CONTAMINATION OF THE ENVIRONMENT BY PATHOGENIC BACTERIA IN A LIVESTOCK FARM IN LIMPOPO PROVINCE, SOUTH AFRICA

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Abstract. The aim of this study was to investigate the occurrence and diversity of pathogenic bacteria in the environment at a livestock farm in Limpopo Province. Environmental samples were collected from three sampling locations: cattle camp (CC), sheep camp (SHC), and goat camp (GC). Samples were processed and analysed for total bacterial counts in the Biotechnology Laboratory, University of Limpopo, South Africa. Identifications were done with Matrix Assisted Laser Disorption Ionisation Time of Flight Mass Spectometry (MALDI-TOF MS) using the simplified on plate technique. The colony forming unit formula per gram of environmental sample (CFU/g) was used to determine total bacterial counts. High counts were observed in CC and low counts in SHC for both soil and faecal samples. *Bacillus species* were the most dominant across the sampling locations for soil samples. *Bacillus cereus* occurred in 36 samples (33%), however, the frequency of isolation dominated in the CC with (44.4%), GC (22.2%) and SHC (13%). *Staphylococcus aureus* had the least frequency (2.2%) followed by *Listeria monocytogene*s (4.4%). In faecal samples, the occurrence and level of contamination with bacterial species varied across sampling locations with *Escherichia coli* dominating in the CC with 20% frequency, and abscent from SHC and GC.
Keywords: *MALDI-TOF MS, livestock diseases, soil, Escherichia coli, Bacillus cereus*

Introduction

The rural communities of South Africa depend on livestock farming in various ways such as the provision of meat, milk, manure, improved livelihoods and socioeconomic relief (Meissner et al., 2013; Becker, 2015; Madziga et al., 2013). However, livestock diseases present a major threat to animal health. The increase in the circulation of infectious agents over the past decade as well as the emergence of antibiotic resistance and environmental pollution has placed human and animal health at risk. This highlights the importance of human-animal ecosystem interfaces in the evolution of emergence of pathogens (Destoumieux-Garzon et al., 2018). A better knowledge of causes and consequences of certain human activities, lifestyles, and behaviour in ecosystems is crucial for a rigorous interpretation of disease dynamics.

Threats from old and new pathogens continue to emerge as a result of changes in the climatic conditions. Young growing animals on pasture are especially prone to spore forming bacteria of the *Bacillus* and *Clostridium spp*. Livestock acquire the spores

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during grazing on contaminated pastures (Lange et al., 2010; Cooper and Valentine, 2016). Once the bacteria are in abundance on pasture, cases of the disease usually occur frequently in susceptible herds. Affected farms are usually those with irregular vaccination programs as farmers seek to minimise production costs (Maas, 2012). Limited studies focused on the ecology of transmission of livestock diseases (Morand and Figuié, 2016; Morand and Lajaurie, 2017; Cantor et al., 2017). In endemic areas, *Bacillus* and *Clostridium spp* may be present in soil and faeces (Dharmasena and Jiang, 2018). Christianssonn et al. (1999) reported bacterial contaminants of soil particularly *Bacillus cereus* to contaminate teats and udders of pasture fed cows leading to milk contamination and food borne diseases. Over the last few decades, several pathogens have either emerged or re-emerged. Livestock from small scale production systems in Limpopo Province suffer endemic diseases as they are kept under scavenging conditions which expose them to virulent factors.

In most cases, there is little attention to disease control and prevention in these livestock production systems (Gibbs, 2005). The extent of the diversity of microorganisms in soils determines the quality of pastures, since a wide range of pathogenic bacteria are involved in pasture contamination from livestock manure (Bagge et al., 2010). Bacterial species have always been of considerable medical and economic importance and the world is faced with changing landscape of infectious diseases due to pathogenic bacteria that affect man and animals posing significant threats to health and welfare of livestock (King et al., 2006). According to the 'one health', 60% of all human infections are directly or indirectly linked to livestock disease outbreaks (FAO et al., 2007). The documents and publications on the "One Health" approach, and the strategic framework developed around it, have largely focused on the battle against emerging zoonoses originating in domestic (Day, 2011) or wildlife (Dantas-Torres et al., 2012) and/or their interactions (Mencke, 2013). Bagge et al. (2010) reported manure to be the common pasture contaminant resulting in livestock infections. Based on the farm records, diseases and conditions such as blackleg, pulpy kidney, pink eye and mastitis are endemic at the farm. Therefore, identification of the causative agents in the farm is crucial for implementation of prompt therapeutic and control measures (Neumann et al., 2002). Therefore, the objectives of the study were to determine the total bacterial counts from environmental samples collected from the cattle and small stock camps in the farm using the colony forming unit (CFU) formula per gram of environmental sample and to identify the pathogenic bacteria isolated from soil and faecal samples.

Literature review

Arable land and pastures contaminated with the faeces of sick animals contribute considerably to pathogen transfer resulting in farm environment pollution (Trawińska et al., 2006; Nageswaran et al., 2012; Czekalski et al., 2012). Furthermore, the soil and manure in high producing farms provide diverse biological and physicochemical environments to microorganisms which pose a significant threat to humans and animals (Godwin and Moore, 1997; Whitman, 1998; and Douglas et al., 2003). Stecher and Hardt (2008) reported that bacteria in the gastro-intestinal tracts of animals can be excreted at high levels in faeces thereby contaminating soils and pastures. These bacteria may persist due to differences in local soil conditions (Dapilly and Neyrat, 1999; Gale, 2004). Extensive livestock farming poses a threat to the environment, as

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livestock shed millions of bacteria through their faeces and contaminate the soil and pastures thereby elevating the risk of disease breakouts. Nonetheless, disease outbreaks can be prevented through vaccination when local strains are recognised and safeguarding of human and animal health can be ensured (Radostitis et al., 2000; Amin et al., 2013; Manyi-Loh et al., 2016).

Livestock practices influence the microbial composition of faeces shed by animals (Wang et al., 2004; Manyi-Loh et al., 2016). The ability of these pathogens in manure to pollute, contaminate and infect the environment and livestock depends on the pathogen's ability to survive in manure following excretion or the hygiene practices to prevent its entry into the food chain (Wang, 2004). Studies on diversity of spore forming bacteria in cattle manure reported pathogenic bacteria of particular concern for animal health as, *Clostridium spp,* particularly (*C. chauvoei*, *C. botulinum*, *C. tetani*) and *Bacillus spp* (*B. anthracis* and *B. cereus*) (Bagge et al., 2010; Adak et al., 2002*;* Pachepsky et al., 2006; Pell, 1997; Plaut, 2000; Chauret et al., 1999). *Campylobacter spp* (*C. jejuni* and *C. coli*), *Salmonella enterica* (Adak et al., 2002*;* Pachepsky et al., 2006; Pell, 1997; Plaut, 2000), *Yersinia spp* (Y. enterocolitica and Y. pestis), *Leptospira spp* (*L. interrogans*) and *Coxiella burnetii* (Aitken et al., 2005; Nightingale et al., 2004), *Mycobacterium avium* sub species *paratuberculosis, Listeria monocytogenes, Escherichia coli O157* (Robert et al., 2017) are also reported as major threats to animal and human health. These bacterial species can cause serious clinical diseases in farm animals, when they are ingested in feed and have also been implicated in cases of food borne diseases in humans such as *L. mocytogenes* in human listeriosis.

Salihu et al. (2009) reported that *Ca. jejuni* and *Ca. fetus* cause abortion, stillbirths and birth of weak lambs in sheep during late pregnancy. The campylobacter species has highly been associated with feed contamination mostly in poultry as a result of faecal contamination. This alarms the need to identify these species at farm level so as to put hygiene strategies like proper manure management in place to prevent cross contaminations. Moreover, the depletion of medicinal plants due to increased population alarms the need to recognise local pathogen strains so that reliable drugs and vaccines can be produced for proper management of livestock diseases (Nishteswar, 2014). Thus, it is necessary that local pathogen strains be recognised so that vaccines can be developed for better disease management at farm level.

There is ample evidence showing that soil amendments that harbor enteric pathogens, such as raw animal manure or incompletely composted manure, are a means of introducing these pathogens in crop production systems (Kim et al., 2009; Nicholson et al., 2005; Ziemer et al., 2010). Products from mixed farming, including meat, eggs, and fresh produce, are at greater risk of cross-contamination as they are grown in the same facility and are currently considered to be high-risk foods (Adl et al., 2011). The source of contamination of fresh produce with enteric pathogens can frequently be traced back to environmental reservoirs associated with farm animals such as poultry, cattle, swine, goat, and sheep (Brinton et al., 2009; Park et al., 2014).

Materials and methods

Description of the study area

The study was carried out in a livestock farm in Limpopo Province, South Africa, coordinates: 23°49' S; 29°41' E. During the study period, no animal diseases or mortalities were reported. The total number of livestock kept at the farm was 225

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comprising 82 cattle, 77 sheep and 66 goats. The farm practices mixed livestock farming. Extensive livestock production system increases the chances of disease outbreak as livestock will have access to pastures and water supplies that may be contaminated with disease causing agents such as bacteria (McGuirk, 2015). The farm relies on chemoprophylaxis as a disease management tool, however, the practice is irregular. Environmental samples were collected from different resting points within camps in the farm. *Figures 1-4* depict the environment from where samples were collected.

Figure 1. *Collection of soil samples from resting points in the cattle camp in a livestock farm in Limpopo Province*

Figure 2. *Collection of faecal samples from resting points in the cattle camp in a livestock farm in Limpopo Province*

Figure 3. *Livestock water source in the vicinity of resting points from where environmental samples were collected*

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Figure 4. *Collection of faecal pellets from small stock camps*

Collection of environmental samples

Environmental samples (soil and faeces) analysed in this study were collected from the vicinity of sheep and goat housing facilities as well as cattle grazing pastures. Samples are collected over a period of three months, from January to March 2018. The samples were labelled according to the site of collection as cattle camp samples (CCS), sheep camp samples (SHCS) and goat camp samples (GCS). Hundred soil samples comprising of sixty CCS, twenty SHCS and twenty GCS were collected from locations that were perceived as high risk areas for bacterial contamination in a random zigzag pattern. Unpolluted and polluted top soil samples in the vicinity of faecal droppings were collected at different depths. Samples taken from below the soil surface were collected using a soil auger according to the procedure described by Brooks (2016). Seventy faecal samples comprising of thirty CCS, twenty SHCS and twenty GCS were collected. Cattle faecal samples were collected as cowpats on grazing land while sheep and goat faecal samples were collected as pellets. Approximately 100-200 g environmental samples were collected in sterile sealable plastic bags. The samples were then transported on ice, in a Styrofoam cooler box and transported to the University of Limpopo, Biotechnology Laboratory for microbial analysis

Microbial analysis of environmental samples

All the required consumables for the study were procured from Prestige Laboratories (Pty) Ltd, Durban, South Africa. Microbial analyses were performed under sterile conditions in a laminar flow with a Bunsen burner to avoid contamination from the environment. The collected samples were cultured on selective and general media for isolation of pathogenic bacteria.

Reinforced clostridia agar, Reinforced clostridia broth, sheep blood agar, anaerobic basal broth and nutrient agar were prepared and used for detailed investigation of pathogenic bacteria according to the manufacturer's instructions. Environmental samples were serially diluted to reduce the bacterial concentration of the original soil sample to levels low enough for single colonies to be grown on agar plates, allowing for calculation of the initial counts of bacteria in the environmental samples (Koch, 1883).

Purification of bacteria and determination of total counts

Bacterial culture and purification were carried out according to the methodology of Bagge et al. (2009) while colony counts were determined according to Brugger (2012) (*Eq. 1*). Samples cultured on sheep blood agar grew and triggered haemolyses within

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24 h of incubation at 37 °C. The isolates appeared flat and raised with the shape being filamentous to irregular, round and punctiform. They were smooth or wrinkled and the colour ranged from grey, cream to white and with few colonies being yellow (*Figs. 5-7*). Different colonies subcultured on nutrient agar for purification were able to grow under anaerobic conditions within 24 to 36 h of incubation at 37 °C.

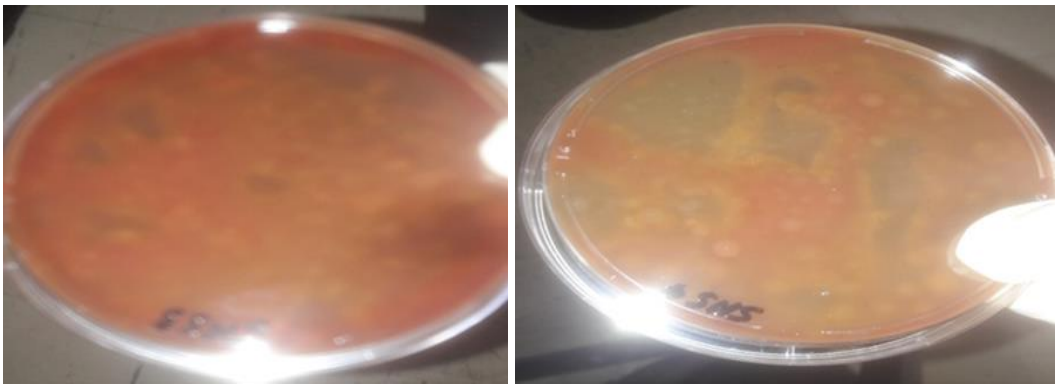

Figure 5. *Plates of colonies cultured on sheep blood agar*

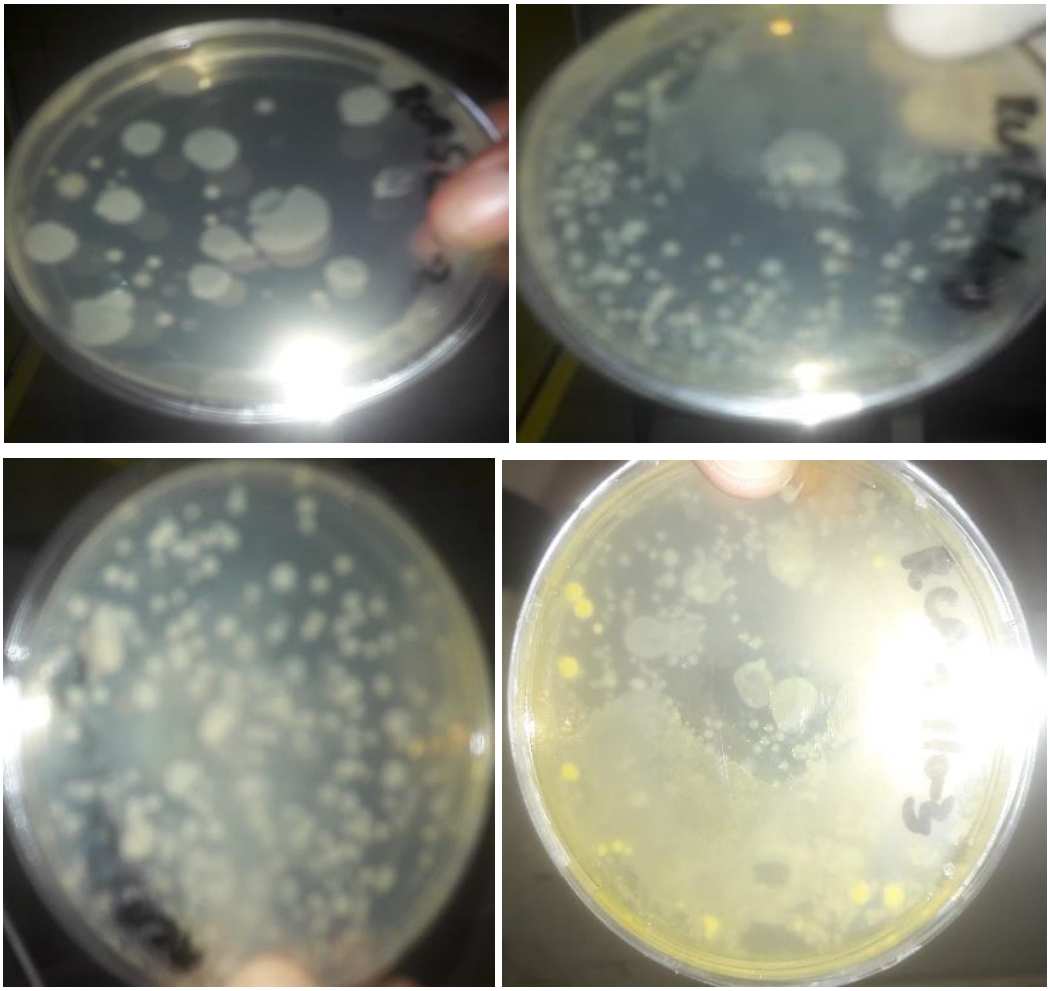

Figure 6. *Plates of colonies cultured on Reinforced Clostridia and nutrient agar*

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Figure 7*. Plates with a four-way streaking of pure cultures on nutrient agar*

Proteomic identification of colonies

All bacterial isolates reported in this study were analysed by MALDI-TOF MS, using a Microflex LT bench top mass spectrometer (Bruker Daltonics, Maldi Biotyper, Bremen, Germany). The software for the control of the instrument was FlexControl 3.3 and Maldi Biotyper 3.1 (Bruker Daltonics) for the analysis of the spectra and comparison with the database. A bacterial test standard provided by the manufacturer was included in every run for calibration purposes. Default settings (acquisition of mass spectra in the linear positive mode within the 2e20 kDa range, ion source 1 (IS1) 20 kV, IS2 18.05 kV, lens 6.0 kV, linear detector 2,560 V) were applied for all the detections. A rapid, on-plate method that requires less time and reagents for its performance was followed in this study (Matsuda et al., 2012).

Data presentation

The numbers of viable bacteria per gram were obtained using a mathematical formula for colony forming units (CFU) per gram of environmental sample. Diversity, occurrence and frequency of isolation of pathogenic bacteria identified in environmental samples using MALDI-TOF MS are presented with descriptive statistics. Microsoft Excel, 2013 was used to compare the level of contamination in faecal and soil samples.

$$\text{CFU} / g = \frac{\text{Number of colonies per agar plate} \times \text{Dilution factor}}{\text{Volume of culture plated}} \qquad \text{(Eq.1)}$$

Results

Colony forming units per gram of environmental sample

Colony forming units were calculated using the formula of Harley (2005) (*Eq. 1*) based on average colony counts per plate. This was done to estimate the number of viable bacteria per gram of environmental sample. All samples were of similar weight (1 g) and exposed to same dilution factors of 10^{-5}, 0.1 ml of bacterial culture were plated per agar plate. Based on these calculations, there were differences in the average colony forming units of different sampling locations. The results indicated that the cattle

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camp had a higher level of contamination as compared to the goat and sheep camps for both soil and faecal samples. The highest counts were observed in samples from the cattle camp and the lowest counts were observed in samples from the sheep camp for both soil and faecal samples (*Figures 5* and *6*).

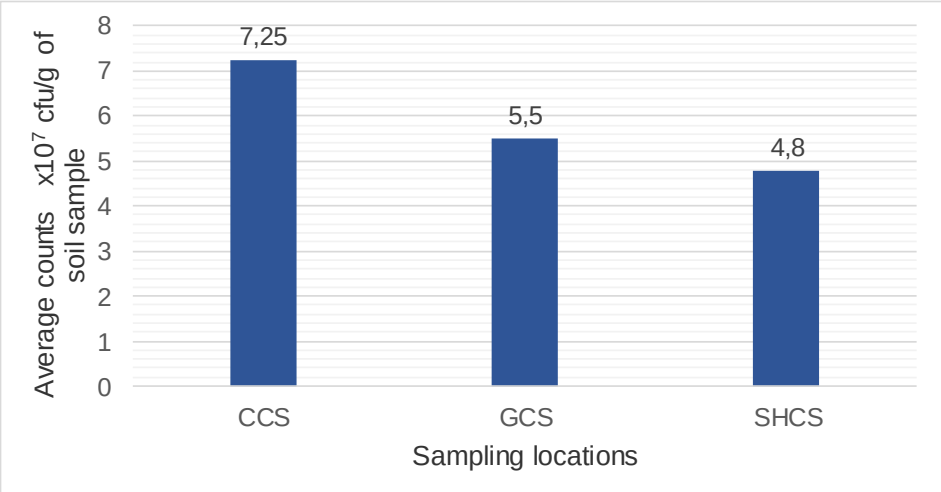

Figure 5. *Average colony forming units (CFU) per gram of soil sample from different camps within the farm*

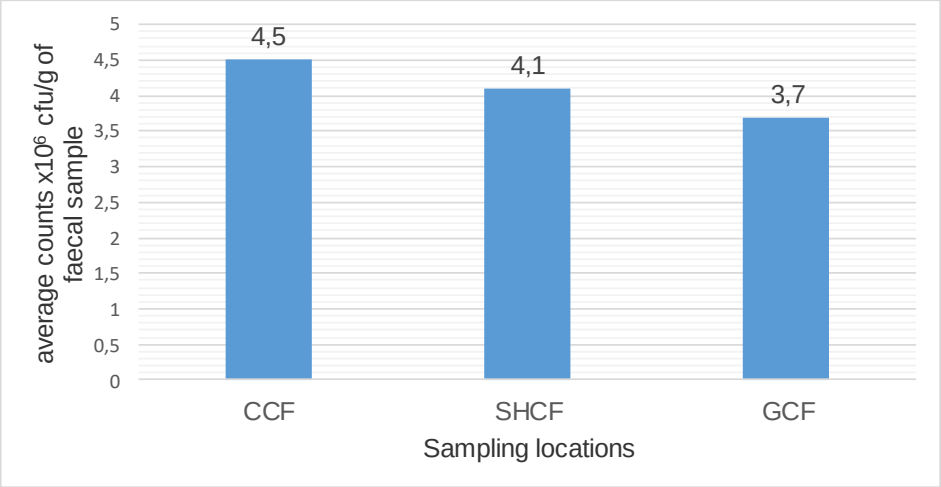

Figure 6. *Average colony forming unit per gram of faecal sample from different camps in the farm*

Diversity of bacterial species identified in soil and faecal samples

During the study period, a total of hundred and seventy environmental samples collected for microbial analysis of pathogenic bacteria were analysed by MALDI-TOF MS using the simplified on-plate method. Hundred and thirty-four (79%) of the samples comprising of 108 soil and 26 faecal samples were successfully analysed and tested positive for different bacterial species. The remaining thirty-six samples (21%) could

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not be identified by the technique used. *Tables 1* and *2* indicate the bacterial isolates identified upto genus/species level for faecal and soil samples respectively. Based on these results, it was observed that different farm environments harbour different bacterial species. However, minor similarities were observed with few bacterial species where *B. cereus* and *E. coli* were isolated in both manure and faecal samples. Using (*Eq. 2*) *Bacillus cereus* were identified as the most dominant species isolated from soil samples in 36 positive samples (33%), followed by *B. mycoides* in 24 positive samples (22%) (*Table 3*). Regarding faecal samples, 26 samples tested positive with *E. coli* being the most prevalent species, identified in 9 positive samples (34.6%), followed by *Acinetobacter baumannii* identified in 8 samples (30.8%), *Acinetobacter baumanii* in 5 samples (19.2%), *B. cereus* and *B. psedomycoides* were the least dominant with (7.7%) each (*Table 4*).

Table 1. *Bacterial species identified from faecal samples by MALDI-TOF MS using the simplified on-plate method (n = 26)*

Bacteria species	No of positive samples	Cut off values proposed for this study			
		≥3	2.00-2.999	1.699-1.999	0.00-1.599
*Escherichia coli**	9	-	8	1	-
*Bacillus cereus**	2	-	-	2	-
Bacillus pseudomycoides	2	-	-	-	2
Acinetobacter genomospecies	8	-	2	6	-
Acinetobacter baumanii	5	-	4	1	-
Total	**26**	**0**	**14**	**10**	**2**

*Pathogenic bacteria

Table 2. *Bacterial species identified from soil samples by MALDI-TOF MS using the simplified on-plate method (n = 108)*

		Cut off values proposed for this study			
Bacteria species	**No of positive Samples**	**≥3**	**2.00-2.99**	**1.6-1.99**	**0.00-1.599**
*Bacillus cereus**	36	-	6	30	-
*Listeria monocytogens**	2	-	-	-	2
Lysinibacillus fusiformis	4	-	-	2	2
Bacillus megaterium	1	-	-	1	-
*Staphylococcus aureus**	1	-	-	1	-
Enterobacter cloacae	4	-	-	3	1
Enterobacter cancerogenus	1	-	-	-	1
Bacillus simplex	1	-	-	1	-
Enterobacter absburiae	2	-	-	2	-
Bacillus mycoids	24	-	-	20	4
Bacillus weihenstephanesis	12	-	-	8	4
*Escherichia coli**	4	-	3	1	-
*Seratia marcescens**	5	-	2	2	1
*Bacillus licheniformis**	4	-	-	3	1
Bacillus endophyticus	1	-	-	1	-
Pseudomonas resinovarans	1	-	-	-	1
*Pseudomonas aeruginosa**	2	-	-	2	-
Pseudomonas monteilii	2	-	-	1	1
Pseudomonas corrugate	1	-	-	1	-
Total	**108**	**0**	**11**	**79**	**18**

*Pathogenic bacteria

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Table 3. *Occurrence and percentage frequency of bacteria from soil of different camps in the farm (n = 108)*

Bacterial species	No. of positive samples in the farm	% frequency in the farm	Occurrence			No of positive samples in the camp and % frequency					
			CCS	SHCS	GCS	CCS		SHCS		GCS	
						$^{+}$NSC	%FC	$^{+}$NSC	%FC	$^{+}$NSC	%FC
*Bacillus cereus**	36	33.3	+	+	+	20	28	6	50	10	41.6
*Listeria monocytogenes**	2	1.9	+	-	-	2	2.8	0	0	0	0
Lysinibacillus fusiformis	4	3.7	-	+	+	0	0	1	8.3	3	12.5
Bacillus megaterium	1	0.9	+	-	-	1	1.4	0	0	0	0
*Staphylococcus aureus**	1	0.9	+	-	-	1	1.4	0	0	0	0
Enterobacter cloacae	4	3.7	+	-	-	4	5.6	0	0	0	0
Enterobacter cancerogenus	1	0.9	+	-	-	1	1.4	0	0	0	0
Bacillus simplex	1	0.9	+	-	-	1	1.4	0	0	0	0
Enterobacter absburiae	2	1.9	+	-	-	2	2.8	0	0	0	0
Bacillus mycoides	24	22.2	+	-	-	24	33	0	0	0	0
Bacillus weihenstephanesis	12	11.1	+	-	-	12	17	0	0	0	0
*Escherichia coli**	4	3.7	+	-	-	4	5.6	0	0	0	0
*Serratia marcescens**	5	4.6	-	-	+	0	0	0	0	5	20.8
*Bacillus licheniformis**	4	3.7	-	+	-	0	0	4	33.3	0	0
Bacillus endophyticus	1	0.9	-	-	+	0	0	0	0	1	4.2
Pseudomonas resinovarans	1	0.9	-	+	-	0	0	1	8.3	0	0
*Pseudomonas aeruginosa**	2	1.9	-	-	+	0	0	0	0	2	8.3
Pseudomonas monteilii	2	1.9	-	-	+	0	0	0	0	2	8.3
Pseudomonas corrugate	1	0.9	-	-	+	0	0	0	0	1	4.2
Total	**108**	**100**				**72**	**100**	**12**	**100**	**24**	**100**

CCS = Cattle camp soils; SHCS = Sheep camp soils; GCS = Goat camp soils; $^{+}$NSC = Number of positive samples in camp; % FC = Percentage frequency in the camp; *Pathogenic bacteria; + = present; - = absent

Table 4. *Occurrence and percentage frequency of bacteria from faecal samples of different camps in the farm (n = 26)*

Bacteria	No. of positive samples in the farm	% frequency in the farm	Occurrence			Number of positive samples in the camp and % frequency					
			CCF	SHCF	GCF	CCF		SHCF		GCF	
						$^{+}$NSC	% FC	$^{+}$NSC	% FC	$^{+}$NSC	% FC
*Escherichia coli**	9	34.6	+	-	-	9	64.2	0	0	0	0
*Bacillus cereus**	2	7.7	+	-	-	2	14.3	0	0	0	0
Bacillus psedomycoides	2	7.7	+	-	-	2	14.3	0	0	0	0
Acinetobacter baumannii	8	30.8	+	+	+	1	7.1	3	100	4	44.4
Acinetobacter genomospecies	5	19.2	-	-	+	0	0	0	0	5	55.6
Total	**26**	**100**				**14**	**100**	**3**	**100**	**9**	**100**

CCF = Cattle camp faeces; SHCF = Sheep camp faeces; GCF = Goat camp faeces; + = Present; - = Absent; *Pathogenic bacteria; $^{+}$NSC = Number of positive samples in the camp; % FC = Percentage frequency in the camp

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In the present study, the levels of environment contamination by pathogenic bacteria varied with respect to sampling location. However, there were some similarities with respect to the bacterial isolates prevailing across sampling locations (*Tables 3* and *4*).

Percentage frequencies in *Tables 3* and *4* were calculated using *Equation 2:*

$$\%Frequency = \frac{\text{Number of samples from a camp positive for a bacterial species}}{\text{Total number of positive samples from the farm/camp}} \times 100 \quad \text{(Eq.2)}$$

Discussion

This study utilised MALDI-TOF MS to identify bacterial isolates from environmental samples. The results indicated that 134 (79%) environmental samples out of the 170 collected tested positive for various bacterial species with MALDI-TOF MS identification. Urwlyer and Glaubitz (2015) reported MALDI-TOF MS to have a revolutionized speed and precision of microbial for clinical isolates to outperform conventional methods. This is evidenced in their study were MALDI-TOF MS showed the lowest number of false identification (4%) and 60% accuracy at genus level. In contrast, the biochemical-based system assigned 25% of genera incorrectly. In this study, MALDI-TOF MS provided identification at the genus level of 67% of the bacterial isolates. This agrees with Dupont et al. (2010), Justeen et al. (2011), Nagy et al. (2012), Wieser et al. (2012), Lee et al. (2015) and Florio et al. (2018) who reported the capacity of MALDI-TOF MS to identify bacterial microorganisms at the genus and species level according to the cut of values in this study (score value 2.000 - 2.299) ranging from 65.2 to 83.9%. Average colony forming units in both soil and faecal samples fell within the range reported by previous researchers for environmental samples (Okoh et al., 1999; Ogunmwonyi et al., 2008). Total bacterial counts were relatively higher in soil samples suggesting high contaminations as compared to faecal samples.

These results agree with the reports of Trawińska et al. (2006) who indicated that soil in the vicinity of high-production farms is commonly microbial-contaminated as arable land and pastures contaminated with the faeces of sick animals, especially, contribute considerably to pathogen transfer into the soil. Boes et al. (2005) and Ngole et al. (2006) reported that micro-organisms survival in the soil environment is favoured by high temperature and moisture. Oliver et al. (2006) stated that rainfall events result in faecal microbes being washed from the cowpats into the surrounding soils where their survival could be enhanced. This finding is further supported by the results of Muirhead (2009) who reported an increase in soil *E. coli* concentrations on the grazed camps which they believed coincided with an increase in rainfall during their study period. Furthermore, Kress and Gifford (1984) and Stoddard et al. (1998) reported that heavy rain or irrigation on a fresh cow pat is likely to result in far greater microbial mobilization and leaching. Mobilization and leaching of bacteria during rainfall could be the reason for high percentage prevalence of bacteria species in soil compared to faecal samples recorded in the current study. Bacteria leach with water into the soil, where they incubate and multiply resulting in contamination of pastures were animals graze (Kress and Gifford, 1984).

In the present study, *Bacillus species* was identified to be the most prevalent species in soil samples. This is in agreement with Gutiérrez- Mañero et al. (2003) and Amin (2015), who reported bacillus as naturally occurring soil bacteria and most abundant

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genus in the rhizosphere of soil. From this genus, the most dominant species appeared to be *B. cereus,* which when allowed an opportunity to invade mammalian tissues is an opportunistic pathogen that may cause severe or local systematic infections such as endophthalmitis and septicaemia (Kotiranta et al., 2000). *B. cereus* has been reported to cause serious clinical diseases in farm animals, thereby causing economic loses to farmers. Songer and Post (2005), Nieminen et al. (2007) and Salih (2015) reported *B. spp* to occasionally cause mastitis in cattle. Furthermore, *B. cereus* has been reported as an important food borne pathogen. Tewari and Abdullah (2015) reported highly toxic strains of *B. cereus* responsible for food-related fatalities. Ranieri et al. (2009) and Gundogan and Avci (2014) reported *Bacillus spp* to be ubiquitous in nature. The ability of its spores to tolerate different environmental conditions including elevated temperatures result in these species being the most common isolated bacteria from food, air, soil, and faecal samples. However, the reports of Bavykin et al. (2004) indicating that bacillus is the most common genus in faecal samples are in contrast with the findings of this study as these bacteria tested positive only in faecal samples of cattle with a frequency occurrence of 6.6%.

In faecal samples, *E. coli* was the most frequently isolated pathogen. The dominance of *E. coli* in faecal samples reported in our study agrees with reports by previous researchers (Tahamtan et al., 2006; Raji et al., 2006; Hiko et al., 2008; Hashemi et al., 2010). Hogan et al. (1999) and Abakpa et al. (2015) reported *E. coli* to be naturally present in faeces of warm blooded animals. *E. coli* occurrence has been previously used as an indicator of faecal contamination, signaling the possible presence of faecal pathogens such as *Salmonella* and *Shigella* species (Odonkor and Addo, 2018). In a study by Rodrigues et al. (2015), MALDI TOF-MS identified 83% *E. coli* prevalence in faecal samples, however, the pathogenicity of the strains were not reported. Schmidt et al. (2015) reported *E. coli* to be free-living commensals in animal intestines. The prevalence of this bacterial species was found to be high in the cattle camp in comparison with the small stock camps. Sima et al. (2009), Kiranmayi et al. (2010) and Rahimi et al. (2012) reported domestic animals as the sources of *E. coli*. However, the major animal carriers seem to be healthy domesticated ruminants, primarily cattle and, to a lesser extent sheep.

These findings agree with the results of our study as *E. coli* were only identified in cattle faeces with zero prevalence in sheep and goat faecal samples. However, large variations have been described in the shedding patterns of individual animals, the proportion of shedding animals on farms that harbor them and, over time, in the amount of shedding on the same farm (Smith et al., 2010). Among cattle, shedding occurs intermittently (Hancock et al., 1997; Kulow et al., 2012; Sharma et al., 2012), and it was reported that, at any time, up to 50% of the healthy animals excrete *E. coli* in their stool (Lim et al., 2010). Freshly deposited faeces contain the nutrients required by bacteria, and replication presumably depends on the faeces retaining water and attaining suitable temperatures for growth. Cow pats are able to retain moisture when exposed to sunlight. The pats quickly form a skin, which thickens to a well-defined crust within 48 h favouring bacterial growth (Van Kessel et al., 2007). However, exposure of sheep and goat pellets to the sun results in drying of the pellets due to size and less moisture contained making the environment unfavourable for bacterial growth. Topp et al. (2003) studied the relationship between soil moisture content and prevalence of *E. coli* bacteria and concluded that elevated soil moisture levels were associated with increased number of bacteria in the soil. This could explain the low counts of *E. coli* in soil samples of the

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current study as soil samples were collected in early summer where moisture content in soil is believed to be relatively low. Of the twenty-two bacterial isolates isolated from the environmental samples in this study, some species have been reported previously to carry virulent strains which are responsible for livestock infections particularly in cattle, sheep and goats. These pathogenic bacteria include: *E. coli* (Olson, 2001; Chekabab et al., 2013), *B. cereus* (Nieminen, 2007; Manyi-Loh, 2016; Robert et al., 2017), *L. monocytogens* (Nightingale et al., 2004; Nicholson et al., 2005), *Staphylococcus aereus* (Toroitich, 2013), *Pseudomonas aeruginosa* (Radostits et al., 2000), *Seratia marcescens* and *B. licheniformis* (Olson, 2001).

Pathogenic bacteria in farm environments pose a major epidemiological threat (Amin et al., 2013). Although *E. coli* have been reported as harmless commensals of the intestines of warm blooded animals, the strain *E. coli* O157:H7 has been reported to be harmful (Titilawo et al., 2015). Shearer et al. (2003) reported *E. coli* as one of the major bacterial pathogens associated with livestock infections in farms. Healthy colonized cattle and other ruminants are the most significant animal reservoir harboring *E. coli* (Ferens and Hovde, 2011). Previous studies linked approximately 75% of the human *E. coli* outbreaks to food products of bovine origin (Callaway et al., 2009; Munns et al., 2016). Other reservoirs that may impact transmission include sheep (La Ragione et al., 2012; Soderlund et al., 2012; Gencay, 2014), goats Pao et al.(2005), La Ragione et al.(2009), Mersha et al.(2010), Alvarez-Suarez et al.(2016) and Swift et al.(2017).

According to Tomita and Hart (2001) *E coli* bacteria possess several pathogenic factors responsible for their pathogenicity, among which exotoxin A (*tox*A) and exoenzyme S (*exo*S) are the two major fatal toxins which are associated with subclinical mastitis infection in bovines (Toroitich, 2013) in a cross-sectional study to determine the prevalence of mastitis and identify the associated risk factors reported the most predominantly isolated bacterium to be *Staphylococcus aureus* with a prevalence of 36% followed by *E. coli* with a prevalence of 27.2% and *Pseudomonas* were least isolated with less than 1% prevalence.

In the present study, *L. monocytogenes* tested positive in cattle camp soils (4.4%). This falls in the same range as the findings of Mohammed et al. (2009) (5.4%) and less compared to the findings from previous studies by Moshtaghi et al. (2003) (17.7%) and Nightingale et al. (2004) (22.2%) and Locatelli et al. (2013) (38.1%). Several animal derived *L. monocytogenes*-contaminated food products, including raw milk, pasteurized milk, chocolate milk, butter, soft cheeses, and processed meat and poultry products, have been implicated as sources of human listeriosis cases and outbreaks (Buchanan et al., 2017). It has been reported that manure from infected or shedding animals represent direct links between human infections and *L. monocytogenes* in farm animals and farm environments as a result of consuming animal-derived food products that are not processed before consumption such as raw milk and raw foods of plant origin (Nightingale et al., 2004).

Previous studies reported *Campylobacter* species and *Clostridium* species to be pathogenic bacteria of livestock commonly isolated from environmental samples (Baserisalehi et al., 2007; Bagge, 2009). However, in the present study, all samples tested negative for *Campylobacter* species. *Clostridium species* were detected in 2 samples (5%). Bandelj et al. (2016) reported a 10% prevalence of *clostridium difficile* in cattle faecal samples. The use of selective media may have disadvantaged growth of other bacteria species in the samples. Olson (2001), reported the *Campylobacte*r genus to have fastidious growth requirements making conventional detection and

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identification difficult. This could be the reason why all samples in the present study tested negative for *Campylobacter species*. The differences in bacterial contamination in the sampling locations in this study may be due to different management practices in the livestock camps were cattle are kept in their camp continuously resulting in piling of faecal samples increasing microbial habitat. In comparison with sheep and goats which are housed at night resulting in few fresh pellets in their camps. Spiehs and Goyal (2007), Hutchison et al. (2005) and Manyi-Loh et al. (2016) indicated that the levels and types of pathogens occurring in livestock faeces vary with animal species, dietary sources, health status and age of the animal.

Conclusions and recommendations

This study showed that diverse bacterial species contaminate the livestock grazing environment at the study farm. Although samples were collected from different locations within the farm, the bacteria isolated from soil samples were generally similar. However, with regard to faecal samples, bacterial isolates differed from one camp to the other entailing that different livestock harbour different bacteria in their faeces. Of the bacterial species isolated in the study, some genus have been reported to be highly pathogenic. This study demostrated contamination by opportunistic, food-borne bacteria like *B. cereus* and *L. monocytogenes* in the farm environment and the need for good hygiene practices to prevent its entry into the food chain. Rapid microbial identification is necessary for quick implementation of relevant disease management strategies at farms. The findings of this study show that MALDI-TO MS can identify bacteria rapidly in environmental samples. This method can be used to identify potential disease risk in an environment and allow for appropriate control measures.

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