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Short Communication

Effect of Soil Chemistry on Distribution of *Listeria monocytogenes* Across Punjab, Pakistan

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Abstract

A comparative study was conducted in Punjab province, Pakistan to ascertain any correlation between various physico-chemical characteristics of soil and presence of *Listeria monocytogenes* (LM) DNA in soil. For this purpose, 34 soil samples (n=17 positive for LM and n=17 negative for LM) were collected from nine districts of Punjab province. Atomic absorption spectrophotometer was used for assessing the levels of several factors like phosphorous (P), copper (Cu), chromium (Cr) nickel (Ni), manganese (Mn), cobalt (Co), lead (Pb), cadmium (Cd), iron (Fe), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and nitrogen (N). Contrarily pH, moisture, electrical conductivity, organic matter, and texture of soil (silt, sand and clay) were determined by following standard protocols. Upon statistical analysis, a significant association was observed only for clay (0.000) and organic matter (0.001) whereas all other factors did not prove any positive association (P>0.05) with presence of LM DNA in soil. Hence it is concluded that the composition of soil does influence the existence of LM DNA in environment.

Key words: Chemical factors; *Listeria monocytogenes;* Soil; Metal; Pakistan

Introduction

Listeriosis, also named as silage disease is an infectious condition caused by soil borne *Listeria monocytogenes* which is prevalent worldwide. It has wide distribution range and is isolated from water, soil, fruits, vegetables, milk, meat and dairy products (Locatelli *et al.* 2013). Listeric infections usually lead to abortion in ruminants and humans also. Besides abortion, other clinical manifestations are depression, loss of appetite, fever, septicemia, encephalitis and ultimately death. The most frequent manifestation of listeriosis in ruminants is circling in one direction and is referred as circling disease (Clark *et al.* 2004). The infection rate of LM in ruminants is 10 percent while morbidity ranges up to 30 percent (Peter 2000). Across the world listeriosis occurs in epidemic form and mostly the nature of infection is subclinical in animals (OIE 2014). In Indian sub-continent, Malik *et al.* (2002) has reported some sporadic cases of listeriosis in both humans and animals. The estimated prevalence of LM in buffaloes by Shakuntala *et al.* (2006) is 4.4% in India. In humans, annual endemic disease rate across the world varies from 2 to 15 cases per million of population (McLaughlin *et al.* 2011). In the past few years incidence of listeriosis has increased in elderly people in European countries (Fierer *et al.* 2001). Australia has constant incidence range of listeriosis which is 0.2–0.4 cases/100,000 population from 1991–2000 (Botzler *et al.* 1974), whereas, USA has seen remarkable rate of 37% decrease in incidence of listeriosis during 1996–2001 (Locatelli *et al.* 2013). From Asian countries very few cases are being reported including 48 cases from Taiwan during 1996–2008 (Kulesh 2017) and 479 cases from China during 1964–2010 (FAO 2004).

Soil is considered as main source of transmitting several pathogens due to presence of microbe rich areas named macropores in it (Bundt *et al.* 2001). In Pakistan very little work has been documented relevant to LM and no study has ever been conducted to ascertain role of soil chemistry with presence of LM in soil. There is no baseline data related to the effects of land management practices on the abundance of this bacterium and in which soil types or under which environmental conditions it is more prevalent. this study was designed to correlate data of the prevalence of *L. monocytogenes* in the soil with data of soil chemistry for establishing any association.

Materials and Methods

A total of 34 soil samples from LM positive (n=17 and negative sites (n=17) were taken (unpublished data). Briefly, 200 g of soil sample was collected from 3 inches below the ground surface with the help of electronic weighing balance. All the samples were placed into clean, pre-labeled zipper

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bags and were transported at room temperature to Department of Environmental Sciences, University of Veterinary and Animal Sciences, Lahore and Department of Plant Sciences, Quaid-i-Azam University, Islamabad.

Soil texture was determined by following the protocol of Robert and Friedrick (1995). The moisture content was measured by placing sample (10 g) in hot air oven for 4 days at 72°C (Mclean 1982). Digital pH meter was used for measuring the pH of soil samples (Committee CSS 1978). Ammonium bicarbonate-diethylenetriaminepenta acetic acid (DTPA) method was used for measuring concentrations of Mg, Cu, Cr, Ni, Mn, Co, Pb, Cd, Na, Fe, Ca, N (Fierer *et al*. 2001) and P (Warncke and Brown 1998). The wavelength for measuring concentration of various analytes in atomic absorption spectrophotometer SpectrAA-100 (Varian, Springvale Australia) was 880 nm Total soluble salts were determined by following protocol of Magistad *et al.* (1945) Whereas organic matter content was measured by following Nelson and Sommers (1982) protocol.

Results

The effect of soil composition in relation to presence or absence of LM was assessed by compiling data into Microsoft Excel spreadsheet. As the data violated the normal distribution pattern so a non-parametric test named Mann-Whitney test (95% confidence interval and 5% level of significance) by S.P.S.S. version 20.0 (S.P.S.S. Inc., Chicago, IL, USA) was utilized. Considerable variations among concentrations of various analytes were recorded in soil samples of both LM positive and negative groups (Table 1). A positive association was observed in case of clay (0.000) and organic matter (0.001) with *L. monocytogenes.* Contrarily analytes like silt (0.918), sand (0.617), pH (0.570), soluble salts (0.318), Nitrogen (0.364), Phosphorus (0.535), Nickel (0.278), Cadmium (0.959), Copper (0.502), Manganese (0.570), Calcium (0.270), Magnesium (0.582), Lead (0.052), Sodium (0.263), Zinc (0.547) and Potassium (0.654) showed no significant association with LM in soil (Table 2).

Table 1: Characteristics of various physio-chemical factors of soil in LM positive and negative groups

Soil analyte	LM positive soil Mean SD	LM negative soil Mean SD
pH	8.528,0.4203	8.569,0.4839
Soluble salts%	2.584, 0.6442	2.804,0.7500
Organic matter	5.503,3.819	11.484, 5.161
Calcium (mg/kg)	0.3131,0.354	0.417,0.339
Sodium (mg/kg)	0.119,0.162	0.173, 0.144
Potassium (mg/kg)	0.281, 0.3535	0.307, 0.3165
Nitrogen (mg/kg)	0.0687, 0.017	0.111, 0.160
Phosphorus (mg/kg)	18.63,2.211	18.41, 2.44
Magnessium (mg/kg)	0.292, 0.3535	0.307, 0.1165
Managnese (mg/kg)	4.329, 2.065	3.836,1.468
Zinc (mg/kg)	0.932, 0.367	0.865, 0.344
Lead (mg/kg)	6.503,2.819	10.184, 7.261
Electrical conductivity	247.80, 88.33	269.85,98.8
Nickle (mg/kg)	0.495, 0.585	0.247, 0.332
Copper (mg/kg)	0.051, 0.095	0.0591, 0.059
Clay (mg/kg)	4.503,4.009	10.484, 4.161
Silt (mg/kg)	0.624, 0.214	0.497, 0.312
Sand (mg/kg)	0.262, 0.4535	0.207, 0.2165
Cadmium (mg/kg)	0.613, 0.2256	0.507, 0.3208

Table 2: Characteristics of soil and their association with Positive LM soil samples

Soil analyte	Mann Whitney U	*Significance
pH	128.000	0.570
Soluble salts (%)	115.500	0.318
Organic matter (%)	51.500	0.001
Calcium (mg/kg)	112.500	0.270
Sodium (mg/kg)	112.500	0.263
Potassium (mg/kg)	131.500	0.654
Nitrogen (mg/kg)	118.500	0.364
Phosphorus (mg/kg)	126.500	0.535
Magnesium (mg/kg)	116.500	0.582
Manganese (mg/kg)	128.000	0.570
Zinc (mg/kg)	127.000	0.547
Lead (mg/kg)	88.000	0.052
Electrical conductivity	116.500	0.335
Nickle (mg/kg)	113.000	0.278
Copper (mg/kg)	125.000	0.502
Clay (mg/kg)	31.000	0.000
Silt (mg/kg)	141.500	0.918
Sand (mg/kg)	130.000	0.617
Cadmium (mg/kg)	143.000	0.959

*$P < 0.05$ *positively associated and* $P > 0.05$ *vice versa*

Discussion

Soil is fortified with bacterial DNA, which is liberated actively from bacteria or after its death the autolytic changes releases it into the environment (Palmen and Hellingwerf 1995, 1997). Bacterial DNA prevails in the environment for different range of time period depending upon physical conditions of soil or type and presence of nucleases in the soil (DeSalle *et al.* 1992). The persistence of bacteria in soil is also determined by the texture of soil. According to study conducted by Marshall (1975), more clay content in soil enhances the survival rate of bacteria. Locatelli *et al.* (2013) in his study also supported clay soil for enhanced bacterial survival rate due to more ratio of organic content in it. One other possible reason for bacterial stability in clay soil is its significant relationship between Base cation saturation ratio (BCSR). BCSR represents the cations in soil along with the anions provided from soil. Clay and organic matter both are negatively charged and fix the nutrients having positive charge which are mandatory for survival of bacteria (Dowe *et al.* 1997). Moreover many studies revealed that fine textured soils are more favorable to growth of bacteria because fine textured soils have more pore spaces which protects them from various protozoans (Botzler *et al.* 1974; McLaughlin *et al.* 2011). Rate of DNA adsorption is greatly influenced by soil texture and according to Lorenz and Wackernagel (1994) 100 folds more DNA adsorption is observed in case of clay soil as compared to sandy soil.

In the present study, soil elements like P, N, Mg, Mn, Zn, Cd, Ni, Ca, Na, K and Cu did not show any association with presence of LM in soil. Heavy metals like copper, zinc, cadmium, arsenic, mercury and nickel are reported to have deleterious effects on bacteria (Lorenz *et al.* 1991). The untreated industrial waste, continuously discharged into the surrounding environment, has significantly increased the level of heavy metals. These elevated levels of heavy metals have detrimental effects on DNA by inducing cytotoxic effects on living cells. Hence the soil contaminated with these metals has higher activity of DNAse enzyme which accelerates the destruction of DNA (Tsuzuki *et al.* 1994; Mukherjee and Das 2002) .Chromium is well known to cause fragmentation of DNA (Patlolla *et al.* 2009) whereas higher levels of Mg and Ca interfere with the DNA adsorption level by soil (Nguyen and Chen 2007) .Cadmium is also lethal but various bacteria have developed cadmium resistance mechanisms like enzyme systems which render these metals non-toxic. They protect themselves by stopping their entry within the bacteria cell by chelating these metal ions and by adopting efflux mechanism. Besides industrial effluents seepage, soil is contaminated by heavy metals from the fecal material of birds and animals and decaying vegetation (Basta *et al.* 2005). In many villages, the practice of adding animal manure in fields as fertilizer also leads to heavy metal contamination of soil (Chaney and Oliver 1996).

Conclusion

Clay and organic matter are associated with the existence of *L. monocytogenes* specific DNA in soil. In future studies, controlled environment and soil type can be used to ascertain the possible role of each analyte in more efficient way.

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Author Contributions

M Rabbani, A Ahmad and MZ Shabbir planned the project while R Tahir and Y Tipu conducted the research and statistical analysis. All the authors significantly contributed in writing this manuscript.

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