STUDY OF *SALMONELLA* SPP. CARRIAGE IN FECAL OF BOVINE ORIGIN IN THE WILAYA OF TIZI OUZOU (ALGERIA)

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Abstract: The objective of this study was to evaluate the importance of the reservoir of *Salmonella* spp. in dairy cattle through the study of asymptomatic carriage in feces in some municipalities of the Wilaya of Tizi Ouzou. 548 fecal samples from 65 farms were carried out, and the ISO 6579 method was used for the analysis of the samples. The results showed that 13.48% of the farms and 2.18% of the samples were positive for the search of *Salmonella* spp. Most of the strains (75%) were resistant to a maximum of two antibiotics and 25% of the strains were multidrug resistant. No relationship between practices observed in the field and the excretion of *Salmonella* spp. could be demonstrated.

Keywords: Antimicrobial resistance, Asymptomatic carriage, Dairy cattle, *Salmonella* spp.

INTRODUCTION

Pathologies caused by *Salmonella* spp. constitute a real public health problem in the world, by their frequency and the serious of the disorders which they can cause. Added to this is, the ability of this germ to resist to antibiotics Nyman et al. (2013), like *Salmonella Typhimurium* DT 104 which can resist to six antibiotics (phenotype ACSSuT). Numerous outbreaks of *Salmonella* spp. gastroenteritis have been declared worldwide. In Algeria, epidemics of *Salmonella* spp. multidrug resistant have been recorded since the end of the sixties (Weill, 2008). The animal origin has been demonstrated several times in these outbreaks, of which a dozen animal species have been implicated (Weill, 2008). Cattle infected with *Salmonella* spp. are recognized as an important source of infection for humans (Aubry, 2010). Fecal excretion is the main source of *Salmonella* Callaway et al. (2005), with titers which can reach 108 bacteria per gram of feces in cattle (Aubry, 2010). The information provided on this cattle reservoir, will result in suitable measures that would limit the impact of this germ in human pathology, particularly in foodborne infections, where it is frequently associated (3 cases of foodborne infections out of 1000 inhabitants in the Netherlands) and can cause serious disorders (600 deaths per year in the United States) Hendriksen et al. (2004).

Consequently, the classification of an animal, a herd or a region would help to limit the spread of this germ, limit the control measures to a restricted area, and finally this information could answer certain preoccupation in human pathology.

MATERIAL AND METHODS

Study area: The Wilaya of Tizi Ouzou is located in central Algeria, it consists of 67 municipalities and has a large cattle herd. The majority of these cattle are held by the private sector with a workforce of 127 224 head including 54 103 improved dairy cattle, a hundred cattle raised in the pilot farms, namely the pilot farm of Draa Ben Khedda (SOWT-O, 2020).

Our study focused on dairy farms in some municipalities of the Wilaya of Tizi Ouzou (Ouaguenoun, Freha and Draa Ben Khedda). Once the three municipalities were targeted, we took samples during several visits.

Sampling: Given the difficulty of carrying out a random sampling due to the lack of logistical means (Impossibility of reaching all the farms selected at random), the absence of a precise database on the size of the farms and their locations exact, moreover the risk of refusal of certain owners constitutes a real problem. For this, we are resigned to make a reasoned choice sampling, in which we contact one or two breeders who were favorable to participation in our study through veterinarians practicing in these municipalities, and once on site, these breeders are in charge to direct us to other farms.

Collection of samples: Once inside the farms, the animals sampled are the accessible animals (dairy cows present inside the stable). At least 50 grams of fecal matter directly taken from the rectum per animal in a sterile jar or sterile plastic bags, and put on melting ice for a maximum of 48 hours prior to culture.

Collection of information: we drew up a survey sheet for each farm, in which we noted certain farming practices in relation to the problem posed.

Bacteriology: The fecal samples were analyzed with the bacteriological method ISO 6579 (2002), in which we carried out the following steps:

-Pre-enrichment: in a non-selective liquid medium, this step was intended to revive the bacterial cells. For this, we proceed to the preparation of a solution by adding to 25g of feces 250 ml of Buffered Peptone Water (Pasteur Institute of Algiers, Algeria), and incubated for 18-20 h at 37°C.

-Selective enrichment: Subsequently, the sample was subjected to two selective enrichments in parallel, using MSRV (Oxoid) medium (with Novobiocin added) and Muller Kauffmann Novobiocin Broth (Oxoid) and incubated for 24 h at 37°C.

-Isolation on solid media: For the Muller Kauffmann medium, the inoculation has been done systematically on XLD (Bio-Merieux, Frances) and Hektoen agar (Pasteur Institute of Algiers, Algeria). However, for the MSRV medium, which is a semi-solid medium, the boxes are read by examining the migration zones, which must be greater than 20 mm. If this migration is confirmed, an inoculum is taken from the migration zone and inoculated on XLD and Hektoen agar.

-Identification: Each presumptive colony is re-isolated and subjected to biochemical identification (API 20E) and serological confirmation (serological agglutination on slide according to the Kauffmann-White scheme).

Antimicrobial susceptibility test: The antibiotic sensitivity profiles of the strains were determined by disc diffusion method on Mueller-Hinton agar medium

(Pasteur Institute of Algiers, Algeria), as recommended by the Clinical Laboratory Standards Institute guidelines (CLSI, 2017).

The following antibiotics were tested: Amoxicillin (10 µg), Ticarcillin (75 µg), Piperacillin (100 µg), Amoxicillin / Clavulanate (20 µg / 10 µg), Cefoxitin (30 µg), ceftazidime (30 µg), cefotaxim (30 µg), ceftriaxone (30 µg), cefepime (30 µg), aztreonam (30 µg), Meropenem (10 µg), Streptomycin (10 µg), Nalidixic acid (30 µg), Ciprofloxacin (5 µg), Colistin (10 µg), Chloramphenicol (30 µg),
Trimethoprim (5 µg), Sulphonamides (300 µg), Trimethoprim / Sulfamethoxazole (1.25 µg / 23.75µg), Tetracycline (30 µg) and Furans (300 µg).

Echerichia coli ATCC® 25922 obtained from American Type Culture Collection was used as quality control organism.

Statistical analysis: Chi-square test and Exact Fisher's test were used to examine association between prevalence of the germ, the antibiotic sensitivity profile and farming practices.

RESULTS AND DISCUSSIONS

Occurrence of *Salmonella* spp.: 65 farms were visited and a total of 548 fecal samples taken from 548 dairy cows were realized.

A region is considered to be positive if at least one farm is positive and a farm is considered to be positive if at least one animal from the farm in question is positive for *Salmonella* spp. We isolated *Salmonella* spp. in the three municipalities visited (Table 1).

Table 1

Prevalence of isolation of *Salmonella* spp.

	Farm results		Individual results		
Municipalities	N	N positive	N	N positive	Serovars
Ouaguenoun	38	4 (10.52%)	300	5 (1.66%)	*Salmonella* Kedougou (n=2) *Salmonella* Virginia (n=3)
Freha	16	1(6.25%)	143	2 (1.39%)	*Salmonella* Thyphimurium (n=2)
Draa-Ben Khedda	11	4 (36.36%)	105	5 (4.76%)	*Salmonella* Thyphimurium (n=1) *Salmonella* Kedougou (n=1) *Salmonella* Enteritidis (n=1) *Salmonella* Agbeni (n=1) *Salmonella* Indiana (n=1)
Total	65	9 (13.48%)	548	12 (2.18%)	

***N :** number of isolates.*

Research of risk factors related to farm practices and individual: A higher rate of isolation of *Salmonella* spp. was observed in farms exposed to external contamination through the possibility of contact with other cattle (pasture, common paths), the non-practice of quarantine of new animals and the practice of boarding or loaning, but without statistically significant difference (Exact Fischer Test) (Table 2).

Table 2

Prevalence of *Salmonella* spp. according to the practices put in place in the farms.

Municipalities	Practice of quarantine of new acquisitions				Residential or loan practice				Possibility of contact with other farms during common grazing			
	Yes		No		Yes		No		Yes		No	
	N	N positive	N	N positive	N	N positive	N	N positive	N	N positive	N	N positive
Ouaguenoun	12	2 16.16%	26	2 7.66%	24	3 12.5%	14	1 7.14%	27	3 11.11%	11	1 9.09%
Freha	7	0	9	1 11.11%	7	1 14.28%	9	0	9	1 11.11%	7	0
Draa-Ben -Khedda	5	1 20%	6	3 50%	5	3 60%	6	1 16.66%	6	2 33.33%	5	2 40%
Total	24	3 12.5%	41	6 14.63%	24	7 29.16%	41	2 4.87%	42	6 14.28%	23	3 13.04%

N : *number of isolates.*

Regarding individual risk factors, a higher isolation rate of *Salmonella* spp. was observed in improved breeds, older than 5 years and lactating cows, but without statistically significant difference (Exact Fisher Test) (Table 3).

Table 3

Prevalence of *Salmonella* spp. according to individual risk factors.

Municipalities	Breed				Age				Lactation stage			
	Improved		locales		<5 years old		>5 years old		In lactation		at the drying-up	
	N	N positive	N	N positive	N	N positive	N	N positive	N	N positive	N	N positive
Ouaguenoun	271	5 1.84%	29	0	191	2 1.04%	109	3 2.75%	197	4 2.03%	103	1 0.97%
Freha	103	2 1.94%	40	0	103	2 1.94%	40	0	102	2 1.96%	41	0
Draa-Ben -Khedda	90	4 4.44%	15	1 6.66%	78	2 2.56%	27	3 11.11%	79	5 6.32%	26	0
Total	464	11 2.37%	84	1 1.19%	372	6 1.61	176	6 3.40%	378	11 2.91%	170	1 0.58%

N : *number of isolates.*

Antimicrobial resistance profiles: In the present study, 25% (n = 3) of the strains are resistant to penicillins (amoxicillin, ticarcillin, piperacillin, Amoxicillin and Clavulanate). 16.6% (n = 2) are resistant to first, second and third generation of cephalosporins, these resistances are present only in *S.* Typhymurium. For other antibiotics, a resistance rate of (50%) was recorded for Nalidixic acid, as well as low resistance rates were recorded for Streptomycin (8.3%, n = 1), Chloramphenicol (8.3%, n = 1) and Furans (8.3%, n = 1) (Table 4).

The three strains of S, Typhymurium show a multidrug resistance (25% of the isolated strains). For the other serovars, at most two resistances to antibiotics were noted (75% of the isolated strains) (Table 5).

Table 5

Phenotypic resistance profiles of strains of *Salmonella* spp. isolated in feces.

Serovars	Profiles	Total no. of isolates
S. typhymurium	AMX, TIC, PRL, KZ, AMC, FOX, CAZ, CTX, CR, ATM. AMX, TIC, PRL, AMC, S, NA, C, TE, SSS.	2/3 (16.6%) 1/3 (8.3%)
*S.*virginia	NA.	2/3 (16.6%)
*S.*enteritidis	NA, F.	1(8.3%)
*S.*indiana	NA.	1(8.3%)
*S.*agbeni	NA.	1(8.3%)
Total		08

Table 4

Antibiotic resistance of strains of *Salmonella* spp. isolated in the feces

SEROVARS	ANTIBIOTICS																					
	N	Amx	Tic	Prl	Amc	Fox	Caz	Ctx	Cr	Fe	Atm	Me	S	Na	Cip	Cs	C	w	Ss	Sxt	Te	Fu
S.Typhymurium	3	3	3	3	3	2	2	2	2	0	2	0	1	1	0	0	1	0	1	0	1	0
S.Virginia	3	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0
S.Kedougou	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.Eteritidis	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
S.Indiana	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
S.Agbeni	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
TOTAL	12	3/12 25%	3/12 25%	3/12 25%	3/12 25%	2/12 16.6%	2/12 16.6%	2/12 16.6%	2/12 16.6%	/	2/12 16.6%	/	1/12 8.3%	6/12 50%	/	/	1/12 8.3%	/	1/12 8.3%	/	1/12 8.3%	1/12 8.3%

N : *number of isolates,* ***Amx*** *:Amoxicillin,* ***Tic*** *: Ticarcillin,* ***Prl****: Piperacillin,* ***Amc****: Amoxicillin and Clavulanate,* ***Fox*** *: Céfoxitin,* ***Caz****: ceftazidim,* ***Ctx:*** *Céfotaxim,* ***Cr*** *: Ceftriaxon ,* ***Fe*** *: Céfépim,* ***Atm*** *: Aztreonam,* ***Me*** *: Meropenem,* ***S*** *:*

*Streptomycin, **Na** : Nalidixic acid, **Cip** : Ciprofloxacin, **Cs** : colistin, **C** : chloramphénicol, **w**: Triméthoprim, **Ss** : Sulphonamides, **Sxt** : Triméthoprim and Sulfaméthoxazole, **Te** : Tetracylline, **Fu** : Furans.*

In the present study, 65 farms in three municipalities of the Wilaya of Tizi Ouzou were visited, and 548 samples were collected. The number of farms visited reflected the degree of cooperation, the number of contacts and the number of people who could be reached at the time of collection of the samples, resulting in a greater number of farms sampled in some municipalities.

13.48% of farms were positive for *Salmonella* spp., the municipality of Draa Ben Khedda seems the most contaminated in which a higher rate was recorded but without statistical significance between the three municipalities. This result is less important than that obtained in the United States by Callaway et al. (2005) and Murinda et al. (2002) with a prevalence of 56% and 25.3% respectively.

Overall individual prevalence was found to be around 2.18%, higher than that recorded by Bordonaro et al. (2015) and Mohamed et al. (2011) with a prevalence of 1.7% and 1.54% respectively, lower than that obtained in Algeria by Nouichi et al. (2018) with a prevalence of 6.87%. However, Nouichi et al. (2018) worked on samples taken at slaughterhouses, and according to Wells et al (2001), *Salmonella* excretion is higher in cull cows or cows in markets than at the farms level.

These differences in prevalence between these studies can also be attributed to the intermittent excretion of this germ (Present in the mesenteric lymph nodes and released during stress) or to bacteriological methods which are less sensitive than molecular techniques. This problem being even more acute when it comes to detecting asymptomatic carriers Jensen et al. (2013).

Regarding the isolation rate of *Salmonella* spp., compared to the facts and practices observed in the field, we were interested in factors specific to the farm and to individual factors.

In the present study, the rate of *Salmonella* isolation is slightly higher in farms where animals are more exposed to contact with foreign cattle, through the possibility of contact with other cattle (grazing, common paths), the practice of quarantine of new animals and the practice of boarding or loaning. However, our results do not allow us to conclude that these factors are implicated in a higher rate of excretion (Exact Fisher Test).

However Wells et al. (2001), reports a greater fecal excretion in cattle present at the market level. Vanselow et al. (2007), Davison et al. (2001) have questioned the new acquisitions as risk factors in favor of a higher percentage of fecal excretion of *Salmonella* spp. in dairy farms.

This demonstrates the importance of quarantining new animals and the risk incurred by animals when frequenting common areas (market, path).

Concerning the individual factors associated with a higher rate of excretion of *Salmonella* spp., we compared the percentage of excretion in relation to breed (local, improved), Age (more than 5 years and less than 5 years) and finally the physiological status (in lactation, at drying up), but no significant differences could be found (Exact Fisher Test).

Huston et al. (2002) concluded that lactating cows were significantly associated with increased fecal excretion of *Salmonella* spp.

Seven different serovars of *Salmonella* spp. have been isolated. However *Salmonella* Kedougou, Virginia and Thyphimurium represent the most isolated

serovars (50%). In Algeria Nouichi et al. (2018) reports the isolation of other serovars, in Egypt Mohamed et al. (2011) reports the isolation of *S*. Typhimurium and Enteritidis. In the study by Mcevoy et al. (2003) the most widespread serotypes are the Dublin serotype and the Typhimurium serotype.

In our study, most strains (75%) were resistant to up to two antibiotics. Wells et al. (2001) and Charles et al (1981) point out that the multidrug resistant character of *Salmonella* spp. was more present in strains of human origin than those isolated from animals.

Our results show that 25% of the strains express a phenotype of multidrug resistance to antibiotics, with even third-generation cephalosporin resistance in two strains of *S*. Typhimurium. This antibiotic resistance may be due to the intensive use of antibiotics for curative and preventive purposes Nouichi et al. (2018), or the ability of certain serovars to develop a multidrug resistant phenotype, such as *S*. Typhimurium DT 104, which can be resistant to six antibiotics (ACSSuT phenotype).

CONCLUSIONS

In conclusion, our study showed that dairy cattle represent a reservoir of multidrug resistant *Salmonella* spp. through their excretion in feces. This excretion could not be associated with individual factors or factors related to farm practices.

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