**Detection and characterization of *Salmonella* in recreational aquatic environments in the Northeast of Argentina**

**ABSTRACT**

The aim of this work was to detect the presence of *Salmonella* in recreational aquatic environments in the Northeast of Argentina and to relate it with water and environmental parameters. Sixty eight samples of water from recreational aquatic environments in the provinces of Chaco and Corrientes, Argentina, were studied. Salmonellae were detected in 6 samples (8.8%). *Salmonella* isolates belonged to the following species and serovars: *S. enterica* ser Give, *S. enterica* subespecie IV, *S. enterica* ser Bredeney, *S. enterica* ser Rubislaw, and *S. enterica* ser Enteritidis (2 isolates). None of the isolates were resistant to tested antimicrobials. Significant differences did not exist between *Salmonella* isolation and site of sampling and the rest of the variables. The presence of *Salmonella* spp in our recreational aquatic environments reaffirms the need for surveillance in order to minimize the risks of infection of the exposed susceptible people.

**Key words**: foodborne pathogen, surface water, enteropathogens

**Detecção e caracterização de *Salmonella* em ambientes aquáticos para uso recreativo no Nordeste da Argentina**

**Resumo**

O objetivo deste trabalho foi detectar a presença de *Salmonella* em ambientes aquáticos para uso recreativo no Nordeste da Argentina e relacioná-la com parâmetros ambientais e das águas, Foram estudados sessenta e oito amostras de água de ambientes aquáticos de lazer nas províncias de Chaco e Corrientes, Argentina. *Salmonella* foi detectada em seis amostras (8,8%). *Salmonella* isolados pertenciam às espécies e sorovares *S. enterica* ser Give, *S. entérica* subespécie IV, *S. enterica* ser Bredeney, *S. enterica* ser Rubislaw e *S. enterica* ser Enteritidis (dois isolados). Nenhum dos isolados testados foram resistentes aos antimicrobianos. Não existiam diferenças significativas entre os isolamentos de *Salmonella* e os locais de amostragem e o restante das variáveis. A presença de *Salmonella* spp em nossos ambientes aquáticos para uso recreativo reafirma a necessidade de vigilância, a fim de minimizar os riscos de infecção das pessoas suscetíveis expostas.

Palavras-chave: patogénicos de origem alimentar, água de superfície, enteropatógenos

1. **INTRODUCTION**

The emergence and spread of infectious disease in plant, animal and human populations is a problem around the world; water is a common element in the ecology of many pathogens affecting these populations and waterborne pathogens can pose threats to drinking water supplies, recreational waters, source waters for agriculture and aquaculture, as well as to aquatic ecosystems and biodiversity (Edge et al., 2001).

The phenomenon of “emergence” and “re-emergence” of infectious diseases in general is now well recognized and up to 75% of emerging pathogens may be of zoonotic origin (WHO, 2004).

There is consistency in the overall body of evidence concerning health effects from faecally polluted recreational waters and the most frequent adverse health outcome associated with exposure to faecally contaminated recreational water is enteric illness (WHO, 2009).

To scientifically evaluate pollutants and to develop protective public policies, risk assessment is the accepted approach. This approach, however, is no better than the database on which we subsequently build public health strategies. A usable database must include information about sources, occurrence, concentrations, frequency, survival, and transport of specific microorganisms in the environment (Rose et al., 1999).

Water sources are vulnerable to contamination from many origins. Humans and animals are all sources of faecal contamination (Dechesne and Soyeux, 2007).

There are several well documented waterborne zoonotic bacterial pathogens, including *Salmonella*, *E. coli* O157:H7, *Campylobacter*, and Yersinia. The prevalence of these organisms depends on the nature of the source and the water supply, excreta and other waste disposal processes, and environmental and climatic factors (WHO, 2004)

Surface water quality is subject to frequent, dramatic changes in microbial quality as a result of a variety of activities, because discharges of municipal raw (untreated) water, treated effluents from processing facilities, storm water runoff, or other non-point source runoff all affect surface waters (Anderson and Davidson, 1997).

The region influenced by the cities of Resistencia (Province of Chaco) and Corrientes (Province of Corrientes) have several sites used as recreational environments by the local and visiting populations mostly during the warm seasons (September to March), when the lagoons and rivers are used for bathing, windsurfing, and rowing; however, through all the year, aquatic environments are used for fishing.

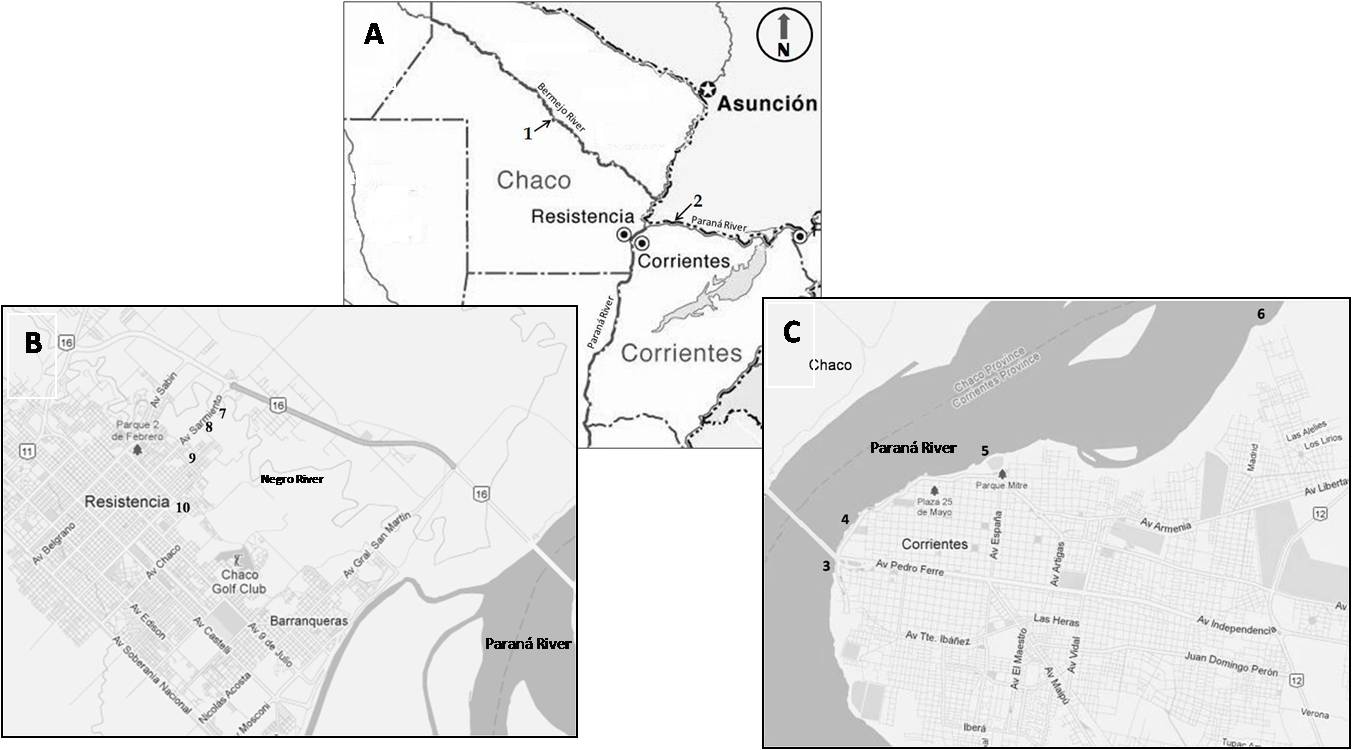
The aim of this work was to detect the presence of *Salmonella* in recreational aquatic environments in the Northeast of Argentina and to relate it with water and environmental parameters.

1. **MATERIALS AND METHODS**
   1. **Sampling sites**

Water samples were collected in the sites indicated in the Fig. 1, all of them used as recreational environments. They were lagoons (Argüello, Colussi, and Francia) and rivers (Negro and Bermejito) located in the province of Chaco, and the following beaches along the Paraná River in the Province of Corrientes (Arazatí, Regatas Club, Molina Punta, Malvinas Argentinas, Canotaje Club, and Paso de la Patria).

**2.2. Sample collection**

Water was collected at least one time through different seasons in the year between April 2008 and April 2011 as grab samples (5 liters) in sterile polypropylene bottles. Samples were kept at 4°C until microbiological analyses were completed. Water was analyzed *in situ* for temperature (ºC) and pH.

**Fig 1.** Sampling sites in the provinces of Chaco and Corrientes, Argentina. Ref.: A. Northeast of Argentina. B. City of Resistencia. C. City of Corrientes. 1. Bermejito River, 2. Paso de la Patria Beach, 3. Arazatí Beach, 4. Canotaje Club Beach, 5. Regatas Club Beach; 6. Molina Punta Beach; 7. Negro River, 8. Colussi Lagoon; 9. Francia Lagoon; 10. Argüello Lagoon.

**2.3. Rainfall data**

For each time of sampling, four days previous rainfall were recorded.

**2.4. Microbiological analyses**

**2.4.1. Fecal indicator bacteria**

Within 30 minutes of collection, samples were screened for total coliform bacteria and *Escherichia coli* by filtration of two aliquots of 100 ml tenfold diluted sample through two 0.45 µm-pore-size membranes (HPA, 2007). Enumerations were carried out by placing the membranes on m-ENDO® medium and m-ColiBlue24® medium, respectively.

**2.4.2. Salmonellae**

Within 30 minutes of sample collection, *Salmonella* spp. was detected by filtering 1 liter of water through 0.45 µm-pore-size membranes. Then, the membrane was placed into 50 ml of Rappaport-Vassiliadis broth (RVB) and incubated at 42°C for 24 h for selective enrichment of *Salmonella* (HPA, 2004). Ten microliters of the RVB enrichment were then streaked onto *Salmonella*-Shigella agar for isolation at 35°C for 24 h and colonies presumptively identified as *Salmonella* were identified to the genus level by biochemical tests (Caffer et al., 2008). Colonies that were positively identified as *Salmonella* were shipped to the National Reference Laboratory (Instituto Nacional de Enfermedades Infecciosas ANLIS “Dr. Carlos G. Malbrán”, Buenos Aires, Argentina) for serotyping. Antimicrobial susceptibility tests for Salmonellae were performed by an agar diffusion disk method according to the standards outlined by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2008; CLSI, 2009). The commercial disks used were: ampicillin 10 μg, cephalothin 30 μg, cefotaxime 30 μg, neomycine 30 μg (NEO) gentamicin 10 μg, tetracycline 30 μg, furazolidone 300 μg, nalidixic acid 30 μg, chloramphenicol 30 μg, trimethoprim/sulfamethoxazole 1.25/23.75 μg, ciprofloxacin 5 μg, colistin 10 μg, and fosfomycin 50 μg. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212 were tested as quality control organisms.

**2.5. Statistical analyses**

Data were recorder and analyzed using Epi Info 2000 software (Centers for Diseases Control and Prevention, Atlanta, GA). For all bacterial counts, a value of zero was used for any sample with concentrations below the limit of detection; Chi Square an t-student test were determined to describe the relationships between the presence of *Salmonella* and other variables. For all measures of association, *p* values <0.05 were considered significant.

1. **RESULTS**

Sixty eight samples of water were studied. Origins and amounts of samples in each one of them are presented in the Table 1. Salmonellae were detected in 6 samples (8.8%). The site with highest contribution was the Argüello Lagoon in the city of Resistencia, where the organism was recovered in 4 samples, followed by Regatas Club Beach and Negro River with one strain each one; nevertheless, significant differences did not exist between *Salmonella* isolation and site of sampling.

**Table 1:** Origins and numbers of samples.

|  |  |  |
| --- | --- | --- |
| **Site of sampling** | **Type of site** | **Number of samples** |
| Argüello | Lagoon | 20 |
| Colussi | Lagoon | 4 |
| Francia | Lagoon | 4 |
| Negro | River | 16 |
| Bermejito | River | 4 |
| Arazatí Beach | River | 4 |
| Club de Regatas Beach | River | 4 |
| Molina Punta Beach | River | 4 |
| Club de Canotaje Beach | River | 4 |
| Paso de la Patria Beach | River | 4 |
| **Total** |  | **68** |

Previous rainfalls were recorded in the 28.6% of positives samples for *Salmonella* spp and in the 33.3% in the negative samples, this difference was not significant.

The average values of water´s parameters are shown in the Table 2. There were no significant differences between these parameters and the possibility of recovering *Salmonella*.

*Salmonella* isolates belonged to the following species and serovars: *S. enterica* ser Give, S. enterica subspecie IV, *S. enterica* ser Bredeney, *S. enterica* ser Rubislaw, and *S. enterica* ser Enteritidis (2 isolates). None of the isolates were resistant to tested antimicrobials.

**Table 2:** Environmental parametersof the sampling sites related to thepresence/absence of *Salmonella* spp.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Environmental**  **and**  **water parameters** | **Presence of *Salmonella*** | | | | | |
| **Positive**  **samples**  **(n=6)** | | **Negative**  **samples**  **(n=62)** | | **Total**  **samples**  **(n=68)** | |
| **Average** | **Range** | **Average** | **Range** | **Average** | **Range** |
| pH of water | 7.7 | 7.4-8.7 | 7.2 | 4.6-9.6 | 7.2 | 4.6-9.6 |
| Temperature  of water (ºC) | 24.0 | 15.0-28.5 | 26.2 | 17-29 | 26.0 | 15.8-32.6 |
| Total Coliforms  (CFU/100 ml) | 1.8x106 | 5.1x103-  1x107 | 3.3x105 | 1.1x102-  1x107 | 4.8x105 | 1.1x102-  1x107 |
| *E. coli*  (CFU/100 ml) | 1.2x104 | 0-1x105 | 1.1x104 | 0-1x105 | 1.2x104 | 0-1x105 |

CFU: Colony forming units

1. **DISCUSSION**

*Salmonella* is a recognized human pathogen and the waterborne transmission has been well documented (CABRAL, 2010). *Salmonella* detection in waterways indicates the spread of the agent in the environment, highlighting the importance of fecal contamination of the water environment in the spread of salmonellosis (Winfield and Groisman, 2003).

Although this is the first work about detection of this bacterium in recreational aquatic environments in Northeast of Argentina, *Salmonella* was the enteric pathogen more frequently recovered in coastal waters in Hong Kong and several rivers in Japan and another countries (Yam et al., 2000; Jokinen et al., 2011; Giménez Martí et al., 1990; Gorski et al., 2011); that is why it is very important the surveillance of this organism due to the possibility of infection through direct contact with superficial waters.

Salmonellae has been detected for several authors in surface waters in different percentages, as varied as 8.5% (Jokinen et al., 2011), 15.4% (Adingra et al., 2012), 18.0% (Yam et al., 2000), 62.9% (Anselmo et al., 1999), 79.2% (Haley et al., 2009) and 96.0% (Rajabi et al., 2011). This may be due the presence and the abundance of Salmonellae in aquatic environments vary temporally (Haley et al., 2009) and is related to one or a combination of sewage effluents; agricultural run-off and direct faecal contamination from natural fauna (Abulreesh, 2012). Additionally, the possibility for intermittent findings or for detecting different serovars in the same site of sampling suggests the heterogeneity of the aquatic environment (Rolland and Block, 1980).

One factor that might explain the differences in the abundance and diversity of *Salmonella* isolates between different locations is the climate (Gorski et al., 2011).

The intermittent detection of Salmonellae in aquatic environments may be due to the assumption that enteric bacteria do not survive very long after cells are introduced in oligotrophic aquatic environments; about this fact, Santo Domingo et al published that a considerable decrease in plate counts of *Salmonella* was observed after 7 days of being suspended in untreated and filtered river water (Santo Domingo et al., 2000).

This shows that monitoring should be continuous and should not rule out the possibility of contamination of the environment only in the presence of a negative sample.

The climate in our region is subtropical without dry season, with small differences in the temperature wideness throughout the year (Gobierno de la República Argentina, 2013); this fact could explain that the presence of *Salmonella* in our aquatics environments does not depend on environmental parameters. Although, in other studies, the presence of *Salmonella* increased with the high levels of rainfall that occurred at the study locations and its prevalence was substantially higher than in the dry summers (Polo et al., 1999; Adingra et al., 2012)

Regarding the presence of faecal indicators, such as the count of *Escherichia coli* and total coliforms, in this study there was no correlation with the presence of *Salmonella*, in agree with a previous work (Dechesne and Soyeux, 2007). This is because surface water and reservoirs are particularly liable to pollution from animals and birds, and *Salmonella* spp. may be detected even when only a small number of indicator organisms are present, e.g. *Escherichia coli* (POLO et al., 1999).

Additionally, some authors highlighted that the different rates of survival of *Salmonella* and *E. coli* in nonhost environments suggest that *E. coli* may not be an appropriate indicator of *Salmonella* contamination (Winfield and Groisman, 2003).

Among the more than 2,500 known *Salmonella* serotypes, in agree with our findings, S. Enteritidis, S. Rubislaw, S. Give and S. Bredeney were the most common serovars isolated from river water and from wild bird species (Anselmo et al., 1999; Gorski et al., 2011; Polo et al., 1999; Jokinen et al., 2011; Rajabi et al., 2011).

This small range of the environmentally recovered serotypes may reflect the relatively small assortment of serotypes that commonly infect humans and animals as well as differential environmental persistence among serotypes at different temperature ranges (Haley et al., 2009).

Similarly to that found in our work, *Salmonella* spp. isolated from the environment in previous works are those with low invasiveness; patients infected by them usually experience a milder type of diarrhea and do not require hospitalization. Consequently, these low invasive *Salmonella* spp. may enter into the coastal waters through domestic sewage discharges (Yam et al., 2000).

Strains of *Salmonella* spp. with resistance to antimicrobial drugs are now widespread in both developed and developing countries. In developed countries it is now increasingly accepted that for the most part such strains are zoonotic in origin and acquire their resistance in the food-animal host before onward transmission to humans through the food chain (Threlfall, 2002).

An important issue in public health is the emergence of multidrug-resistant strains of *Salmonella*. Nevertheless, we did not find resistant strains. These results are different from the results from other surveys characterizing *Salmonella* antibiotic resistance, possibly because these other surveys targeted regions with reported high incidences of *Salmonella*, areas affected by animal agriculture, or feedlots and diseased animals (Gorski et al., 2011).

It has been reported that some strains of *Salmonella* enter the viable but nonculturable (VBNC) state when they encounter environmental stresses; these strains fail to grow and develop colonies on culture media, but their metabolic activity capabilities indicate that they are still alive and certain conditions could resuscitate the VBNC forms, becoming them in pathogens again (Zeng et al., 2013).

**5. CONCLUSIONS**

According to the results of this study, temperature, pH, presence of previous rains, counts of total coliforms and *Escherichia coli* seem do not influence on detection of Salmonellae in aquatic environments in our region.

Further studies are needed to examine the complex environmental parameters, especially in relationship to wildlife distribution, human activities, and other factors that may impact the microbial diversity and survival of *Salmonella* in our recreational aquatic environments.

The presence of *Salmonella* spp in our recreational aquatic environments reaffirms the need for surveillance in order to minimize the risks of infection of the exposed susceptible people.

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