ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Serotyping Isolated Strains of Salmonella for Consomption in the Eastern Logone Province in Chad

T. Maoudombaye[1], A. Bodering[1], Mahamat Seid Ali Malloum[2*], G. Ndoutamia [1], A. Ngakou [3]

[1]Faculty of Science and Technology, University of Doba, Chad
[2]Faculty of Exact and Applied Sciences, University of Moundou, Chad
[3]Faculty of Sciences, University of Ngaoundere, Cameroon

Abstract

The present work was carried out in the Doba petroleum basin, Eastern Logone Province, and focused on the serotyping of strains of salmonella isolated in well water, boreholes and rivers for the consumption of the population. The sampling was carried out according to a complete randomized device with 10 samples per source of water, making a total of 30 samples. Salmonellae were detected according to the French standard ISO 6579: 2002, followed by serotyping. The results of the biochemical identification test of the API 20 E gallery led, thanks to the Apiweb Tm-API 20E V4.1 site to Salmonella spp. The serotyping results revealed, according to the White-Kauffmann-Le Minor table, serovars Anatum, Mbandaka and Idikan. S. Anatum was detected in P4 well waters and F6 wells; S. Mbandaka, in F9 and R8 river water; and finally, S. Idikan, in F5 and R4 river water. These results show a homogeneous distribution of these serotypes in the different water sources of the study area. The presence of these pathogenic serotypes in drinking water sources attests that these waters are unhealthy in accordance with WHO's guidelines for drinking quality. Corrective measures are needed to improve the quality of these water rich in germs that may cause food poisoning.

Keywords: Serotyping; Salmonella spp.; Drinking water; oil basin; Chad.

Introduction

Salmonella were first isolated in 1886 by Salmon and are intestinal pathogènes (D'Aoust, 1991) found in the intestines of humans and animals. Their release into the environment comes mainly from faecal contamination (Murray 2000 ; Hanes 2003). They are responsible for salmonellosis, zoonoses very widespread in tropical and worldwide (Cardinale et al., 2004). Animal reservoirs of salmonella are numerous. In industrialized countries, livestock are the main reservoir (Rostagno and al., 2006 ; Sánchez-Vargas and al., 2011). The transmission of salmonella infections is mainly through ingestion of water or contaminated food. Salmonellosis is one of the most common food-borne diseases reported worldwide (WHO, 2000). The Salmonella genus has never ceased to be of considerable importance in the veterinary and medical

Cite this article as:

T. Maoudombaye et al. (2019) Int. J. Appl. Sci. Biotechnol. Vol 7(1): 43-48. DOI: 10.3126/ijasbt.v7i1.23297

***Corresponding author**
Mahamat Seid Ali Malloum,
Faculty of Exact and Applied Sciences, University of Moundou, Chad
Email: seidaligarga@gmail.com

Peer reviewed under authority of IJASBT

fields, as much by the economic losses due to diseases, as by the high incidence in humans of typhoid fevers and food poisoning (Grimont and Weill, 2007 ; O'Brien and De Valk, 2003). All salmonellae are potentially pathogenic, but the severity of the disease is a function of the strain and the amount of bacteria ingested (Van Asten and Van Dijk, 2005). Salmonella gastroenteritis is reported annually at about 1.3 billion cases worldwide, resulting in 2 million deaths and many economic losses (WHO, 2015). This number varies according to the health status of the population, the level of knowledge, the rules of hygiene, but is generally higher in developing countries (Hendriksen et al., 2012). data exist on the epidemiology of salmonella (Vlieghe and al., 2009).

In Chad, the first Salmonella studies date from the work of Perpezat and al. (1964) and Le Minor and al. (1969) and Guard and al. (1973), which demonstrated the epidemiological importance of salmonellosis. humans and animals, as well as the diversity of serotypes encountered. Recent work has also resulted in a distribution of Salmonella serovars in human stools and layers in N'Djamena (Djim-Adjim and al., 2013) and in broiler and traditional chicken farms in N'Djamena and Doba (Bodering and al., 2017). On the other hand, there are no data on the distribution of salmonella in drinking water. To this end, the objective of this study is to group by serotype Salmonella strains from well water, boreholes and rivers intended for consumption in the Doba oil basin in Chad.

Material and Methods

Choice of the site

The Province of Eastern Logone is one of twenty-three (23) Provinces of the Republic of Chad. Its surface area is 28,035 km² for a total population estimated at 796,453 inhabitants, and therefore a density of 28.4 inhabitants per km². It finally has 1027 villages, 42 cantons, 23 sub-prefectures distributed in 6 departments (CIRAD, 2005). The choice of the study area was guided by its proximity to oil wells and by the problem of drinking water supply (Maoudombaye et al., 2016). In fact, in 2009, about 24 villages in the oil fields were declared "impacted by the oil project" according to the classification of the socio-economic team of ESSO (oil consortium which exploits the Doba basin) in its 2009 quarterly reports. Most of these villages are in the Nyan Department with the capital, Bébédjia, the second largest city in the oil province. This department shelters most of the oil installations: the wells of Komé, Miandoum, Bolobo, Nyan, Maikiri, Moundouli and Timbré; and the water injection wells that ESSO had to drill next to the extraction wells to reinject the water to maintain the level of production. There are the two major ESSO bases where staff and equipment are housed, as well as the facilities of the contracting companies. It is also where migrants who have come to search for employment have failed, and who have settled there in other trades. In addition to drilled wells, crude oil collection plants and other oil installations, the area is traversed by innumerable tracks, some leading to collection centers, the others following the lines of the pipes. Beside this gigantic device, one can see all over the quarries which had been opened for the needs of the constructions and which are well that is said rehabilitated currently abandoned for the majority because unfit for agriculture. These quarries have become water retention basins of dubious quality. As a result, four oxen and two pregnant cows perished after drinking the dirty water from the mud of a drilled well (Djéralar, 2010). Given these environmental degradations, the Department should benefit in return from substantial compensation. Unfortunately, since most villages do not have drinking water supply systems, people often use water that is within their reach (Maoudombaye, 2017).

Collection, Conservation and Transport of Water Samples

The sampling was carried out according to a complete randomized device with three treatments (well water, manual well water and river water) and ten repetitions each, for a total of 30 samples. The samples were taken at each water point according to WHO standards for the bacteriological analysis of drinking water.

For river water, samples were taken at the level of agglomerations in places where human activities are frequent (Maoudombaye, 2017).

In order to ensure the representativeness of the samples, the sampling of the boreholes was carried out after draining the casing (Lallemand-Barrès 1993 ; Vaute 1998) at least 5 minutes before sampling.

For well water, sampling was done under natural conditions of consumer levy (Maoudombaye, 2017).

The samples were put into 1000 mL glass vials previously sterilized (Belghiti and al., 2013). Before sampling, each bottle was rinsed with the water to be analyzed. The conditioned samples were sent to the laboratory for analysis within 48 hours (CEAEQ, 2011) in an isothermal box at 4 ° C accompanied by a sheet providing information on the origin and date of sampling and the conditions. sanitary facilities at the sampling point (El Ouali and al., 2014).

Bacteriological Analysis

The isolation of salmonella strains was carried out according to the reference method NF / EN ISO 6579 (2002) (ISO, 2002) and serotyping by slide agglutination technique according to the Kauffmann-White scheme (Kauffmann, 1996).

Culture and isolation of Salmonella strains

It required 4 phases, according to the reference method NF EN ISO 6579 :

- A phase of pre-enrichment in a non-selective medium where the samples are pre-enriched at 1/10 with buffered peptone water, homogenized with a vortex for 2 min, left for revivification at room temperature during 30 minutes then incubated at 37 °C for 18 to 20 hours.

- An enrichment phase in liquid selective media in which 0.1 ml of the pre-enrichment is used to inoculate 10 ml of Rappaport Vassiliadis Soy (RVS) medium and 1 ml per 10 ml of Mueller-Kaufmann tetrathionate (MKTTn) medium; . Subsequently, the RVS medium was incubated at 42 °C and the MKTTn at 37 °C for 18 to 24 hours.

- An isolation phase: Hektoën and Xylose Lysine Deoxycholate (XLD) agar selective media are seeded from the enrichment products by the quadrant method and incubated at 37 °C for 24 h. Subsequently, 5 characteristic colonies were then removed and seeded on Hektoen agar for first purification and 24 h after on nutrient agar for a second purification (ISO, 2002).

- A biochemical identification during which the following media were seeded with pure colonies, typical of *Salmonella*:

• Kligler-Hajna agar: typical Salmonella cultures correspond to an alkaline slope (red) and an acidic (yellow) pellet, with formation of gas (about 90% of cases) and hydrogen sulphide (blackening of the agar).

• Urea-indole medium: in this medium, the characteristics of salmonella are urease (-) and indole (-).

• Galerie Api 20E: after inoculation and incubation according to the manufacturer's recommendations, the reactions are translated by spontaneous color changes, revealed by the addition or not of reagents. The reading is done using the analytical catalog.

For all these identification steps, the incubation is done at 37 ° C for 18 to 24 h.

Serogrouping of Salmonella Isolates

The Salmonella strains were serotyped by the direct slide agglutination technique, based on a combination of somatic O and flagellar H antigens, according to the Kauffmann and White scheme (Kauffmann 1996 ; IFT 2006 ; Grimont et al., 2000).

The determination of the serotypes is the combination of the antigenic formulas corresponding to the "O" and "H" antigens expressed during the different agglutinations on pure culture plate obtained in 24 hours on ordinary agar medium.

Beforehand, it has been verified that the strains in question are not in the R phase (self-agglutinable). For the non-autoagglutinable strains, the search for agglutination was carried out successively with the polyvalent anti-O serum (OMA, OMB, OMC), anti-O monovalent, then anti-H (HMA, HMB, HMC and H1), following the Kaufmann-white scheme (Kauffmann, 1996). The strain possessing the antigen corresponding to the tested antiserum forms agglutinates visible to the naked eye.

In practice, the OMA and OMB multipurpose sera make it possible to determine 99% of Salmonella strains. The use of monovalent anti-O sera clarifies the group to which Salmonella belongs. The Kauffman-White-Le Minor table (Grimont and Weill 2007 ; Guibourdenche and al., 2010) is used for the determination of the antigenic formula and the reading of the serotyping results (Huneau and al., 2007).

Results and Discussion

Salmonella Culture

The different colors that indicate the greater or lesser suspicion of the waters studied in Salmonella are illustrated in Fig.1.

Fig. 1. Results of cultures in Rappaport-Vassiliadis

After incubation at 42 ± 1 °C for 24 h ± 2 h, the broths in the tubes (1 ; 3 ; 5 and 6) that have kept their dark blue color are negative and those in the tubes (2 and 4) that have a light blue color indicate a suspicion of salmonella. Thus the pure colonies of salmonella on the Hektoen agar medium are represented in Fig. 2.

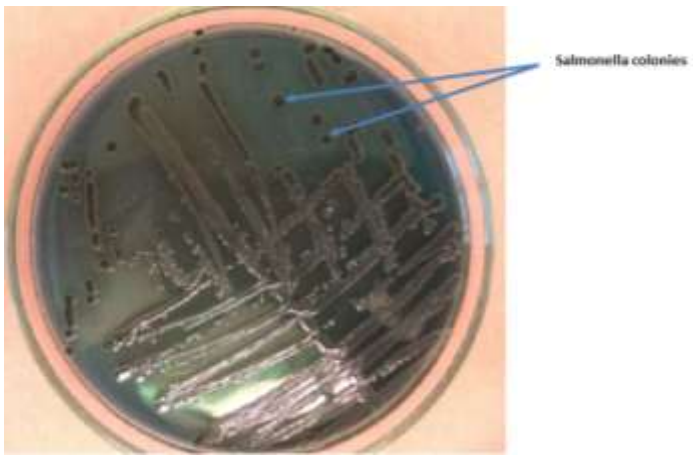

Fig. 2. *Salmonella* colonies isolated on Hektoen agar

Salmonella colonies have a shiny, dwarf, greenish color with a black center.

The results of the Kligler biochemical identification test appear in Fig. 3. After incubation at 37 ° ± 1 ° C for 24 h ± 2 h, the yellow pellet of the tubes (2, 3 and 5) indicates the fermented glucose; the sloping red slope of the tubes (1, 4, 5 and 6), unfermented lactose; the appearance of gas bubbles in the base of the tubes (1, 3, 4 and 6), a production of gas; the production of a black coloration between the base and the slope or along the spike of the tubes (1, 4, 5 and 6), formation of H2S.

On the basis of the typical reactions obtained in the different tubes, the examination of the Kligler test panel allowed identification of the following species: *Salmonella Paratyphi*, *Salmonella typhimurium* and *Salmonella enteridis.*

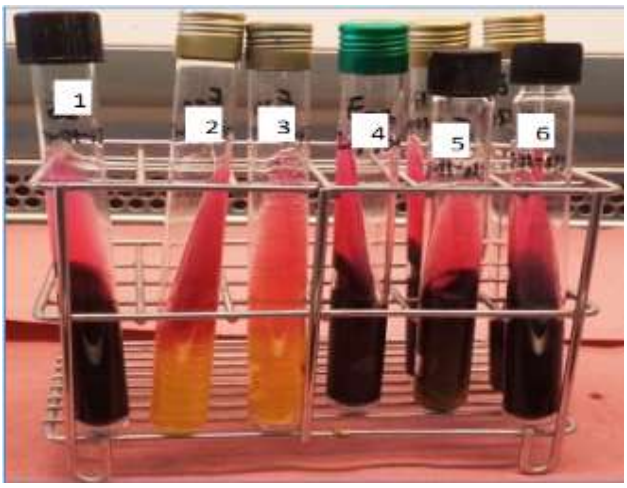

Fig. 3. Results of the Kligler Biochemical Identification Test.

Biochemical Test of salmonella by the Gallery API 20

The reading result of the API 20 E library by the Apiweb Tm-API 20E V4.1 site is given in Table I. The reactions produced during the incubation period result in spontaneous color turns or revealed by the addition of reagents in the microtubes. These reactions were read using the Reading Table and identification was obtained using the Analytical Catalog and identification software on the Apiweb Tm-API 20E V4 site. 1. The resulting Reading Table results in the following 7 digits: 6 7 0 6 7 5 2.

- Analytical catalog: reading from the analytical catalog gave Salmonella spp.

- Api web Tm-API 20 E V4.1 identification software: the reading of the result on this site is given by the table XI.

Biochemical Test of Salmonella by Serotyping

Figure 4 illustrates the biochemical identification test images of *Salmonella* serotyping

The reading of the results of the biochemical Salmonella identification test by serotyping gave, according to the White-Kauffmann-Le Minor table (Guibourdenche et al., 2010), three serotypes, namely the serotypes Anatum, Mbandaka and Idikan recorded in Table II.

The *Salmonella anatum* serotype was identified in well water (P4) and in drilling water (F6). The serotype *Salmonella Idikan* has been identified in drilling water (F5) and in river water (R4), while the serotype Salmonella Mbandaka has been found in river water (R8) and in water. drilling (F8). All three serotypes were found in borehole, river and well water. This shows their homogeneous distribution in the different water sources of the study area.

Table 1: Biochemical identification by the Apiweb Tm-API 20E V4.1 site

Gallery	API 20 E V4.1	
Profil	6 7 0 6 7 5 2	
Note (s)	**Confirm with serological tests**	
	Germs identified	Percentage of identification
Significatif Taxon	*Salmonella* spp	99,9
Next Taxon	*Salmonella* chileraesuis *ssp arizonae*	0,1

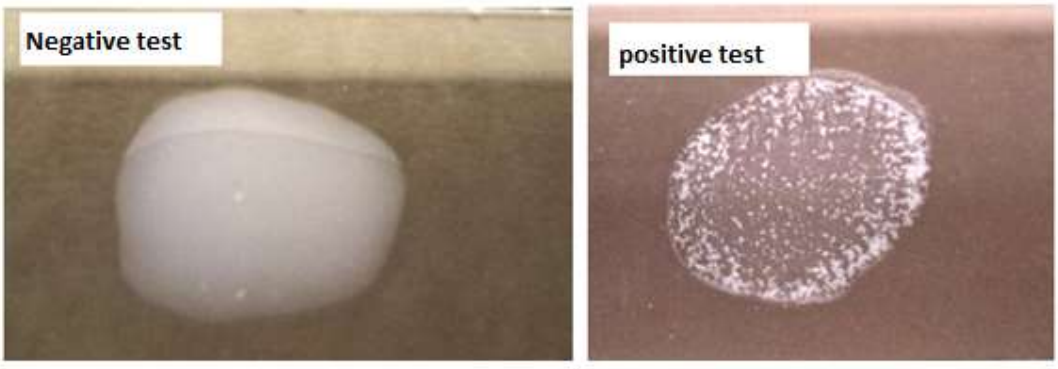

Fig. 4: Result of biochemical identification test by serotyping

Table 2: Salmonella serotyping result.

Will be anti-O versatile	Will be anti-O monovalent	Serogroups	Serotypes
OMA	O 3, 10, 15	O : 3,10 (E1)	*S.* Anatum
OMB	O 13, 22, 23	O : 13 (G)	*S.* Idikan
OMB	O 6, 7, 8	O : 7 (C_1)	*S.* Mbandaka

Bodering and al., (2017) isolated 40 strains of Salmonnella spp. with a prevalence of 3.67%. The serotypes that they reported are with their respective percentages S. Idikan (20%) ; S. Anatum (15%) ; S. Mbandaka (12.5%) ; S. Limete, S. Infantis and S. Derby (10%), S. Virchow (7.5%), S. Gallinarum and S. Choleraesuis (5%) and finally S. Paratyphi A and S. Enteritidis (2.5%). Djim-adjim (2013) identified 28 serotypes of Salmonella in 16 chicken poultry farms with serotype S. Idikan, and 21 serotypes of Salmonella in the human stool of 5 hospitals, including S. Idikan and S. Anatum, in the N'Djamena commune in Chad. 8 serotypes of Salmonella including 4 S. Typhi (50%), 2 S. para Typhi A (20%), 2 S. para Typhi B (20%) were isolated in the stools of cholera in Chad (Béssimbaye, 2014) . Ben Moussa and al., (2014) isolated the serotype S. Butantan (3 ; 10 ;15 ; b ; 1 ;5) in the surface waters of Oued Khoumane in Morocco. Serovars S. Mbandaka and S. Anatum were isolated respectively at 3.6% and 7.3% of Salmonella strains from samples taken from the Food Hygiene sector in 2002 in France (Afssa, 2005). Serovars S. Anatum and S. Mbandaka have been isolated from cows with clinical salmonellosis (Aubry 2010).

Conclusion

At the end of this work, the bacteriological quality of water resources for consumption in the Doba oil basin remains a major concern for the health of the population. The results of serotyping generally show that the consumption of these waters, without any prior treatment, can lead to potential health risks as they are rich in pathogenic microorganisms represented by serovars Anatum, Mbandaka and Idikan. This pollution is the combined effect of bedrock characteristics and factors related to sanitation and environmental health.

Indeed, most water wells are of traditional type, superficial, with wooden copings or tires, without covers or protective fences. The methods of water management in households are not without consequences on the quality of this water. The factors of the bacteriological pollution of these waters are essentially related to the sanitation of the environment, to the hygienic behavior of the populations and the salubrity around the works. A better source of water does not guarantee people a better health when the efficient and improved means of storing drinking water and those concerning hygiene and sanitation are non-existent.

References

Aubry P (2010) Salmonellosis in dairy cattle. Clinical presentation and bacteriological culture. Master's thesis in veterinary medicine. Department of Pathology and Microbiology. University of Montreal, 147 p.

Belghiti L, Chahlaoui A, El Moustaine R and Bengoumi D (2013) Contribution to the study of the bacteriological quality of spring water in the city of Elhajeb (Meknes-Morocco Region). *Larhyss Journal* **14**: 37-47.

Ben moussa A, Chahlaoui A, Rour E, Shahboune M, Aboulkacem A, Karraouan B and Bouchrif B (2014) Prevalence and virulence genes of Salmonella isolated from the surface waters of wadi khoumane, Morocco. *Lebanese Science Journal* 10 p.

Béssimbaye N (2014) Epidemiology of diarrheal diseases in Burkina Faso and Chad. Ph.D. thesis of the University of Ouagadougou. *Option: Applied Sciences* 204 p.

Bodering A, Ndoutamia G, Mopate LY, Ngandolo BN and Ngakou A (2017) Occurrence of Salmonella spp. Serotypes isolated from the poultry holdings in N'Djamena and Doba Towns-Chad. *Int. J. Recent Sci. Res.* **8**(3): 16016-16021. DOI: 10.24327/ijrsr.2017.0803.0056

Cardinale E, Tall F, Gueye E, Cisse M, Salvat G (2004) Risk factors for Salmonella enterica subsp. enteric infection in Senegalese broiler chicken flocks. Preventive Veterinary Medicine, 63: 151-161. DOI: 10.1016/j.prevetmed.2004.03.002

Center of Expertise in Environmental Analysis of Quebec (CEAEQ) (2011) Research and enumeration of faecal coliforms (thermotolerants) and confirmation to the species Escherichia coli: membrane filtration method. MY. 700 - Fec. Ec 1.0, Rev. 3, Quebec Department of Sustainable Development, Environment and Parks, 21 p.

CIRAD (2005) Regional Development Plan for the Petroleum Zone (Chad), final report, Ndjamena, CTNSC, 117 p.

D'Aoust JY (1991) Pathogenicity of foodborne Salmonella. *Inter J Food Microbiol* **12**(1): 17-40. DOI: 10.1016/0168-1605(91)90045-Q

Djéralar M (2010) Living with oil. Study on the living conditions of the villages in the oil zone of Doba in Chad. Report. 90 p.

Djim-adjim T (2013) Study of the contribution of avian salmonella to human salmonellosis in Chad: Case of the capital city, N'Djamena. Thesis of the Institute of Life Sciences and Industries and Environment *AgroParisTech* 151 p.

Grimont PAD and Weill F-X (2007) Annual Activity Report. CNR Salmonella, biodiversity unit of emerging pathogenic bacteria. Institut Pasteur, Paris, pp. 34-36.

Grimont PAD, Grimont F and Bouvet P (2000) Molecular basis of the diversity in the genus Salmonella. In Salmonella in domestic animals. Wray et al. CABI Publishing, British Library, London, U.K. 1-17. DOI: 10.1079/9780851992617.0001

Guard O, Delpy P and Sirol J (1973) Salmonella infections in Chad. About 152 cases observed at the Fort Lamy Hospital in 1970. *Tropical Medicine: French Review of Pathology and Public Health* 750 p.

Guiburdenche M, Roggentin P, Mikoleit M, Fields PI, Bockemuhl J, Grimont PA, Weill FX (2010) Supplement 2003-2007 (No 47) to the White-Kauffmann-The Minor scheme. *Research Microbiology* **161**: 26-29. DOI: 10.1016/j.resmic.2009.10.002

Hanes D (2003) No typhoid Salmonella. In: Miliotis N, Bier J, (Eds) *International Handbock of Foodborne Pathogens*, Marcel Dekker: New York, pp. 137-149. DOI: 10.1201/9780203912065.ch9

Hendriksen RS, Vieira AR, Karlsmose S., Wong DMA, Fo Fo, Jensen AB, Wegener HC, Aarestrup FM (2012) Global Monitoring of Salmonella Serovar Distribution from the World Health Organization Global Foodborne Infections Country Data Bank: Results of Quality Assured Laboratories from 2001 to 2007. *Foodborne Pathog Dis* **8**: 887-900. DOI: 10.1089/fpd.2010.0787

Huneau SA, Chemaly M, Petetin I, Rouxel S, Lalande F & Le Bouquin S (2007) Multidimensional descriptive analysis of layer farms contaminated with Salmonella spp. : Looking for risk factor assumptions. Seventh Journées de la Recherche Avicole, Tours. 505-509 pp.

Institute of Food Technologists (IFT) (2006) Antimicrobial resistance: implications for the food system. Comprehensivereviews in Food Science and Food Safety, 5, 71-136. DOI: 10.1111/j.1541-4337.2006.00004.x

Kauffmann F (1966) On the history of Salmonella research. Zentralbl. Bakteriol., 201: 44-48.

Maoudombaye T (2017) Evaluation of the physicochemical and bacteriological quality of water resources for consumption in the Doba oil basin in Chad. Doctoral Thesis Ph.D. University of Ngaoundere. 182 p.

Maoudombaye T, Ndoutamia G and Ngakou A (2016) Evaluation of the bacteriological quality of well water, boreholes and rivers consumed in the Doba oil basin in Chad. International Journal of Recent Scientific Research (7) 6: 12236-12243.

Minor L, Chamoiseau G, Barbé E, Charie-Marsaines C, Egron L1 (969) Ten new Salmonella serotypes isolated in Chad. Annals of the Pasteur Institute, 116 (6): 775-780.

Murray C (2000) Environmental aspects of Salmonella. In: Wray C., Wray A. (Eds), Salmonella in domestic Animals. CABI Publishing: Oxon. pp. 265-283. DOI: 10.1079/9780851992617.0265

O'Brien SJ, De Valk H (2003) Salmonella " old " organism, continued challenges. Euro Surveillance, 8: 29-31. DOI: 10.2807/esm.08.02.00397-en

Perpezat A, Perreau P, Thomé M, Vigier M (1964) Different Salmonella serotypes isolated in the Republic of Chad. Journal of Livestock and Veterinary Medicine of the Tropics, 17 (1): 35-41.

Rostagno MH, Wesley I, Trampel D and Hurd H (2006) Salmonella prevalence in market-age turkeys on farm and at slaughter.Poultry science, 85 (10): 1838-1842. DOI; 10.1093/ps/85.10.1838

Sánchez-Vargas FM, Abu-El-Haija MA, Gómez-Duarte OG (2011) Salmonella infections: an update on epidemiology, management, and prevention. Travel Medicine and Infectious Disease, 9 (6): 263-277. DOI: 10.1016/j.tmaid.2011.11.001

Van Asten A J, Van Dijk J E (2005) Distribution of classic virulence factors among Salmonella spp. FEMS Immunology Medicine and Microbiology, 44: 251-259. DOI: 10.1016/j.femsim.2005.02.002

Vaute L (1998) Groundwater monitoring of the Lorraine Basin in 1998. *BRGM report R 40789*. 69 p.

Vlieghe E, Phoba MF, Muyembe Tamfun JJ and Jacobs J (2009) Antibiotic resistance among bacterial pathogens in Central Africa: A review of the published literature between 1955 and 2008. *International Journal of Antimicrobial Agents* **34**: 295-303. DOI : 10.1016/j.ijantimicag.2009.04.015

World Health Day (WHO) (2015) Food Safety Are your foods really safe? From farm to plate, you all have a role to play. Awareness guide 12 p.

World Health Organization (WHO) (2000) WHO Surveillance Program for Control of Food Terminal Infections and Intoxications in Europe. *Seventh Report on Disease Surveillance in Europe 1993-998.*