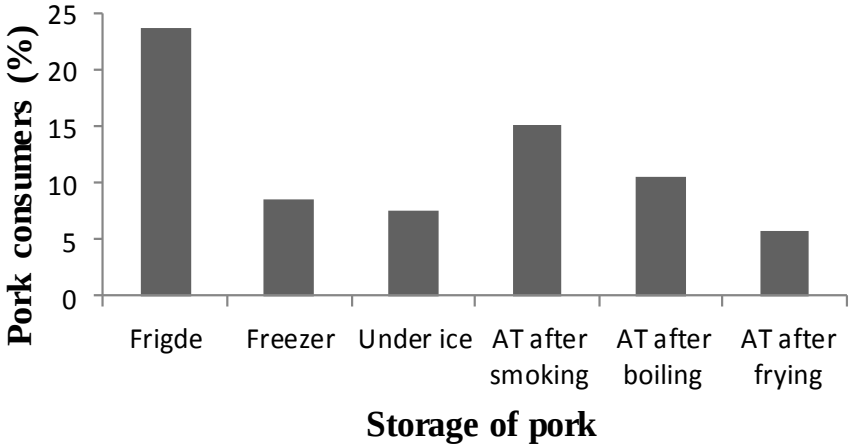

Figure 5. Methods of pork storagebefore preparation cooking.
AT: Ambient temperature.

Digestive disorders related to pork consumption

Respondents (34.3%) reported digestive disorders (diarrhea, abdominal pain, vomiting and nausea) after eating pork. Diarrhea was the most frequently reported (24.3%). Diarrhea with abdominal pain was reported in 21.7% of cases, while fever and vomiting occurred in 8.1 and 10.8% of cases respectively (Figure 6).

Biochemical characteristics of Y. enterocolitica 4/O:3

The biochemical characteristics of *Y. enterocolitica* 4/O:3 strains on API 50CH strips: control (-); Glycerol (+); Erythritol (-); D-arabinose (-); L-arabinose (+); D-ribose (+); D-xylose (-); L-xylose (-); D-adonitol (-); Methyl-βD-xylopyranoside (-); D-galactose (+); D-glucose (+); D-fructose (+); Mannose (+); L-sorbose (+); L-rhamnose (-); Dulcitol (-); Inositol (-); D-mannitol (+); D-sorbitol (+); Methyl-αD-mannopyranoside (-); Methyl-αD-glucopyranoside (-); N-acetylglucosamine (+); Amygdalin (-); Arbutin (-); Esculin + iron citrate (-); D-cellobiose (+); D-maltose (+); D-lactose (-); D-melibiose (-); D-sucrose (+); D-trehalose (+); Inulin (-); D-melezitose (-); D-raffinose (-); Starch (-); Glycogen (-); Xylitol (-);Gentiobiose (+); D-turanose (-); D-lyxose (-); D-tagatose (-); D-fucose (-); L-fucose (-); D-arabitol (-); L-arabitol (-); Potassium gluconate (-); Potassium 2-ketogluconate (-); Potassium 5-ketogluconate (-). The three different strains isolated from tongue and carcass had the same

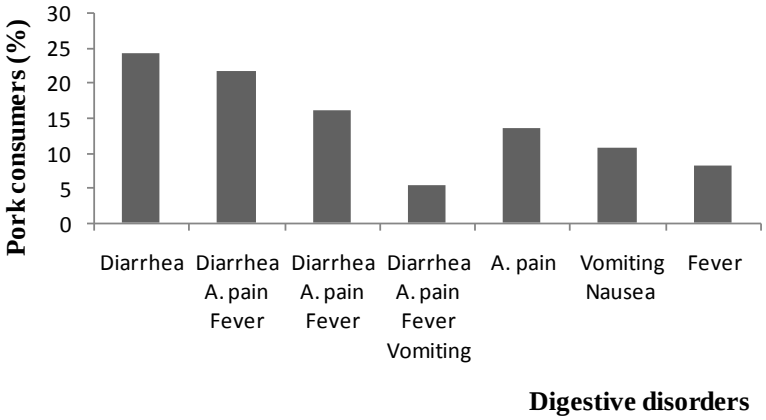

Figure 6. Digestive disorders evoked after pork consumption.
A. pain: Abdominal pain.

Table 5. Frequency of *Yersinia enterocolitica* 4/O:3 in pork.

Nature of samples	Effective (n)	Prevalence of *Y. enterocolitica* 4/O:3 n(%)
Tongue	200	2(1)
Carcass	200	1(0.5)
Total	400	3(0.7)

characteristics.

Frequency of Y. enterocolitica 4/O:3 isolated from pork

In total, 3 (0.7%) strains of *Y. enterocolitica* 4/O:3 were isolated, including 2 (1%) strains in 200 tongues and 1 (0.5%) strain in 200 carcasses (Table 5), that is 2 of 200 tongues and 1 of 200 carcasses were tested positive for *Y. enterocolitica* 4/O:3.

Antibiotic susceptibility of Y. enterocolitica 4/O:3

Y. enterocolitica 4/O:3 strains isolated were resistant to penicillin, ampicillin, amoxicillin, amoxicillin/clavulanic acid, ticarcillin and cefalotin. However, they were susceptible to imipenem, cefoxitin, ceftriaxone, ciprofloxacin, nalidixic acid, gentamicin, trimethoprim/ sulfamethoxazole, sulphonamide and tetracycline. The three different strains isolated from tongue and carcass had the same characteristics.

Virulence markers of Y. enterocolitica4/O:3

All the three *Y. enterocolitica* 4/O:3 strains carried the *ail* and *ystA* chromosomal virulence genes (Figure 7), indicating their pathogenicity.

DISCUSSION

Consumption of pork

In Côte d'Ivoire, studies on foodstuffs consumption are generally rare. However, they provide a better understanding of consumers' preferences, uses made of these food products and the environment of consumption whether at home or out of home. While the food choices reflect the preferences and eating habits of the population, the composition of a meal also relies on the availability and accessibility of food sources (Dindé et al., 2017).

The survey performed in this study revealed that three quarters of the population in Abidjan District consumed pork. Pork consumption is mainly made at home (64.8%). The predominance of women among consumers may be

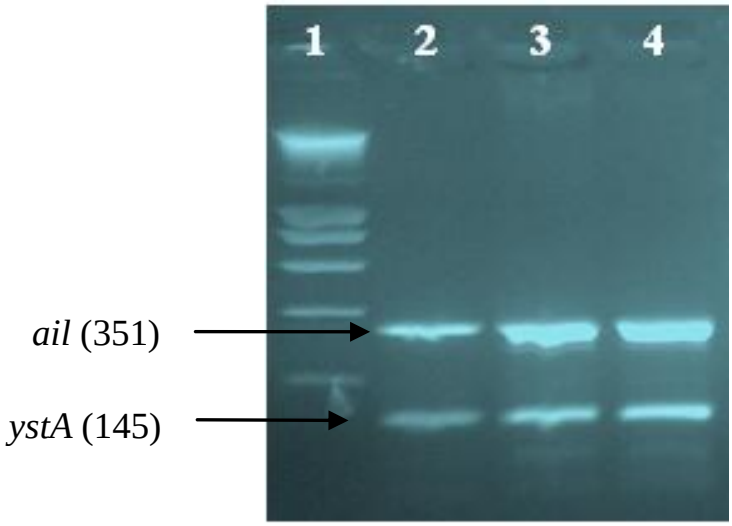

Figure 7. Profile detection of virulence genes *ail* and *yst* in *Y. enterocolitica*4/O:3. **Lane 1:** DNA molecular weight markers; **lane 2:** *Y. enterocolitica* 4/O:3 CCUG (positive control); **Lane 3 and 4:** *Y. enterocolitica* 4/O:3.

related to the way of survey performing. Indeed, the methodological approach during the survey consisted in submitting the questionnaire randomly to the target population. At the time of the survey, people most often found in households were women. In fact, women are usually in charge of the family meal in household (Grace et al., 2015). The pork marketing (promotion, distribution, selling of pork or buying and selling in the market) described as activity, allow women to emerge from poverty. Furthermore, the grouping of women in association allows them to control this sector, take care of themselves, feed their families and educate their children.

In general, pork has a cheap cost and accessible to people with low incomes compared to beef or poultry meat. This result is in accordance with that of Legendre (2008), who showed that households with the lowest incomes and the rural ones buy and consume more pork than other meats. Moreover, with the development of pig breeding in Côte d'Ivoire, pork is increasingly available in the different markets of Abidjan District. Thus, this meat is used as a main component of daily food for consumptiondue to the ease of cooking. The study also showed that pork was consumed most frequently at home, smoked (51.9%) in sauce. Previous studies have justified this preference. According to Stolzenbach et al. (2009) and Chevillon et al. (2009), smoking processing reduces the high fat content, partially masking the unpleasant smell of fresh pork; which contributes to improve the nutritional and organoleptic characteristics of the cooked product. However, for Lawrie and Ledward (2007) slow combustion of sawdust derived from hardwoods produces smoke, which inhibits microbial growth, retards oxidation of fats, and imparts flavor to cured meats.

On the other hand, in the street, pork consumption in baked (51.3%) form is preferred. This preference for consumers outside the home could be explained with greater availability. In addition, consumption of baked pork in association with *attiéké* or *alloco* is of the convenience for most of the consumers. Indeed, culinary preparation in sauce requires a variety of logistics and ingredients that are not always easy to combine in an out-of-home environment. In addition, appearance of baked pork is similar to barbecue, which leads to a product locally called *choukouya* in Côte d'Ivoire.

The survey also highlighted that pork consumption in the street was consumed near the *maquis* (38.5%). Pork meat whether boiled in soup or baked, is often consumed, associated with traditional alcoholic beverages such as *koutoukou* (palm wine liquor) and *tchapalo* (fermented sorghum beer), or industrial beverages (beer, wine). Consumers of these beverages would therefore be the most regular consumers of pork. This observation was also made in Ouagadougou (Mopaté and Kaboré-Zoungrana, 2013), Yaoundé (Koussou and Duteurtre, 2002) and Chad (Paloumi, 2002).

Prevalence of *Yersinia* spp.

The prevalence of *Y. enterocolitica* in pork (tongues and carcasses) in this study was 0.7%. This prevalence is

lower than that reported by EFSA (2010) which is 2% *Yersinia* in fresh pork meat. Similarly, in South Africa, out of 1634 stool examinations, *Y. enterocolitica* was isolated with a frequency of 1% (Jennings et al., 1987).

Several reasons may explain the low prevalence of this pathogen species, such as the nature of the sample, the number of samples analyzed, the season, the geographical location of *Yersinia* strains, and the isolation methods also discussed by Siriken (2004), Nesbakken et al. (1991) and Magras et al. (2008). In addition, one of the major limitations in this study was the isolation methods of *Yersinia* strains from the pig samples because of their slow growth and optimal temperature at 28°C. The same observation was made by Savin et al. (2010) who reported difficulties in isolating strains of *Yersinia* from biologically contaminated samples such as stool. The present study was performed in Abidjan, where the climate is generally warm. Due to the psychrotrophic nature of *Yersinia*, the warm climate does not promote their growth and can become an inhibitory factor for these strains.

Although the isolation rate of *Y. enterocolitica* is higher in countries with a cold climate than those with a warm climate, this study established the presence of *Y. enterocolitica* bioserotype 4/O:3 in pork in Côte d'Ivoire. Similarly, in Nigeria, the bioserotype 4/O:3 was found in fecal samples from a pig and sheep (Okwori et al., 2009). In Europe, the prevalence of strains of *Y. enterocolitica* bioserotype 4/O:3 of slaughtered pigs was estimated at 56% in Finland (Korte et al., 2004) and rate of 60% in Southern Germany (Fredriksson-Ahomaa et al., 2001).

All *Y. enterocolitica* strains isolated belonged to the serotype O:3. No other serotypes were identified in this study. It is important to mention that *Y. enterocolitica* serotype O:3 is isolated with a rate more than 90% during human yersiniosis epidemics (EFSA, 2010). Yersiniosis is the third most frequent bacterial disease causing human enteric infections in Europe (Eurosurveillance-Editorial-Team, 2015), but reports on this disease are extremely non frequent in developing countries. In West Africa, only few countries reported the isolation of *Yersinia* from clinical cases (Simpore et al., 2009; Agbonlahor et al., 1983; Okwori et al., 2009), most likely because an active search for these bacteria is not performed. A survey of the pig reservoir in Senegal (Chambron and Bourdin, 1971) and Burkina Faso (Hakalehto et al., 2014) did not identify any *Yersinia* strains.

In this study, the low prevalence of pathogenic *Y. enterocolitica* in samples of slaughtered pigs may suggest that the slaughterhouse is not the main source of pathogenic strains of pigs. Transmission from other infected pigs seems more likely. In addition, Fukushima et al. (1990) and Sammarco et al. (1997) showed that *Y. enterocolitica* can be transmitted horizontally from infected pigs to other pigs (healthy) in the slaughterhouse. Pigs may be contaminated from the feces of infected pigs or soil contaminated with *Yersinia* strains during transport to the slaughterhouse and during the time spent at the slaughterhouse. Transport to the slaughterhouse is a significant risk factor for contamination by *Y. enterocolitica* (Nowak et al., 2006) as for other pathogens.

Pork production is affected by a variety of risk factors among which are quality of pig feed, number of subclinical carriers within a farm, conditions during transportation and lairage premises before slaughtering, slaughter line contamination by carrier animals, conditions during processing and retailing of pork products, conditions of handling pork products during catering and home-food preparation (Boyen et al., 2008; Lo Fo Wong et al., 2004; Kranker et al., 2001).

Risk factors

Diarrheal diseases are a major public health problem in developing countries, with a high infant mortality rate in Africa (Boschi-Pinto et al., 2008). Consumers reported experiencing digestive disorders such as diarrhea, abdominal pain, nausea, vomiting; consecutive to pork consumption. Although these symptoms are similar to those of yersiniosis (Munk Petersen et al., 1996; Huovinen et al., 2010), there are several reasons for which it cannot be established a direct link between these disorders and the agent of this disease. Indeed, not only the symptoms mentioned above may have a non-infectious origin, but also and in this study, *Yersinia* strains have not been isolated from faeces patients to support this link. Moreover, in the event that these digestive disorders are considered to have an infectious origin, the causative agent may be a microorganism other than *Yersinia*; because pork is known to be an important vehicle of various agents (Fosse, 2008) that can be viral, parasitic or other bacterial sources. Although transmission to human through pork consumption has not yet been proved, *Yersinia enterocolitica* 4/O:3 can be considered as a suspected hazard for it has been detected in food.

It is found, as revealed by the investigation, that pork is also sold and consumed in the street; which exposes the consumer to a contamination whose source may not always be an agent intrinsically transported by pork. But, according to Fredriksson-Ahomaa et al. (2001), in shops and then in kitchens, *Y. enterocolitica* can easily contaminate other foods thorough direct contact with contaminated raw pork and edible offal or via contaminated hands or equipment during handling and preparation.

Acute gastroenteritis accompanied by vomiting, abdominal pain, diarrhea and fever are symptoms that consumers of pork in Côte d'Ivoire may have as previously reported by Rosner et al. (2013). For Munk-

Petersen et al. (1996), *Y. enterocolitica* 4/O:3 is known as a major cause of acute gastroenteritis after ingestion of pork products. *Y. enterocolitica* 4/O:3 isolated from the Abidjan District were susceptible to most antibiotics commonly used to treat Gram-negative enteropathogens, and were resistant to penicillin and first and second-generation cephalosporin, due to the presence of the chromosomal *blaA* and *blaB* genes (Seoane and Garcia Lobo, 1991; Bonke et al., 2011). Moreover, *Y. enterocolitica* 4/O:3 were also positive for chromosomal virulence gene *ail* related to invasion and *yadA* located on the plasmid pYV (Fredriksson-Ahomaa, 2001), indicating their potential pathogenic. Generally, the pathogenicity of *Y. enterocolitica* depends on the presence of several genes, known as virulence markers. These genes facilitate bacteria to enter a susceptible organism, colonize it, evade the immune system and grow under unfavorable conditions (Gierczyński, 2000). Van Damme et al. (2015) found that the initial presence of *Y. enterocolitica* in tonsils and/or in faeces of pigs at slaughter was significantly associated with carcass contamination and the findings of the same genotypes in tonsils, offal, and in minced pork support the assumption that tonsils are the primary source of contamination with pathogenic *Y. enterocolitica* at the slaughterhouse level (Fredriksson-Ahomaa et al., 2001). Therefore, considering the fact that insufficiently thermally processed pork is the main source of infection for humans, public information campaigns are an important element of the preventive measures against *Y. enterocolitica* infections.

Conclusion

Pork products are widely consumed at home and out-of-home. This study indicated that pork can be a source of pathogenic *Y. enterocolitica* 4/O:3 at retail level in Abidjan District. The type of processing undergone by pork affects the choice of consumers according to the location of consumption. Thus, at home, smoked pork ribs in sauce are more highly value. Out of home, baked pork accompanied with beverages are appreciated by consumers. The relatively low cost of this meat and its availability give a prominent place in the diet of consumers. This study showed that consumers of pork are generally aware of the risk of consuming undercooked and raw pork. The survey also found that pork consumption would be a risk factor for *Yersinia* infection. The pathogenic strains of *Y. enterocolitica* 4/O:3 with virulence genes, were isolated from pork in Côte d'Ivoire. Therefore, sufficient heat treatment and prevention of cross-contamination during cooking of food in the kitchen should be recommended. Information and sensitization of population and institutions on *Y. enterocolitica* 4/O:3 risk factors and preventive measures to must be observed.

ACKNOWLEDGEMENTS

The researchers are grateful to the staff of Laboratory of Biotechnologies, especially to Thierry Lessoy and Kambou Sansan for critically proofreading the manuscript.

REFERENCES

Agbonlahor D. E., Odugbemi T. O. & Dosunmu-Ogunbi O. (1983). Isolation of species of *Yersinia* from patients with gastroenteritis in Nigeria. J. Med. Microbiol. 16:93-96. doi: 10.1099/00222615-16-1-93 PMID: 6822994.

Aulisio C. C. G., Mehlman I. J. & Sanders A. C. (1980). Alkali method for rapid recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from foods. Appl. Environ. Microbiol. 39:135-140. PMID: 6986846.

Bauer A. W., Kirby W. M., Sherris J. C. & Turck M. (1966). Antibiotic susceptibility testing by a standardized single disk method. 45:493-496. PMID: 5325707.

Bonke R., Wacheck S., Stuber E., Meyer C., Martlbauer E. & Fredriksson-Ahomaa M. (2011). Antimicrobial susceptibility and distribution of beta-lactamase A (blaA) and beta-lactamase B (blaB) genes in enteropathogenic *Yersinia* species. Microb. Drug Resist. 17:575-581. doi: 10.1089/mdr.2011.0098 PMID: 21970627.

Boschi-Pinto C., Velebit L. & Shibuya K. (2008). Estimating child mortality due to diarrhoea in developing countries. World Health Org. Bull. 86:710-717.

Boyen F., Haesebrouck F., Maes D., Van Immerseel F., Ducatelle R. & Pasmans F. (2008). Non-typhoidal *Salmonella* infections in pigs: A closer look at epidemiology, pathogenesis and control. Vet. Microbiol. 130:1-19.

CA-SFM/EUCAST (2015). Comité de l'Antibiogramme de la Société Française de Microbiologie/European Committee on Antimicrobial Susceptibility Testing. Recommandations 2015 (V1.0 Janvier 2015). 116p. Available online at: http://www.sfm-microbiologie.org/UserFiles/files/casfm/CASFM_EUCAST_V1_2015.pdf.

Chambron J. & Bourdin M. A. (1971). A propos d'une enquête bactériologique sur les ganglions mésentériques de porcs à Dakar. Recherche négative de *Yersinia enterocolitica*. Intérêt épidémiologique d'une telle recherche. Méd. Afr. Noire. 18:823-826.

Chevillon P., Bonneau M., Le Strat P., Guingand N., Courboulay V., Quiniou N., Gault E. & Lhommeau T. (2009). Acceptabilité par les consommateurs des viandes de porc mâle entier transformées en saucisses, lardons, saucissons secs et jambons cuits. Techni Porc. 32, N°5, 2009 - la revue technique de l'IFIP.

CTA (2007). Centre technique de coopération agricole et rurale (CTA). Porcs, Des atouts sous la menace, Élevage de porcs dans les zones tropicales, CTA, ISSN 1011-0054. Spore n° 132. 11p. Available online at: http://spore.cta.int.

Dindé O. A., Mobio J. A., Konan G. A., Fokou G., Yao K., Esso J. C. L. E., Fantodji A., Koussemon M. & Bonfoh B. (2017). Response to the Ebola-related bush meat consumption ban in rural Côte d'Ivoire. Agriculture and Food Security 6:28.DOI 10.1186/s40066-017-0105-9.

Drummond N., Murphy B. P., Ringwood T., Prentice M. B., Buckley J. F. & Fanning S. **(**2012). *Yersinia enterocolitica*: A brief review of the issues relating to the zoonotic pathogen, public health challenges, and the pork production chain. Foodborne Pathog. Dis. 9:179-189. doi: 10.1089/fpd.2011.0938 PMID: 22217012.

European Food Safety Authority, EFSA (2010). The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and food-borne outbreaks in the European Union in 2008.

EFSA J. 8: 1496.
European Food Safety Authority, EFSA (2011). EFSA panels on biological hazards (BIOHAZ), on contaminants in the food chain (CONTAM), and on animal health and welfare (AHAW). Scientific opinion on the public health hazards to be covered by inspection of meat (swine). EFSA J. 9: 198.
Eurosurveillance-Editorial-Team (2015). The 2013 joint ECDC/EFSA report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks published. Eurosurveillance. 20:43-43.
FAO (2014). Sources of meat. Food and agricultural organization, animal production and health. Available online at: http://www.fao.org/ag/againfo/themes/en/meat/backgr_sources.html. Accessed 11.05.2015.
Fosse J. (2008). Valeur informative d'indicateurs*ante* et *post mortem* pour la détection des dangers biologiques pour le consommateur de viande porcine. Thèse de Vie-Agro-Santé, Sciences de la Vie et de l'Environnement, Rennes. 408p.
Fosse J., Seegers H. & Magras C. (2009). Prevalence and risk factors for bacterial food-borne zoonotic hazards in slaughter pigs: a review. Zoon. Public Health. 56:429-454.
Fredriksson-Ahomaa M. & Korkeala H. (2003). Low Occurrence of Pathogenic *Yersinia enterocolitica* in clinical, food, and environmental samples: A methodological problem. Clin. Microbiol. Rev. 16:220-229.doi: 10.1128/CMR.16.2.220-229.2003 PMCID: PMC153140.
Fredriksson-Ahomaa M. (2001). Molecular epidemiology of yad A-positive *Yersinia enterocolitica,* Academic dissertation in Faculty of Veterinary Medicine of the University of Helsinki. 85p.
Fredriksson-Ahomaa M., Bucher M., Hank C., Stolle A. & Korkeala H. (2001). High prevalence of *Yersinia enterocolitica* 4/O:3 on pig offal in southern Germany: A slaughtering technique problem. Syst. Appl. Microbiol. 24:457-463. http://dx.doi.org/10.1078/0723-2020-00055 PMid:11822684.
Fukushima H., Maruyama K., Omori I., Ito K. & Iorihara M. (1990). Contamination of pigs with *Yersinia* at the slaughterhouse. Fleischwirtschaft. 70:1300-1302.
Gierczyński R. (2000). Evaluation of the usefulness of selected virulence markers for identification of virulent *Yersinia enterocolitica* strains. II. Genotypic markers associated with the pYV plasmid. Med. Dośw. Mikrobiol. 52:35-49.
Grace D., Roesel K., Kang'ethe E., Bonfoh B. & Theis S. (2015). Gender roles and food safety in 20 informal livestock and fish value chains. In: Discussion paper 01489. International Food Policy Research Institute. Available online at: http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/129860. Accessed 14 Aug. 2017.
Hakalehto E., Nyholm O., Bonkoungou I. J., Kagambega A., Rissanen K., Heitto A., Barro N. & Haukka K. (2014). Development of microbiological field methodology for water and food-chain hygiene analysis of *Campylobacte*r spp. and *Yersinia* spp. in Burkina Faso, West Africa. Pathophysiology. 21:219-229. doi: 10.1016/j. pathophys.2014.07.004 PMID: 25156815.
Huovinen E., Sihvonen L. M., Virtanen M. J., Haukka K., Siitonen A. & Kuusi M. (2010). Symptoms and sources of *Yersinia enterocolitica*-infection: a case control study. BMC Infect. Dis. 10: 122.
Ibrahim A., Liesack W., Griffiths M. W. & Robins-Browne R. M. (1997). Development of a highly specific assay for rapid identification of pathogenic strains of *Yersinia enterocolitica* based on PCR amplification of the *Yersinia* heat-stable enterotoxin gene (*yst*). J. Clin. Microbiol. 35: 1636-1638.
ISO 10273 (2003). Microbiology of food and animal feeding stuffs-Horizontal method for the detection of presumptive pathogenic *Yersinia enterocolitica* (ISO 10273: 2003). International Organization for Standardization, Geneva, Switzerland.
Jennings D. L., Miliotis M. D. & Koornhof H. J. (1987). Human *Yersinia enterocolitica* infection in the Eastern Cape. A report on 18 and a review of yersiniosis in the RSA. S. Afr. Med. J. 72: 868-870.
Kapperud G. (1991). *Yersinia enterocolitica* in food hygiene. Int. J. Food Microbiol. 12: 53-65. PMID: 2018706.
Korte T., Fredriksson-Ahomaa M., Niskanen T. & Korkeala H. (2004). Low prevalence of pathogenic *Yersinia enterocolitica* in sow tonsils. Foodborne Pathog. Dis. 1:45-52.
Koussou M. O. & Duteurtre G. (2002). Les facteurs de compétivité de la filière porcine dans le bassin du Logone. Communication présenté au colloque« Système agro-alimentaire localisé-Syal », Montpellier, Cirad, Octobre 2002, 13p. Available online at: http://pigtrop.cirad.fr/fr/vie_scientifique/economie_Logone.htm.
Kranker S., Dahl J. & Wingstrand A. **(**2001). Bacteriological and serological examination and risk factor analysis of *Salmonella* occurrence in sow herds, including risk factors for high *Salmonella* seroprevalence in receiver finishing herds. Berl. Munch. Tierarztl. 114(9-10):350-352.
Krauss H., Weber A., Appel M., Enders B., Isenberg H. D., Schiefer H. G., Slenczka W., von Graevenitz A. & Zahner H. **(**2003). Zoonoses infectious diseases transmissible from animals to humans, 3rd. Edition. ASM Press, Washington, DC.
Lawrie R. A. & Ledward D. A. (2007). Lawrie's meat science (7th ed.). Boca Raton, FL: CRC Press.
Legendre V. **(**2008). Les consommateurs de viande de porc frais et de charcuterie: qui sont-ils? Eclairage sociologique. Techni Porc, Vol. 31, N°4- la revue technique de l'IFIP.
Lo Fo Wong D. M. A., Dahl J., Stege H., van der Wolf P. J., Leontides L., von Altrock A. & Thorberg B. M. (2004). Herd-level risk factors for subclinical *Salmonella* infection in European finishing-pig herds. Prev. Vet. Med. 62(4):253-266.
Magras C., Vallée T., Leclercq A. & Fosse J. (2008). Detection de *Yersinia enterocolitica* sur les carcasses de porc: premiers résultats. Journée de Recherche Porcine. 40:79-80.
Miller V. L., 3rd Farmer J. J., Hill W. E. & Falkow S. (1989). The ail locus is found uniquely in *Yersinia enterocolitica* serotypes commonly associated with disease. Infect. Immun. 57:121-131. PMID: 2642465.
Mopaté L. Y. & Kaboré-Zoungrana C. Y. (2013). Production, commerce et consommation de viande porcine dans quelques villes d'Afrique de l'Ouest et du centre. Revue Scientifique du Tchad – décembre. Pp. 43-44.
Munk-Petersen A., Vinther Nielsen S., Meyer D., Ganer P. & Ladefoged K. (1996). Bacterial gastro-enteritis among hospitalised patients in a Danish Country. Scand. J. Gastroentero. 31:906-911.
Nesbakken T., Eckner K., Køidal H. K. & Røtterund O. J. (2003). Occurrence of *Yersinia enterocolitica* and *Campylobacter* spp. in slaughter pigs and consequences for meat inspection, slaughtering, and dressing procedures. Int. J. Food Microbiol. 80:231-240.
Nesbakken T., Kapperud G., Dommarsnes K., Skurnik M. & Hornes E. (1991). Comparative study of a DNA hybridisation method and two isolation procedures for detection of *Yersinia enterocolitica* O:3 in naturally contaminated pork products. Appl. Environ. Microbiol. 57:389-394.
Nowak B., Mueffling T. V., Caspari K. & Hartung J. (2006). Validation of a method for the detection of virulent *Yersinia enterocolitica* and their distribution in slaughter pigs from conventional and alternative housing systems. Vet. Microbiol. 117:219-228.
Okwori A. E. J., Martinez P. O., Fredriksson-Ahomaa M., Agina S. E. & Korkeala H. (2009). Pathogenic *Yersinia enterocolitica* 2/O:9 and *Yersinia pseudotuberculosis* 1/O:1 strains isolated from human and non-human sources in the Plateau State of Nigeria. Food Microbiol. 26:872-875. doi: 10.1016/j.fm.2009.06.001 PMID: 19835774.
Paloumi P. (2002). Contribution à l'étude des principales caractéristiques de la transformation et de la commercialisation de la viande porcine dans la ville de N'Djamena. Mémoire de fin de cycle de Technicien d'élevage, N'Djamena, Tchad, Laboratoire de Farcha. 34p.
Perdikogianni C., Galanakis E., Michalakis M., Giannoussi E., Maraki S., Tselentis Y. & Charissis G. (2006). *Yersinia enterocolitica* infection mimicking surgical conditions. Pediatr. Surg. Int. 22:589-92. PMID: 16770604.
Rosner B. M., Stark K., Höhle M. & Werber D. (2012). Risk factors for sporadic *Yersinia enterocolitica* infections, Germany 2009-2010. Epidemiol. Infect. 140: 1738-1747.
Rosner B. M., Werber D., Hoehle M. & Stark K. (2013). Clinical aspects and self-reported symptoms of sequelae of *Yersinia enterocolitica* infections in a population-based study, Germany 2009-2010. BMC

Infect. Dis.13:236.
Sambrook J. & Russel D. W. (2001). The basic polymerase chain reaction. In: Molecular cloning: A laboratory manual. 3rd ed., Vol. 3. New York: Cold Spring Harbor Laboratory Press. 8:19-24.
Sammarco M. L., Ripabelli G., Ruberto A., Iannitto G. & Grasso G. M. (1997). Prevalence of Salmonellae, Listeriae and Yersiniae in the slaughterhouse environment and on work surfaces, equipment and workers. J. Food Protect. 60:367-371.
Savin C. & Carniel E. (2008). Les diarrhées d'origine bactérienne: le cas de *Yersinia enterocolitica*. Rev. Francoph. Lab. 400:49-58.
Savin C., Leclercq A., Laurent E., Carniel E. & Vaillant V. (2010). National survey on diagnosis of enteropathogenic *Yersinia* infections in metropolitan France, 2003. Bull. Epid. Hebd. 29:307-311.
Seoane A. & Garcia Lobo J. M. (1991). Cloning of chromosomal beta-lactamase genes from *Yersinia enterocolitica*. J. Gen. Microbiol. 137:141-146. doi: 10.1099/00221287-137-1-141 PMID: 2045777.
Simonova J., Borilova G. & Steinhauserova I. (2008). Occurrence of pathogenic strains of *Yersinia enterocolitica* in pigs and their antimicrobial resistance. Bull. Vet. Inst. Pulawy. 52:39-43.
Simpore J., Ouermi D., Ilboudo D., Kabre A., Zeba B., Pietra V., Pignatelli S., Nikiema J. B., Kabre G. B., Caligaris S., Schumacher F. & Castelli F. (2009). Aetiology of acute gastro-enteritis in children at Saint Camille Medical Centre, Ouagadougou, Burkina Faso. Pak. J. Biol. Sci. 12:258-263. PMID: 19579955.
Singer R. S., Cox Jr L. A., Dickson J. S., Hurd H. S., Phillips I. & Miller G. Y. (2007). Modeling the relationship between food animal health and human foodborne illness. Prev. Vet. Med. 79:186-203.
Siriken B. (2004). The Presence of *Yersinia enterocolitica* and Other *Yersinia* Species in Ground Beef in Aydin, Turkey. Turk. J. Vet. Anim. Sci. 28:489-495.
Stolzenbach S., Lindahl G., Lundström K., Chen G. & Byrne D. (2009). Perceptual masking of boar taint in Swedish fermented sausages. Meat Science. 81:580-588.
Thoerner P., Bin Kingombe C. I., Bögli-Stuber K., Bissig-Choisat B., Wassenaar T. M., Frey J. & Jemmi T. (2003). PCR detection of Virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and investigation of virulence gene distribution. Appl. Environ. Microbiol. 69:1810-1816.
Van Damme I., Berkvens D., Vanantwerpen G., Baré J., Houf K., Wauters G. & De Zutter L. (2015). Contamination of freshly slaughtered pig carcasses with enteropathogenic *Yersinia* spp.: Distribution, quantification and identification of risk factors. Int. J. Food Microbiol. 204:33-40. http://dx.doi.org/10.1016/j.ijfoodmicro.2015.03.016 PMid:25835198.