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Full Length Research Paper

Effect of irrigation water on the incidence of *Salmonella* spp. on lettuces produced by urban agriculture and sold on the markets in Dakar, Senegal

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The aim of our survey was to assess the effect of irrigation water of the microbiological quality on the production chain of lettuce in the Dakar area. Microbiological analysis showed that 35% of irrigation water was contaminated by *Salmonella* spp. between the two water-types used for irrigation (groundwater and wastewater), no significant difference ($p>0.05$) in their degree of contamination was found. The incidence of different types of irrigation water on the contamination rate of lettuces from the farm (Pikine and Patte d'Oie) was not different either ($p>0.05$). However, the contamination rate of lettuce from markets of Dalifort and Grand-Yoff that were supplied by the area of Patte d'Oie was greater than those of Sham and Zinc supplied by Pikine ($p<0.05$). Comparison of serotypes of *Salmonella* isolated from irrigation water and lettuce showed that irrigation water may affect the microbiological quality of lettuce. Manures, frequently used as organic amendment in cultivating lettuce are another potential source of contamination. These results showed that lettuce may constitute effective vectors for the transmission of pathogens to consumers. Extensive treatment of the used wastewater and/or composting of manure could considerably reduce these risks.

Key words: Urban agriculture, Dakar, irrigation water, *Salmonella* spp., lettuce, manure.

INTRODUCTION

Foodborne illnesses caused by pathogenic bacteria still occur at unacceptable high frequencies in industrialized nations and developing countries (Lampel et al., 2000). The increasing occurrence of foodborne diseases during the last decades seems to be related to the presence of microorganisms in food (FAO, 2004). According to Sivapalasingam et al. (2004), the proportion of foodborne illness has increased from 0.7 to 6% between 1970 and 1990. In Africa, over 30,000 children die each year due to the consumption of contaminated food (Mensah, 2005). Fresh fruits and vegetables are increasingly recognized as potential sources of diseases (Beuchat, 2002; Ibenyassine et al., 2006; NACMCF, 1999). Fresh vegetables are among the products associated to foodborne disease outbreaks in the Unites States (Sivapalasingam et al., 2004). Vegetables can become contaminated by pathogens while growing, during harvest, by post-harvest handling, or distribution

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Abbreviations: FAO, Food and agriculture organization; **NACMCF,** national advisory committee on microbiological criteria for foods; **UA,** urban agriculture; **PE,** polyethylene; **T,** tonne; **FISH,** fluorescence *in situ* hybridization.

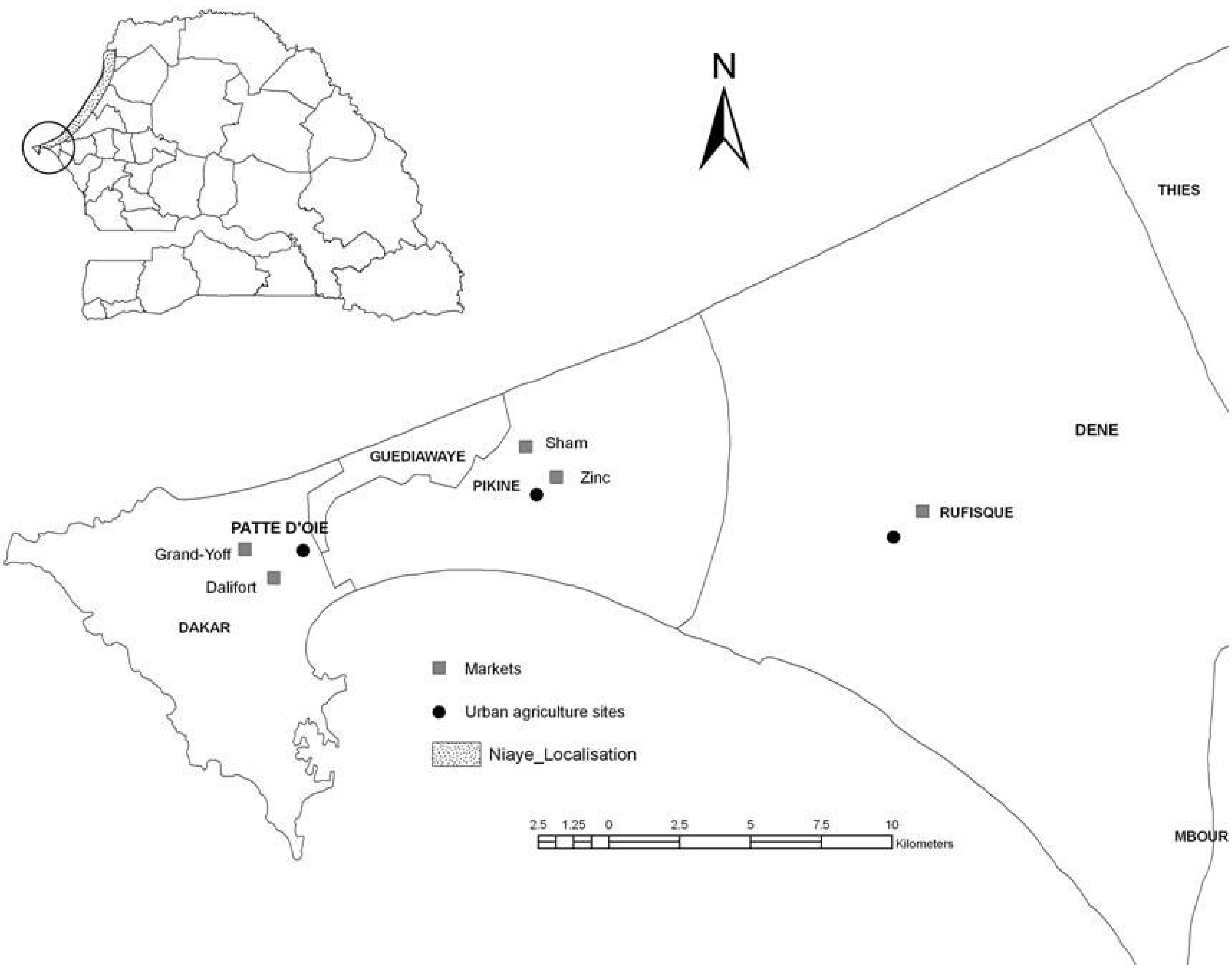

Figure 1. Maps showing the studied urban agriculture sites and markets.

(McMahon and Wilson, 2001). Numerous studies have shown that vegetables from fields irrigated with polluted water and amended with manure are contaminated (Al-Lahham et al., 2003; Amoah et al., 2005; Ibenyassine et al., 2006; Rosas et al., 1984).

Study background

Senegal, like most developing countries, is facing demographic expansion. The capital Dakar hosts one fifth of the population on an area of 0.28% of the national territory. During the last ten years, urban agriculture has played an increasingly important role in food security control, assuring more than 70% of the vegetable supply in Dakar (Mbaye, 1999). However, increasing water scarcity and the poor agronomic value of shallow well water ('*Céanes*'), are at the origin of the frequent use of wastewater and animal manures, such as poultry or horse droppings, in urban agriculture. Therefore, sanitary risks related to the consumption of fresh vegetables from urban agriculture have become an important issue. In fact, the use of wastewater is believed to have contributed to cholera epidemics and typhoid occurring in Senegal during the last decades (Gaye and Niang, 2002). In that regard, it is important to assess the microbiological quality of vegetables (especially raw vegetables) for the whole process chain, that is from the production to market sale, and to determine the sources of contamination, in order to propose alternatives to prevent consumers from disease outbreaks.

MATERIALS AND METHODS

Sampling of the irrigation water

Our survey took place in the two areas of urban agriculture (UA) of Pikine and Patte d'Oie in Dakar (Figure 1 and Table 1). In 2008, six field experiments were carried out. 78 samples of three types of irrigation water, that is wastewater, shallow groundwater ("*Céanes*")

Table 1. Characteristics of the studied urban agriculture sites and their water source.

Characteristic	Study site	
	Pikine	Patte d'Oie
Irrigated area (ha)	49.9	12
Annual lettuce yield (T/year)	3544	560
Number of farmers	830	161
Water source for irrigation	Wastewater, shallow groundwater	Wastewater[1] , shallow groundwater, well water

[1]Wastewater was forbidden since May 2008.

and normal well water have been sampled. For each type, one litre has been collected in a sterile polyethylene (PE) bottle, and placed in an icebox at 4°C for microbiological analysis in the laboratory.

Lettuce sampling

Lettuce sampling in the field was based on 17 and 25 plots respectively in Pikine and Patte d'Oie, with various types of irrigation water. In each plot, three mature lettuce plants were taken (in certain cases less than 3, depending on the farmer found in place). In total, 123 samples were taken. The lettuces were put in separate and sterile plastic bags, and then placed in an icebox containing freezer packs for the conservation at 4°C during transport to the laboratory. Sampling was accompanied by an inquiry upon farmers.

For the sampling on the markets, the choice of the studied markets was made based on the results of an earlier investigation to identify the most important selling points of vegetables produced in the two studied UA areas (Ndiaye, 2009). For Pikine, the Zinc and Sham markets were selected, and for Patte d'Oie, the markets of Grand-Yoff and Dalifort (Figure 1). In each market, three lettuce plants were taken per saleswoman. Conditioning was made as for those sampled in the plots. In total, 205 samples were collected and analysed. As in the UA areas, sampling was accompanied by inquiry upon the saleswomen (13 to 20 according to the market, in total 88).

Analyses

For *Salmonella* spp. analysis of irrigation water, 500 ml of were filtred through an acetate cellulose filter with 0.45 µm pore size (for wastewater ~10 ml because of clogging). The filter was then transferred in a test tube containing 9 ml Rappaport Vasilliadis R10 Broth and incubated at 43°C during 24 h. 100 µl of the mixture were inoculated on the Hecktoen medium and incubated at 43°C for 24 h. Suspect colonies were re-suspended in 1 mL of physiological water (9% NaCl). Biochemical tests were performed using Kligler Hajna, Citrate of Simmons, Mannitol-mobility, urea/indole media and ONPG (o-nitrophényl-β-D-galactopyranoside).

Confirmation for the presence of microbial cells belonging to *Salmonella* in the original sample was performed by the FISH method (Amann et al., 1990) using the Sal3 probe (Nordentoft et al., 1997) labelled with Cy3 dye. Serotyping of *Samonella* spp. was performed using the agglutination method with antiserum for *Salmonella* on a glass slide. The strains that could not be identified were serotyped by the « Nationales Zentrum für enteropathogene Bakterien » of Luzern (Switzerland).

To assess the microbiological quality of lettuces, 10 g of fresh material were weighed and put into a sterile Stomacher bag, containing 90 mL of Rappaport Vasilliadis R10 Broth. After homogenization with a Stomacher mixer at 200 rpm during 5 s, the bag was incubated at 43°C during 24 h. Analysis of *Salmonella* spp. was performed in the same manner as for irrigation water.

The resulting data were analysed using the statistical software SPSS for Windows 17.0. Chi-square tests ($\chi 2$) were performed to evaluate statistical difference of the data and the results are quoted at $p<0.05$ levels of significance.

RESULTS AND DISCUSSION

Salmonella spp. from irrigation water

Salmonella spp. has been isolated in 35% of the irrigation water analyzed. The difference in the rate of contamination between natural water (*Céanes* and well water, 40%) and wastewater (19%) was not significant ($p>0.05$). In Pikine, the rate of contamination of *Céanes* water was 33% and for the wastewater 22% (Table 2). In Patte d'Oie, more than half of *Céane* waters (57%) analyzed contained *Salmonella* spp. and 27% of well waters. Wastewater was found *Salmonella*-free. These results seem paradoxical, that is that *Céanes* and well water (local groundwater) are too contaminated than wastewater ($p>0.05$). A recent study on these two sites showed that the use of manure as fertilizer was systematic whatever the type of irrigation water (Ndiaye, 2009). However, plots irrigated with *Céane* and well water received important amounts of manure because of the water's low-nutrients quality for plants. A comparison of *Salmonella* serotypes from *Céanes* water used as irrigation water at Pikine and Patte d'Oie (*S.* Chester, *S.* Virchow, *S.* Corvallis, *S.* Give) to those that can be found in animals manure (poultry, horses) suggested a contamination of the shallow groundwater by manure spread on fields (Table 3). Indeed, the stagnant shallow groundwater of the ''Céanes'' had high loads of faecal bacteria due to the fact that farmers often walk in them to fill their watering cans, after having walked over their plots treated with animal manure (Ndiaye et al., 2010). In the same manner, runoff can also contribute an important part. According to Amoah et al. (2005), the shallow wells used in urban agriculture in Kumasi (Ghana) are contaminated because they are not protected against runoff. Another important source of contamination was the washing of lettuce in the *Céanes* by saleswomen to remove sands from roots after harvest. The excessive

Table 2. Contamination of irrigation water for *Salmonella* spp.

Type of irrigation water	Study site	
	Pikine	Patte d'oie
Shallow groundwater "*Céanes*" (%)	n[1]=18 (33)	n=21 (57)
Wastewater (%)	n=18 (22)	n=3 (0)
Well water (%)	-	n=18 (27)

[1]Number of samples analysed.

Table 3. Serotype of *Salmonella* isolated from irrigation water.

Type	Group	Somatic (O) Antigen	Flagellar (H) Antigen		Detected in the animals**
			Phase 1	Phase 2	
S. Paratyphi A[2,a]	A	1, 2, 12	a	[1, 5]	
S. Chester[1,a ; 1,b]	B	1, 4, [5], 12	e, h	e, n, x	Po, Sh
S. Kunduchi[2,a ; 3,b]		1, 4, [5], 12, 27	i, z_{28}	1, 2	
S. Kimuenza[2,a]		1, 4, 12, 27	l, v	e, n, x	
S. Kingston[3,b]		1, 4, [5], 12, 27	g, s, t	1, 2	
S. Lomita[1,b]	C_1	6, 7	e, h	1, 5	
S. Arizonae[1,b]		6, 7	l, v	z_{53}	
S. Montevideo[3,b]		6, 7	g, m, [p], s	-	
S. Bonn[1,a]		6, 7	l, v	e, n, x	
S. Virchow[1,b,*]		6, 7	r	1, 2	Po, B, Sh, Go
S. Colindale[2,a,*]		6, 7	r	1, 7	
S. Manchester[2,a ; 1,b]	C_2	6, 8	l, v	1, 7	
S. Corvallis[1,a ; 1,b,*]	C_3	8, 20	z_4, z_{23}	-	H, B, Go
S. Molade[3,b,*]		8, 20	z_{10}	z_6	
S. Typhi[1,b]	D_1	9, 12, [Vi]	d	-	
S. Javiana[3,b]		1, 9, 12	l, z_{28}	1, 5	
Salmonella[1,a]		9, 12	e, n, x	1, 6	
S. Give[1,a]	E_1	3, 10	l, v	1, 7	B, Sh
S. Muenster[1,a]		3, 10	e, h	1, 5	H, B, Sh, Go
S. Stendal[2,a ; 1,b]	F	11	l, v	1, 2	
S. Maracaibo[1,b]		11	l, v	1, 5	B
S. Marseille [2,b,*]		11	a	1, 5	
S. Tilène[3,b,*]	R	1, 40	e, h	1, 2	B, Sh
S. Teshie[1,a,*]	X	1, 47	l, z_{13}, z_{28}	e, n, x	
S. Dougi[2,a ; 1,b,*]	Z	50	y	1, 6	
S. Aarhus[1,b,*]					

[1]Shallow groundwater water; [2] Wastewater; [3] Well water; [a] Site of Pikine; [b] Site of Patte d'Oie
* Species are serotyped by the *Nationales Zentrum für enteropathogene Bakterien*, Luzern (Switzerland)
** Species isolated from Po=Poultry, Sh=Sheep, B=Beef, Goat=Go; H=Horse in Senegal. Source (Doutre and Buisson, 1984)

use of poultry manure as fertilizers (100 and 70% of plots irrigated with *Céanes* and well water at Patte d'Oie site, respectively) seems to be the cause of the contamination of *Céane* and well waters. In fact, several authors have shown that poultry manure is a reservoir of *Salmonella* (Himathongkham et al., 1999; Orji et al., 2005), which can survive for a long time in the soil amended with such type of manure (Islam et al., 2004).

Pathogens such as *S.* Paratyphi A and *S.* Typhi were isolated from wastewater from Pikine and *Céanes* water from Patte d'Oie, respectively.

Salmonella in lettuce

The difference between contamination rates of lettuce from Pikine (6%) and Patte d'Oie (7%) was not significant (Table 4). The method of irrigation (watering cans) used

Table 4. Rate of contamination of lettuce from the fields and markets.

Location	Origin	n*	% of positive samples	χ^2	P**
On-farm lettuce	Pikine	53	6	0.08	0.77
	Patte d'Oie	72	7		
Lettuce from markets	Pikine (Sham and Zinc markets)	99	3	4.34	0.03
	Patte d'Oie (Dalifort and G. Yoff markets)	106	10		

* Number of samples. ** P-value: significant if p<0.05.

Table 5. Serotype of *Salmonella* isolated from on-farm lettuce and those from markets.

Type	Group	Somatic (O) Antigen	Flagellar (H) Antigen		Detected in the animals and/or irrigation water**
			Phase 1	Phase 2	
S. Kunduchi[2]	B	1, 4, [5], 12, 27	i, z_{28}	1, 2	§
S. Caledon[3]		4, 12	g, m, t	[e, n, x]	
S. Kingston[4]		1, 4, [5], 12, 27	g, s, t	1, 2	Ψ
S. Schwarzengrund[a,*]		1, 4, 12, 27	d	1, 7	
S. Banana[3,*]		4, 5, 12	m, t	-	
S. Montevideo[3, 4]	C_1	6, 7	g, m, [p], s	-	H, B, Go, Ψ
S. Mbandaka[a],*		6, 7, N	z_{10}	e, n, z_{15}	Po
S. Manchester[3]	C_2	6, 8	l, v	1, 7	Ψ
S. Molade[b]	C_3	8, 20	z_{10}	z_6	§, Ψ
*S*almonella[a, 1]	D_1	9, 12	e, n, x	1, 6	§
S. Ekotedo[1],*	D_2	9, 46	z_4,z_{23}	-	
S. Sinstorf [3]	E_1	3, 10	l, v	1, 5	
S. Westhampton[b]		3, 10	g, s, t	-	
S. New-haw[4]	E_2	3, 15	e, h	1, 5	
S. Gaminara[2],*	I	16	d	1, 7	H, Go
S. Tilène[4,*]	R	1, 40	e, h	1, 2	B, Sh, Ψ

[a] Site of Pikine; [b] Site of Patte d'Oie. [1]Zinc, [2]Sham, [3]Grand-Yoff, [4]Dalifort. * Species are serotyped by the *Nationales Zentrum für enteropathogene Bakterien*, Luzern (Switzerland). ** Species isolated from Po= Poultry, Sh= Sheep, B= Beef, Go= Goat; H=Horse in Senegal. Source (Doutre and Buisson, 1984). § Serotype isolated in irrigation water from Pikine. Ψ Serotype isolated in irrigation water from Patte d'Oie

in both UA sites plays an important role in the contamination of lettuce. Studies on the effect of irrigation methods on the contamination level of vegetable have shown that even for strongly contaminated irrigation water, drip or furrow methods substantially reduced the risk of crop contamination (Ensink et al., 2007; Keraita et al., 2007). According to Rosas et al. (1984), the contamination level of lettuce, compared to other type of vegetable, is due to its small size which promotes the deposition of soil bacteria and manure under the effect of watering or other mechanisms. Moreover, the serotype of *Salmonella* isolated from irrigation water (that is *S.* Salmonella in Pikine, *S.* Molade in Patte d'Oie), which have been found in lettuce from Pikine and Patte d'Oie, respectively, suggested that the quality of the irrigation water had indeed an impact on the lettuce produced (Table 5). In addition, manure used as fertilizers could affect the quality of lettuce since *S.* Mbandaka which can be isolated from poultry (Doutre and Buisson, 1984) was found on lettuce (Table 5). Manure pathogens can be transferred to the surface of vegetables through conta-minated soils (Islam et al., 2004; Natvig et al., 2002). According to Islam et al. (2004), survival profiles of *Salmonella* on vegetables and soil samples contaminated by irrigation water were similar to those observed when contamination occurred through compost.

Lettuces from Grand-Yoff and Dalifort markets (from Patte d'Oie) were more contaminated by *Salmonella* spp. than those from Zinc and Sham markets (from Pikine) (p<0.05, Table 4). Serotypes isolated in lettuce from Zinc markets (*S.* Salmonella), Sham (*S.* Kenduchy*, S.* Gaminara), Grand-Yoff (*S.* Montevideo*, S.* Manchester) and Dalifort (*S.* Montevideo*, S.* Kingston*, S.* Tilène) are either present in irrigation water and can be found in manure (Table 5). Numerous studies have shown that pathogenic bacteria such as *S.* Typhimurium could survive on lettuce during growth until harvest (Ercolani, 1976; Islam et al., 2004). According to Pescod (1992), *Salmonella* spp. can survive on fresh vegetables up to 30 days.

Conclusion

Results of our survey showed that the use of the contaminated local groundwater (*Céanes* and well water) and wastewater as irrigation water is a greater risk for vegetable contamination.

The presence of *Salmonella* spp. in lettuce shows that they can be an important vector for the transmission of pathogenic bacteria to consumers. Therefore, an adequate training for farmers and saleswomen may have a positive impact and reduce contamination. Similary, awareness among consumers about the need of disinfection of vegetables eaten raw is essential. The use of wastewater for irrigation, at least treated by lagooning before its use, may be an attractive alternative to groundwater because it would allow reducing the amount of manure used, which seems to be the main source of the microbiological contamination.

The method of irrigation (watering cans) plays an important role in the contamination of lettuce. Therefore the use of other methods like drip or furrow irrigation can considerably reduce the risk of crops contamination.

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