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Prevalence, virulence and antibiotic susceptibility of *Salmonella* spp. strains, isolated from beef in Greater Tunis (Tunisia)

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ABSTRACT

The aim of this work was to investigate the presence of *Salmonella* spp. in 300 beef meat samples collected from cattle carcasses of different categories (young bulls, culled heifers and culled cows). The detection of *Salmonella* spp. was performed by the alternative VIDAS Easy *Salmonella* technique and confirmed by PCR using *Salmonella* specific primers. *Salmonella* serotypes were determined by slide agglutination tests. The resistance to 12 antibiotics was determined by the diffusion method on Mueller-Hinton agar antibiotic discs. The overall contamination rate of beef by *Salmonella* spp. was 5.7% (17/300). This rate varied from naught (0/100) in bulls' meat to 14% (14/100) in culled cows' meat ($p < 0.001$). The prevalence of *Salmonella* spp. was higher in summer and in cattle with digestive disorders: chronic gastroenteritis (6/17), traumatic peritonitis (3/17) and intestinal obstruction (2/17) ($p < 0.0001$). Of the 17 *Salmonella* isolates, 6 serotypes were identified, namely *Salmonella* Montevideo (8/17), *Salmonella* Anatum (3/17), *Salmonella* Minnesota (2/17), *Salmonella* Amsterdam (2/17), *Salmonella* Kentucky (1/17) and *Salmonella* Brandenburg (1/17) ($p < 0.05$). Unlike other serotypes, *S.* Montevideo was present during the whole year except winter. Almost all of the strains (16/17) were resistant to at least one of the 12 tested antibiotics. Multidrug-resistance concerned 14/17 of the strains, including Amoxicillin (13/17), Tetracycline (12/17), Streptomycin (10/17) and Nalidixic acid (6/17). All the strains were sensitive to the association (Amoxicillin + Clavulanic acid), Cefoxitin and Ceftazidime. In addition, our study showed that all *Salmonella* strains (17) were positive for invasion gene *invA* and negative for the virulence gene *spvC*. Only one isolate (*S.* Kentucky) harbored the *h-li* virulence gene.

1. Introduction

Salmonellosis is a bacterial disease with a rising prevalence in the cattle industry. It is frequent in calves of one to ten weeks age, but also reported in dairy and beef cattle (Randall, Cooles, Osborn, Piddock, & Woodward, 2004). Typical clinical signs of acute *Salmonella* infection include fever associated to diarrhea. Infected animals can be asymptomatic and shed to one billion *Salmonella* a day in their feces (Millemann, 1998). Salmonellosis is a major food-borne disease worldwide (WHO, 2013). The pathogenicity of *Salmonella* depends essentially on its chromosomal and/or plasmatic virulence genes (Okamoto, Filho, & Rocha, 2009; Montero, Herrero, & Rodicio, 2012). The chromosomal *invA* gene enables the invasion of epithelial cells (Galan & Curtiss, 1989). *H-li* gene is involved in the control of phase change and motility of *Salmonella*. In addition, *h-li* gene is required for optimal transcription of several genes of invasion (Millemann, 1998). The virulence plasmid *spv* genes play a role in multiplication of *Salmonella* in its host cell (Rotger & Casadesus, 1999), increase the severity of enteritis. It allows both infection and persistence in extra-intestinal sites (Libby, Lesnick, & Guiney, 2000) and under hostile conditions (Valone & Muller, 1993).

Ciprofloxacin and Cefotaxime are the most commonly used antibiotics for the treatment of invasive *Salmonella* infections in humans (Su & Ou, 2004; Bertrand, Weill, & Vrints, 2006; Whichard, Gay, & Cooper, 2007). However, multidrug resistant *Salmonella* strains are becoming a real worldwide threat (Weill, Grimont, & Cloeckaert, 2006; Bouchrif, Ennajii, & Timinouni, 2008). Since 2002 in Europe an emergence of ciprofloxacin-resistant *Salmonella* spp. isolates has been reported in travelers returning from Northeast and Eastern Africa (Collard, Place, & Denis, 2007). In African countries, multidrug-resistant *Salmonella* spp. strains exhibiting resistance to Ciprofloxacin have been reported in several studies (Aragaw, Molla, Muckle, & Poppe, 2007; Bouchrif et al.,

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Table 1
Characteristics of *Salmonella* spp. positive animals and corresponding virulence and antibiotic resistance profile.

Serotype	Animals' ages (years)	Culling motif	Virulence gene			Antibiotic resistance profile											
			invA	*spvC*	*h-li*	1	2	3	4	5	6	7	8	9	10	11	12
Salmonella Montevideo	5	Chronic gastroenteritis	+	-	-	R	S	S	S	S	S	S	R	S	S	R	R
	6	Chronic gastroenteritis	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	6	Chronic gastroenteritis	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	3	Digestive occlusive syndrome	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	6	Chronic lameness	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	7	Chronic lameness	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	7	Chronic lameness	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
	10	Chronic mastitis	+	-	-	R	S	S	S	S	S	S	S	S	S	R	R
Salmonella Anatum	3	Chronic gastroenteritis	+	-	-	R	S	S	S	S	S	R	S	R	R	S	S
	6	Traumatic peritonitis	+	-	-	S	S	S	S	S	S	R	S	R	R	S	S
	6	Chronic lameness	+	-	-	R	S	S	R	S	S	R	R	R	S	S	S
Salmonella Minnesota	6	Chronic gastroenteritis	+	-	-	S	S	S	S	S	S	S	S	S	S	S	S
	6	Traumatic peritonitis	+	-	-	S	S	S	S	S	S	S	S	S	S	S	R
Salmonella Amsterdam	3	Traumatic peritonitis	+	-	-	R	S	S	S	S	S	R	S	R	R	S	R
	6	Digestive occlusive syndrome	+	-	-	R	S	S	S	S	S	S	R	S	S	R	R
Salmonella Kentucky	5	Chronic gastroenteritis	+	-	-	R	S	R	R	S	S	R	R	R	R	R	R
Salmonella Brandenburg	9	Chronic mastitis	+	-	-	S	S	S	S	S	S	S	S	S	R	S	S

S: Sensitive; R: Resistant

1: Amoxicillin; 2: Amoxicillin + Clavulanic acid; 3: Chloramphenicol; 4: Cephalothin; 5: Cefoxitin; 6: Ceftazidime.
7: Ciprofloxacin; 8: Gentamicin; 9: Kanamycin; 10: Nalidixic acid; 11: Streptomycin; 12: Tetracycline.

2008; Molla, Berchau, & Kleer, 2006). For these reasons, food-borne diseases caused by *Salmonella* spp. are a significant public health concern around the world (Sire & Garin, 2008; Marrero-Ortiz, Han, & Lynne, 2012). Non-typhoid serovars are increasing in importance as significant pathogens of both humans and animals (Mukhopadhyay & Ramaswamy, 2012). Indeed, there are about 17 million cases of acute gastroenteritis or diarrhea annually due to non-typhoid salmonellosis causing 3 million deaths (Rabsch, Tschäpe, & Baumler, 2001). Over 1 million cases of salmonellosis are attributed to consumption of contaminated foods (meat, eggs…) each year in the United States of America. The most common serotypes associated with human diseases in the U.S.A. are *S.* Typhimurium, Enteritidis, Newport, Heidelberg and Javiana (Scallan et al., 2011). *Salmonella* infections lead to approximately 19,000 cases of hospitalizations and nearly 400 deaths each year in the U.S.A. (Scallan et al., 2011), with an economic burden due to lost work, increased medical costs and deaths (Scharff, 2010). Undercooked beef is the most involved food in gastroenteritis, after eggs and egg products (ANSES, 2011). In France, 35% of food-borne *Salmonella* infections were attributable to the consumption of undercooked beef mainly in children under fifteen (Gauchard, Brisabois, & Espie, 2002). In Tunisia, *Salmonella* food-borne infections constitute an emerging public health problem (Ben Aissa, Troudi, & Belhadj, 2007). The most frequently isolated serotypes, in 2010, were *S.* Enteritidis (45%) and *S.* Typhimurium (27%) (R.T.S.R.B.A, 2012). In 2011, two episodes of *Salmonella* food-borne infections occurred in South Tunisia following the consumption of beef; it caused one death and more than 117 clinical cases (Hamza, 2013).

The deep contamination of meat can be related to either clinical or asymptomatic infections (Vieria-Pinto & Tenreiro, 2012). This phenomenon is increased by the slaughtering stress, especially in the absence of respect of water diet and the animals' rest (Radostits, Blood, & Hinchcliff, 2000). However, surface meat contamination occurs also during the slaughtering from the intestinal contents (Buncic & Sofos, 2012). This fecal contamination is due to poor hygiene during animal slaughtering. There is a high risk of beef meat deep contamination by *Salmonella* spp. especially in sanitary culled animals (Labadie, 1999). The present work aimed to on the one hand estimate the prevalence of *Salmonella* meat deep contamination in both healthy bulls and culled females (heifers and cows) and on the other hand study the isolated *Salmonella* strains.

2. Materials and methods

2.1. Samples

The present study was carried out during the four seasons from September 2013 to August 2014; it concerned a total number of 300 beef meat samples designated to human consumption collected in Greater Tunis (Tunisia) from carcasses. During each season, 75 animals (25 bulls, 25 culled heifers and 25 culled cows) were sampled.

Information about age, sex, disease history and cause of culling of the animals was collected. But, there is no information about the health care provided to the animals in their respective farms. Bulls were healthy at the day of slaughtering but culled females were affected by at least one