# The risk of carriage of Salmonella spp. and Listeria monocytogenes in food animals in dynamic populations

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#### Abstract

Salmonella spp. and Listeria monocytogenes are foodborne pathogens of global importance. We assessed their risks and associated factors in a highly dynamic population of animals. Animal and environmental samples were collected from dairy cattle, sheep, camel and chickens at either the farms or the abattoirs. The pathogens were detected using a combination of bacterial enrichment culture and real-time polymerase chain reaction (PCR). Data on putative risk factors were collect and analysed for their significance of association with these pathogens.

Salmonella spp. were detected at higher proportions in sheep faeces and sheep carcasses in comparison to cattle faeces (odds ratio = 2.4 and 2.2, respectively). This pathogen was less common in milk or carcasses samples from cattle or chickens. Sheep and camel carcass samples were highly contaminated with Salmonella spp. Faecal samples from cattle had the most diverse serovars of Salmonella enterica including S. Newport, S. Haifa, S. Kedougou, S. Kentucky, S. Mbandaka and S. Goettingen. Exotic serovars in sheep included S. Eastbourne, S. Chester and S. Kottnus. Serovars that were shed in camel faeces included S. Newport, S. Bovismorbificans and S. Infantis. In all sampled populations, detection of Salmonella spp. was more likely during warmer months than cold months. Listeria monocytogenes was not common in the targeted populations and was detected at a rate of 2.4%, mainly from sheep carcasses. The study highlights the role of food animals as reservoirs of pathogens across boundaries since all feed are imported in that population from different parts of the world.

Keywords: Foodborne, foodborne pathogens, Listeria monocytogenes, preharvest, Salmonella spp..

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## Introduction

The development of cost-effective strategies for riskbased food depends largely on identification of specific hazards and factors that lead to their introduction and perpetuation on particular food matrices (Batz et al. 2012). Salmonella spp. and Listeria monocytogenes are among the high-ranked foodborne pathogens that predispose humans to severe health sequelae (Batz et al. 2012; Havelaar et al. 2012). Although the rates of contamination of food products by these two pathogens are highly variable,

different estimates of burden of disease consistently indicate a high cost per episode (Hoffmann et al. 2012; Havelaar et al. 2012). The risk of presence of foodborne pathogens is worsened by globalization of trade and ease of international travel (Kendall et al. 2012; Tighe et al. 2012). Because of the need to import animals, feed and food products from around the world and high levels of population turnover, the state of Qatar is a country where these factors intersect.

Food animals and their products are known to be reservoirs for Salmonella spp. and hence pose a

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constant risk of gastroenteritis (de Knegt *et al.* 2015; Gorski *et al.* 2011; Wahlstrom *et al.* 2011). Perpetuation of the pathogen in the environment may exacerbate the risk of salmonellosis by direct exposure of humans to the pathogen through occupational practices, recreational activities or cross-contamination between meat handlers and carcasses in processing plants (Gomes-Neves *et al.* 2012; Moller-Stray *et al.* 2012). Knowledge of the occurrence and distribution of *Salmonella* spp. in these populations is needed to devise cost-effective strategies to mitigate associated risks.

*Listeria monocytogenes* has been incriminated in several food outbreaks of gastroenteritis, meningoencephalitis and/or abortion in various countries (Danielsson-Tham *et al.* 2004; Gillespie *et al.* 2010). *Listeria monocytogenes* has been associated with major economic losses and consistently listed among the most important foodborne pathogens around the world (Batz *et al.* 2012; Havelaar *et al.* 2012; Kamleh *et al.* 2012; Doublet *et al.* 2008).

The current study is part of our long-term objective in developing valid risk-assessment models for foodborne pathogens in hope of making sciencebased recommendations to mitigate their healthadverse consequences. The focus is on *Salmonella* spp. and *L. monocytogenes* in food animals and their products in Qatar – a country inhabited by a highly diverse and dynamic population. We assessed the threat to the food-supply system posed by these bacteria and investigated factors that contributed to their perpetuation in the targeted populations.

# Material and methods

## Target and study populations

The target population consisted of food animals (cattle, sheep, camel and chicken) either at their production sites or when entering the food-supply chain, that is, at the abattoir. The dairy and camel farms were privately owned and located throughout the country. Only dairy that had over 100 animals and camel farms had over 30 she-camels were enrolled. Chicken and sheep carcasses were sampled at the processing plant or abattoir, after final wash and before entering the food chain. Letters of solicitation were sent to all owners of the farms and to the managers of the abattoirs explaining the objectives of the study and requesting their participation. Animals were sampled both in hot (April–October) and cold (November–March) months of the year to capture potential seasonal variations in shedding of pathogens.

#### Sample collection

Individual animal and environmental samples were collected from lactating cows and camels on the selected farms. Each herd was visited once, and relevant farm and herd data were recorded in a questionnaire. Faecal samples (100 g/animal) were collected per rectum and stored in sterile plastic vials with caps. Composite milk samples (100 mL/animal) were collected from each animal and stored in sterile vials. Each individual animal's teat and udder were swabbed with sterile gauze  $(4 \times 4 \text{ inches})$ , without previous disinfection, and the gauze swab was placed in vials. Approximately 100 g of bedding were collected from various locations in the pens and placed into sterile Fisher brand bags. Water and feed troughs in the pens corresponding to sampled animals were swabbed with sterile gauze  $(4 \times 4 \text{ inches})$ and placed individually in the vials. All of the samples were transported in iceboxes to Weill Cornell Medical College at Qatar (WCMC-Q) microbiology laboratory for processing.

Approximately 50 g collected from the large intestine of slaughtered sheep into the vials as described above. Four swabs were collected from each carcass including sheep, cattle, camel and chickens after dressing, washing and inspection by the authority. In sheep carcasses, exterior parts, left and right, including the breast, thorax lateral, brisket, flank and rump area were sampled. In cattle and camels both left and right parts of the neck, brisket, flank and rump area were sampled. In addition, the interior parts of these sides were also sampled. Chicken swabs were collected two from outside the carcass and two from inside after the final wash and before packing. The swabs included neck, under the wings, breast, thigh and legs and the visceral cavity.

#### Sample processing and detection

#### Salmonella spp.

Samples were screened for the presence of the organism using the BAX<sup>®</sup> System (http://www2. dupont.com/Qualicon/en\_US/products/BAX\_System /bax\_salmonella\_testing.htm). The samples were inculcated into primary enrichment medium according to the manufacturer's protocol. The primary enrichment medium – buffered peptone water inoculated with 4 mg of novobiocin per 1 L – was inoculated with the samples at a ratio of 1:10. The inoculum was then incubated at 37°C for 24 h and 20  $\mu$ L samples were then transferred to 1 mL of brain–heart infusion (BHI); secondary enrichment medium, without the antibiotic, was incubated for 24 h at 37°C before performing the real-time PCR assay.

#### PCR assay detection

Salmonella spp. was detected using the BAX<sup>®</sup> System. Five  $\mu L$  aliquots of the secondary BHI enrichment medium were each added to 200  $\mu$ L aliquots of the prepared lysis reagent provided by the manufacturer. Samples were then heated at 37°C and 95°C in a lysis reagent solution to rupture the bacterial cell walls. PCR tablets, which contain all the reagents necessary for PCR plus fluorescent dye specific for each target, were each hydrated with 50  $\mu$ L of the lysed sample and processed in the cycler/detector [AB 7500 Fast Real-Time PCR (Applied Biosystems, Foster City, CA) with BAX® System interface]. Results were displayed on a monitor screen as positive or negative. A table of results that includes cfu/mL values for Salmonella spp. was also displayed, along with graphs of the amplification curves.

#### Serotyping of Salmonella spp.

Salmonella spp.-positive samples were streaked onto XLT4 agar plates (MOLTOX<sup>®</sup>, Boone, NC) and single colonies were picked and inoculated into TTB broth (Becton, Dickinson and Company, Sparks, MD). The broth was incubated for 24–48 h before being shipped to the National Veterinary Services

Laboratory, USDA, Ames, Iowa, for serotyping. A proportional sampling scheme, proportional to the number of isolates recovered from each sample type, was adopted to identify samples to be sent for serotyping. The sampling was adopted because of cost consideration.

#### Listeria monocytogenes

All of the samples were processed according to the BAX System protocol (http://www2.dupont.com/ Qualicon/en\_US/products/BAX\_System/bax\_listeria) for the detection of *L. monocytogenes*. The samples were pre-enriched with Demi-Fraser broth (Oxoid, Hampshire, England) and incubated for 22–26 h at 30°C. The MOPS-buffered Listeria enrichment broth – BBL Listeria enrichment broth, MOPS free acid and MOPS sodium salt (Fisher Scientific, Pittsburgh, PA) – was used as a selective medium in which the samples were incubated at 35°C for 18–24 h. After enrichment, the samples were heated in a lysis reagent solution. The PCR tablets were hydrated with the lysed samples and processed in the automated cycler/detector as described above.

#### **Data collection**

Data on factors hypothesized to associate with the likelihood of the presence of these pathogens in the samples were collected. Data on the herd, processing plant and individual animals sampled in this study were collected by personal interview of the farm/ abattoir manger and by observations. Herd data included the number of animals, breed and health and vaccination programs. Information on the number and type of animals processed, reasons for culling and common diseases at the abattoir was also collected from the chief veterinarian.

#### Statistical analysis

Descriptive statistics including measures of central tendency and dispersion were performed using the SPSS version 20.0 (IBM Statistical System, New York, NY). The prevalence of *Salmonella* spp. and *L. monocytogenes* in each subpopulation was

computed as the proportion of samples that were positive among all of the samples that were tested for the specific pathogen. Composite milk samples, udder and teat swabs and carcass swabs from each animal are pooled and cultured together. The 95% confidence interval (CI) for zero events was calculated using the Wilson score interval with adjustment for continuity (Wilson 1927). The significance of association between each factor and the likelihood of any serotype species in particular samples was evaluated using the logistic regression analysis and quantified by the odds ratio (OR). All significance of associations were considered at Type I error protection of <5% ( $\alpha$  < 0.05).

# Results

#### Salmonella spp.

A total of 1128 samples were collected from the targeted populations (Table 1). The overall prevalence of *Salmonella* spp. was 14.2% (95% CI = 12.2, 16.4). It was most common in faecal samples collected from sheep carcasses and sheep faeces (26.7% and 22.5%, respectively) and was detected at different rates in all faecal samples collected from food animals (Table 1). Detection of *Salmonella* spp. in faecal samples was twice as likely from sheep as from cows (odds ratio = 2.4, Table 1). Although the rate of detection of *Salmonella* spp. in faecal samples collected from camels was higher than that from cows, the difference was not significant (Table 1).

Although cattle shed *Salmonella* spp. in facces at relatively high rates, the organism was not common in milk samples or udder swabs from the same cow (95% CI = 2.3–9.3 and 0–7.6, respectively) (Table 1). *Salmonella* spp. was prevalent in cattle farms and the rate of detection was not significantly different among different types of samples (facces, udder swabs and milk).

Although camel faecal shedding rate of *Salmonella* spp. was higher than that in cows there was no significant difference in the rates of detection between the two types of animal (Tables 1). *Salmonella* spp. detection rate was higher in faecal in comparison to milk samples collected from the

Table I. The occurrence of Salmonella spp. and Listeria monocyto	-
genes in samples collected from various sources in Qatar.	

Type of sample	Salmonella spp. (n) 95% confidence interval	<i>Listeria</i> <i>monocytogenes</i> ( <i>n</i> ) 95% confidence interval
Cattle faecal samples	11% (172)	0% (172)
· · · · · · · · · · · · · · · · · · ·	6.0-15.7%	0-2.7%
Cattle milk samples	5.8% (173)	0% (173)
1	2.3–9.3%	0-2.7%
Cattle udder swab	3.2% (62)	0% (62)
	0–7.6%	0–7.3%
Cattle bedding	16.7% (12)	0% (12)
C	0-41.9%	0–27%
Water troughs	8.3% (12)	0%(12)
0	0–28.1%	0-30.1%
Feed trough	0%(11)	9%(11)
-	0-32.1%	1-42.9%
Sheep faecal samples	22.5% (142)	0% (142)
	15.7-29.4%	00-3.3%
Camel faecal samples	16% (50)	4.0% (50)
-	5.8-26.2%	0-9.4%
Camel milk samples	0 (15)	0 (15)
	0-25.3%*	0-25.3%
Sheep carcass swabs	26.7% (300)	8.0% (300)
	21.4-31.8%	4.9-11.1%
Cattle carcass swabs	4.0% (25)	0% (25)
	0-13.7%	0-16.6%
Camel carcass swabs	50% (10)	0% (10)
	14.0-86.0%	0-34.5%
Chicken carcass swabs	0% (120)	0% (120)
	0-3.9%	0-3.9%

\*Wilson score with correction for continuity.

same camel; however, there was no significant difference in the likelihood of the organism in the two types of samples.

There was no significant difference in the detection rate of *Salmonella* spp. between faecal and carcass swab samples from sheep at the abattoir (Table 1). The organism was more common in sheep carcasses in comparison to cattle (Table 1). There was no significant difference in the likelihood of the pathogens in samples collected from sheep in comparison to camel.

#### Serotypes of Salmonella spp.

A total of 104 isolates were sent for serotyping out of 159 *Salmonella* spp. recovered, of which 70 were serotyped (Table 2). The most common serovar was

Type of <i>Salmonella</i> (number of samples)	Cattle faeces	Cattle milk (%)	Udder swab	Prevalence (95% confidence interval)					
				Cattle bedding	Cattle swab	Sheep faeces	Sheep swab	Camel faeces	Camel swab (%)
S. Typhimurium (43)	2% (1)	0	0	0%	2% (1)	12% (5)	84% (36)	0%	0
S. Adelaide (1)	100% (1)	0	0	0%	0%	0%	0%	0%	0
S. Goettingen (1)	100% (1)	0	0	0%	0%	0%	0%	0%	0
S. Haifa (2)	100% (2)	0	0	0%	0%	0%	0%	0%	0
S. Jangwani (5)	100% (5)	0	0	0%	0%	0%	0%	0%	0
S. Newport (5)	40% (2)	0	0	20% (1)	0%	20% (1)	0%	20% (1)	0
S. Kedougou (2)	50% (1)	0	0	50%(1)	0%	0%	0%	0%	0
S. Mbandaka (1)	100% (1)	0	0	0%	0%	0%	0%	0%	0
S. Bovismorbificans (1)	0%	0	0	0%	0%	0%	0%	100% (1)	0
S. Kentucky (2)	100% (2)	0	0	0%	0%	0%	0%	0%	0
S. Eastbourne (1)	0%	0	0	0%	0%	100% (1)	0%	0%	0
S. Chester (2)	0%	0	0	0%	0%	0%	100% (2)	0%	0
S. Kottbus (1)	0%	0	0	0%	0%	0%	100% (1)	0%	0
S. Infantis (2)	0%	0	0	0%	0%	0%	0%	100% (3)	0
Total (70)	16	0	0	2	1	7	39	5	0

Table 2. The occurrence of Salmonella serotypes in samples collected from dairy operations, camel farms and an abattoir in Qatar

S. Typhimurium, which was identified in 61% of the samples. Salmonella Typhimurium was identified in faecal samples from cattle and sheep and from swabs from cattle and sheep. Serovars recovered from cattle faeces had the most diverse population of which S. Jangwani was most common (Table 2). Both S. Newport and S. Kedougou were isolated from animal faeces and bedding on the same farm.

Salmonella Typhimurium, S. Newport and S. Eastbourne were common in sheep faeces. Serovars detected in sheep carcasses included S. Typhimurium, S. Chester and S. Kottbus (Table 2). Three different *Salmonella* serovars were identified in faecal samples collected from camel farms: S. Newport, S. Bovismorbificans, S. Infantis. None of the five isolates of *Salmonella* spp. from camel carcasses was successfully serotyped.

#### Putative risk factors for Salmonella spp.

There was significant association between the season of the year and the likelihood of detecting *Salmonella* spp. was higher in the hot months (Table 3). We grouped the samples by the species of the animal from which the samples had been collected (cattle, sheep, camels or chickens). It was twice likely to detect the organism in samples collected from sheep **Table 3.** Risk factors associated with the likelihood of detecting *Sal-monella* spp. or *L. monocytogenes* in the study population as analysed using logistic regression

Type of sample	Regression coefficient	Standard error	Odds ratio (95% CI)
Salmonella spp.			
Season			
Hot months	0		1.0
Cold months	-0.431	0.175	0.7 (0.5, 0.9)
Constant	-1.613	0.110	
Animal species			
Cattle	0		
Sheep	0.744	0.370	2.1 (1.0, 4.3)
Camel	1.458	0.206	4.3 (2.9, 6.4)
Constant	-2.536	0.176	
Listeria monocyt	ogenes		
Animal species			
Cattle	0		1.0
Sheep	3.285	1.022	26.7 (3.6, >50.0)
Camel	1.785	1.420	6.0 (0.4, >50.0)
Constant	-6.116	1.001	

than from cattle (Tables 3). *Salmonella* spp. was more common in samples collected from camel in comparison to cattle samples (OR = 4.3).

#### Listeria monocytogenes

*Listeria monocytogenes* was detected in a relatively small number of the samples (2.4%). Swab samples

collected from sheep carcasses at the abattoir had the highest rate (8%) (Table 1). The organism was detected in 4% of the samples from camel faeces and 9% of feed troughs (Table 1). Since most of the samples yielded zero results for *L. monocytogenes* and we had different numbers of samples from each source, we computed the 95% CI for the estimates (Table 1). The upper limit of the interval estimate ranged from 2.7% in cattle faecal samples to 42.9% in samples collected from water troughs on dairy farms.

#### Putative risk factors for L. monocytogenes

All of the positive samples for *L. monocytogenes* were detected in the hot months of the year. It was more likely to detect the organism in samples collected from sheep in comparison to cattle (OR = 26.7).

## Discussion

In a few of the samples evaluated, neither of the two pathogens was detected. Three interpretations are plausible – the targeted population was truly free of the respective pathogen, the pathogen was present below the limit of detection of the technique and we did not have a sufficient number of samples to estimate the prevalence. The second possibility is unlikely because the testing strategy employs an enrichment step and PCR detection which is known to be comparable, if not superior, to the traditional isolation method (Jimenez *et al.* 2011). The third possibility was addressed by computing the confidence interval to capture the potential variability in the estimates of the presence of the pathogens given the numbers of samples (Wilson 1927; Zelmer 2013).

The difference in the reported prevalence of Salmonella spp. between our study and other could be attributed to the targeted populations – apparent healthy vs. culled or clinical cases (Loneragan *et al.* 2012; Cummings *et al.* 2009), management practices or stress caused by transportation (Ruzante *et al.* 2010; Edrington *et al.* 2009; Lomborg *et al.* 2007; Vanselow *et al.* 2007). Another factor was the type of animal sampled (Bolton *et al.* 2012; Gorski *et al.*  2011; Jimenez et al. 2011; Kidanemariam et al. 2010; Vanselow et al. 2007; Liebana et al. 2002). Although our investigation indicated that sheep had the highest shedding rate other studies reported no differences among species (Bolton et al. 2012; Jimenez et al. 2011; Vanselow et al. 2007). As in other studies, in spite of high faecal shedding of *Salmonella* by cattle, the likelihood of the organism in milk was low (Amagliani et al. 2012; Ruzante et al. 2010; Van Kessel et al. 2008; Kunze et al. 2008). The rate of detection of *Salmonella* in healthy camels was similar to that reported in the literature (Molla et al. 2004; Moore et al. 2002).

As in other studies, our investigation demonstrated a public health threat from consumption of beef and mutton since zoonotic serovars, i.e. Goettingen, Haifa, Jangwani, Kedougou, Kentucky, Mbandaka, Newport and Typhimurium, had been detected in these products (O' Doherty et al. 2013; Allerberger 2012; Petrov et al. 2009; Rodriguez-Urrego et al. 2010; Doublet et al. 2008). Cattle faeces showed more diversity in serovars of Salmonella spp., than has been reported in other studies (Petrov et al. 2009; Schneider et al. 2011). Three of the zoonotic serovar isolated in our study (Bovismorbificans, Infantis and Newport) are not common and particularly rare in camel (Miller et al. 2014; Thai et al. 2012; Rimhanen-Finne et al. 2011; Oloya et al. 2007). The high diversity could be attributed to the dynamics of animal and feed in this population where both are imported from all over the world. One speculative explanation for detecting this serovar in the targeted population is that the sheep may have been fed with or had grazed on, grass contaminated with the serovar.

A high rate of cross-contamination occurs among sheep carcasses at the abattoir where most of the S. Typhimurium serovars were recovered – from swabs rather than from faecal samples. A similar higher ratio of carcass-to-faecal contamination was reported in another study in which it was noted that this ratio varied by location of the abattoir (Davies *et al.* 2004). The cross-contamination could be attributed poor sanitary handling by workers and contamination of equipment (Gomes-Neves *et al.* 2012; Todd *et al.* 2010). In addition, the direct role of the workers could not be ignored especially, in view of the finding of rare zoonotic serovars in the on carcasses and not in animal faeces (Kidanemariam *et al.* 2010; Duffy *et al.* 2009; Purvis *et al.* 2005). Contamination of carcasses during processing at the abattoir represents a major challenge to public health workers because it is difficult to identify the critical control point. Therefore, implementation of good hygiene practices is critical to reduce the likelihood of contamination of carcasses.

The low rate of occurrence of L. monocytogenes in the target population was surprising, and as in other studies, was detected mainly in sheep carcasses at the abattoir (Wahlstrom *et al.* 2011; Antoniollo *et al.* 2003). The only isolate of this pathogen in dairy farms was detected in samples collected from feed troughs. However, the slight differences in the rates could be attributed to differences in the targeted populations and the detection techniques being used. Silage is not a common practice in the target population and this might have played a role.

This is the first report on L. monocytoges in faecal samples from camel. The organism was detected in camel sausages obtained from a retail market (Ozbey et al. 2006), and this could be attributed to postharvest contamination. One plausible explanation for the rare occurrence of L. monocytogenes in camels could be explained by feeding behaviour. Camels generally graze on trees and shrubs and are not fed silage (Mohammed et al. 2010; Nightingale et al. 2004).

Our study demonstrated the potential risk from two of the most common foodborne pathogens, at the preharvest level, among ruminants in Qatar, a country that has a highly dynamic population (expatriates-to-local ratio) from every part of the world and is active in animal-trading commerce. The study also showed that *Salmonella* spp. are common at the preharvest level, especially in faecal samples from sheep and camels. Zoonotic serovars were more diverse in faecal samples from cattle than in any other type of sample. Meat from sheep and camels entering the food-supply chain was more likely to be contaminated with *Salmonella* spp. than meat from cattle or chicken. In spite of the fact that both cattle and camels shed *Salmonella* spp. in their faeces, the likelihood that this pathogen enters the food supply chain through the milk matrix was low. The threat to the food-supply chain from *L. monocytogenes* was low and the main food matrix posing a risk was carcasses of sheep. We believe that the risk associated with those two pathogens would be minimized if better sanitary practices were instituted at the abattoir. The implementation of good hygiene practices and hazard analysis of critical control point schemes will no doubt contribute to reducing the likelihood of contamination of carcasses at the abattoir and hence mitigate the risk to the public health associated with both pathogens.

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# **Conflict of Interest**

None declared.

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