Trop Anim Health Prod
DOI 10.1007/s11250-015-0854-z

REGULAR ARTICLES

Herd- and individual-level prevalences of and risk factors for *Salmonella* spp. fecal shedding in dairy farms in Al-Dhulail Valley, Jordan

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Received: 14 October 2014 / Accepted: 18 May 2015

Abstract Salmonellosis is an important disease frequently associated with diarrhea in calves. From January to September 2009, a cross-sectional study involving 91 dairy farms was conducted to determine the prevalence of *Salmonella* spp. infection in cattle in Al-Dhulail Valley, Jordan. A total of 910 calve and cow fecal samples were collected. Information on farm management practices was obtained through personal interviews using a standardized questionnaire and was tested as risk factors for *Salmonella* spp. positivity in farms by using logistic regression analysis. Standard conventional methods for Salmonella isolation and serotyping were used, and the disk agar diffusion test was used for antimicrobial testing. The herd-level prevalence of *Salmonella* spp. in calves, cows, and dairy farms was 12, 12, and 23 %, respectively, and the individual-level prevalence was 4 % for calves, cows, and dairy farms. Forty-six percent of the dairy farms had calf diarrhea, and 4 % had cow diarrhea. Seven (17 %) of the 42 farms with calf diarrhea had Salmonella. However, only 7 % (95 % CI: 4, 10) of the 221 diarrheic and 1 % (95 % CI: 0.2, 4) of the 234 of non-diarrheic calves had Salmonella. A total of 33 Salmonella isolates were obtained from the fecal samples: 12 isolates were *Salmonella typhimurium*, 6 were *Salmonella montevideo*, 6 were *Salmonella anatum*, 2 were *Salmonella enteritidis*, and 7 isolates were not serotyped. All isolates were susceptible to ciprofloxacin, trimethoprim–sulfamethoxazole, gentamycin, neomycin, colisitin, and amoxicillin at 100, 91, 85, 79, 79, and 70 %, respectively. Out of the 11 variables/categories, the frequency of cleaning every 2 months or more was associated with high odds of infection among calves (OR=5.6) and farms (OR=7.0).

Keywords *Salmonella* · Calves · Diarrhea · Prevalence · Risk factors · Jordan

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Introduction

Salmonellae are common food-borne pathogens (CDC 2013), but they may also be transmitted directly to individuals living and working on farms (Shua et al. 2012). They infect many animal species (Kagambèga et al. 2013) and survive in the environment (Toth et al. 2013), feed, and water (Jones 2011; Levantesi 2012). They are shed in feces by apparently healthy (Kagambèga et al. 2013; Toth et al. 2013) and diarrheic animals (Cummings et al. 2010). The occurrence of salmonellosis in 1- to 8-week-old calves has been reported worldwide (Cummings et al. 2010). Substantial economic losses due to mortality and poor growth of infected animals, as well as constituting a public health hazard, were reported (Fraser et al. 2010). The prevalence of Salmonella infections associated with calf diarrhea could reach 18.7–43.5 % and that in calves showing no diarrhea could reach 27.7 % (Moussa et al. 2010; Youssef and El-Haig 2012). Salmonella infections in cattle have been reported worldwide (Carrique-Mas et al. 2010; Lombard et al. 2012; Cho and Yoon 2014). The most frequently isolated serotypes from cattle are *Salmonella montevideo*, *Salmonella typhimurium*, *Salmonella dublin*, *Salmonella enteritidis*, *Salmonella anatum*, *Salmonella newport*, *Salmonella cerro*, *Salmonella kentucky*, and *Salmonella orion* (Alam et al. 2009; Brichta-Harhay et al. 2011; Strawn et al. 2014).

Published online: 12 June 2015

Several factors associated with the reduction or the enhancing of Salmonella fecal shedding were reported; type of production system (Vanselow et al. 2007), poor hygiene (Boqvist and Vågsholm 2005), large herd size, lack of a clean visitors' parking area (Davison et al. 2006), infected neighboring herds, contact with wild birds and rodents, presence of Salmonella carriers (Nielsen et al. 2007), contaminated feeds (Jones 2011), use of nipple feeders (Svensson and Jensen 2007), and access to surface water (lake, pond, river, or stream) (Fossler et al. 2005) increased the risk of fecal Salmonella spp. shedding in dairy farms. By contrast, herds closed to incoming cattle (Berge et al. 2006), calving in an individual animal area within a building (Fossler et al. 2005) and colostrum ingestion and vaccine technology (Hermesch et al. 2008), decrease the risk of fecal Salmonella shedding.

The prevalence of *Salmonella* spp. shedding in cattle has never been studied in Jordan. Thus, this study aimed to determine the herd- and individual-level prevalences of Salmonella fecal shedding in dairy farms in Al-Dhulail Valley, Jordan. Selected risk factors for Salmonella fecal shedding were studied at the herd level in terms of herd status, i.e., calf, cow, or calf and cow status. Moreover, *Salmonella* serovar isolates were identified, and their antibiotic resistance profiles were studied.

Materials and methods

Animals and study area

In Jordan, most dairy cattle farms are located in Zarqa governorates, Al-Dhulail Valley region and are operated in a semi-intensive system. The maximum cattle herd size is 1350, and the minimum is 25 (quartiles; Q1=50, Q2=75, Q3=135). The maximum number of calves per farm is 250, and the minimum is 5 (quartiles; Q1=8, Q2=12, Q3=16). The Holstein–Friesian cattle breed is the most common. Most of the dairy farms adopt the same management system of the herd being divided into three groups: lactating cows, dry cows, and growing heifers. The stage of lactation is not considered. Pregnant cows are isolated before parturition in individual pens in the barn. After delivery, the calves spend about 3 h with the dam to feed on colostrum and are then they feed on the dam's milk or milk replacer. Two milk meals are given daily by using nipple drinkers or buckets. Feeding on starter ration begins in the second week of age. Calf housing is conducted in two systems, namely individual and group pens. The average weaning age is 3 months. After weaning, calves are transferred to another pen. Dairy farms are adjacent to each other and all are infested with rodents (100 %). Some farms control rodents (40 %) by using poison bait (30 %) and sticky trap (10 %). Cattle farms use formalin, iodophors, and hydrated lime as barn disinfectants.

Al-Dhulail Valley has two sources of water: underground artesian well and municipality pipe. In the farm, the water system includes an underground cistern connected to a farm tank that feeds drinkers. No Salmonella vaccine is used in the studied farms, and the commonly used antibiotics are gentamycin, trimethoprim–sulphamethoxazole, penicillin, amoxicillin, and tetracycline.

Sample size determination

The prevalence of *Salmonella* spp. in dairy farms in Jordan has not been investigated previously, and thus, it is assumed to be 50 %. As 95 % level of confidence and 5 % absolute precision were required, the appropriate sample size was calculated at 73 according to Thrusfield (2007). A total of 91 representative farms were selected from Al-Dhulail Valley. The study had at least 80 % power at the 5 % significance level to detect an odds ratio (OR)≥2 for the risk factors present in 50 % of the control and an OR≥3 for those present in 20 % of the control.

Farm sampling and survey

Systematic sampling was used where a list of the operating dairy farms in the study area (n=160) (Anon 2009) was obtained. Odd numbers were picked aside from the list, and 16 even numbers were picked every 10. A total of 96 farms were contacted, with 91 farms agreeing to participate and 5 declining. Ten fecal samples were collected randomly using random tables from each farm, five from lactating cows, and five from calves under 1 month of age.

The median size of the cattle farm in the studied area was 75 and that of the calves in the farm was 12. The sample size required 95 % certainty that at least one animal in the sample would be positive if the disease was present or above the specified level. The minimum number of diseased animal assumed to be in a herd was one. For a population of 30–10,000 individuals, the number of diseased animals was determined on the basis of the assumed prevalence of 50 % and the minimum sample size of five using Cannon and Roe's (1982) formula (Martin et al.1994). A total of 910 samples (455 from cows and 455 from calves) were collected from January to September 2009 from Al-Dhulail Valley.

Data collection

A survey was conducted with the help of a purposely designed questionnaire to collect information on calf housing, frequency of cleaning, barn disinfection, calf feeding, water source, type of water tank, diarrhea, use of antibiotics, and rodent control (Table 4). Close-ended questions were used. Completing the survey form and sampling were done at the same time of the sample collection.

Salmonella isolation, identification, and serotyping

One gram of each fecal sample was taken aseptically, added to 10 mL Rappaport Vassiliadis broth (Scharlau, Spain) and incubated for 24 h at 43 °C. After incubation, the inoculated broth was streaked onto Brilliant green agar media (DIFCO). Plates were then incubated at 37 °C for 24–48 h, and suspected pink Salmonella colonies were picked and transferred to Kligler's iron agar slants (KIA) (DIFCO). The inoculated KIA slants were incubated at 37 °C for 18–24 h. Slants exhibiting a typical Salmonella pattern were further tested by biochemical tests (Markey 2013). Salmonella serotyping was done according to the method of (Popoff et al. 2004) using Salmonella polyvalent O antisera A-G group, poly H antisera phases I and II, and monovalent O and H antisera factors (National Salmonella Centre-Statens Serum Institute, Copenhagen, Denmark).

Antibiotic susceptibility

The agar disk diffusion test was carried out using the Clinical and Laboratory Standard Institute guidelines (CLSI 2008). Ten antimicrobial agents were used: penicillin G 10 units, amoxicillin 25 μg, gentamicin 10 μg, erthromycin 15 μg, neomycin 30 μg, trimethoprim–sulphamethoxazole 1.25/23.75 μg, ciprofloxacin 5 μg, tetracycline 30 μg, doxycycline 30 μg, and colisitin (10 μg) (OXOID).

Statistical analyses

Data were stored in a database and analyzed using SPSS version 16.0 (Anon 2007) and Epi-Info (CDC, Atlanta, Georgia). The 95 % confidence interval was calculated for the prevalence (Thrusfield 2007). Chi-squared analysis was used to test the significance among the proportions, and the odds ratios were calculated. The dependent variable was *Salmonella* spp. status, coded as 0 (negative) or 1 (positive). Analyses were conducted at the farm level, but the farm status was based on calf, cow, or calf and cow (farm) status. A total of 11 variables/categories were tested (Table 4). The screening of the significant variables to be used in the final logistic regression was conducted using simple logistic regression. The variables that were statistically significant $P \leq 0.2$ (two sided) were included in the multivariable model forward selection. P value of ≤ 0.05 was considered significant.

Results

Prevalence

The herd- and individual-level prevalences of Salmonella infections among calves, cows, and dairy farms are summarized in Table 1. At the herd level, 91 calf, cow, and dairy farms were examined, with prevalences of 12, 12, and 23 %, respectively. At the individual levels, 455, 455, and 910 calf, cow, and dairy farm animals were examined, with a prevalence of 4 % for all groups. A total of 33 Salmonella isolates were obtained, identified, and serotyped from 910 fecal samples. The 33 Salmonella species were isolated from 21 dairy farms (from 16 calves and 17 cows). *S. typhimurium* was isolated from six farms, *S. anatum* was isolated from four farms, *S. enteritidis* was isolated from two farms, *S. montevideo* was isolated from four farms, and non-serotyped Salmonella species was isolated from five farms (Table 2).

Diarrhea and Salmonella infections

Forty-two 46 % (95 % CI: 39, 59) out of the 91 cattle farms had calf diarrhea, and only four 4.4 % (95 % CI: 0.1, 8) had cow diarrhea. Seven (17 %) (95 % CI: 0.7, 15) of the 42 farms with calf diarrhea had Salmonella, and two 4 % (95 % CI: 0, 6) of the 49 non-diarrheic calf farms had Salmonella. The individual-level prevalence of Salmonella among diarrheic calves was 7 % (95 % CI: 4, 10) of the 221 calves and 1 % (95 % CI: 0.2, 4) of the 234 non-diarrheic calves. None of the four diarrheic cows had Salmonella.

Antibiotic susceptibility

Antibiotic susceptibility patterns showed that the 33 Salmonella isolates were susceptible to ciprofloxacin, trimethoprim–sulfamethoxazole, gentamycin, neomycin, colisitin, amoxicillin, tetracycline, and doxycycline at 100, 91, 85, 79, 79, 70, 61, and 49 %, respectively. Erythromycin and penicillin showed 100 and 66 % resistance, respectively, as demonstrated in Table 3.

Multivariable analyses

At the farm level, out of the 11 variables/categories, frequency of cleaning every 2 months or more, type of water tank, antibiotic treatment, and diarrhea were associated ($P<0.2$) with *Salmonella* spp. positivity in calves (Table 4). After forward selection, the model only had frequency of cleaning every 2 months or more (Table 5). Barn disinfection, type of water tank, and antibiotic treatment were associated ($P<0.2$) with *Salmonella* spp. positivity in cows in the screening (Table 4). After forward selection, the model only had barn disinfection (Table 5). Type of calf housing, frequency of cleaning every 2 months or more, diarrhea, gentamicin, and trimethoprim–sulphamethoxazole were associated ($P<0.2$) with *Salmonella* spp. infection in calves in the screening (Table 4). After forward selection, the model only had frequency of cleaning every 2 months or more (Table 5). Every model contains one factor only for calves, cows, and dairy farms; thus, there is no correlation.

Table 1 Herd- and individual-level Salmonella infections among dairy farms from January to September 2009 in Al-Dhulail Valley, Jordan

Species	Herd level				Individual level			
	No. examined	No. positive	Prevalence	95 % CI	No. examined	No. positive	Prevalence	95 % CI
Calf	91	11	12	5,19	455	16	3.6	3,6
Cow	91	11	12	5,19	455	17	3.7	3,6
Dairy farms	91	21	23	14,32	910	33	3.6	3,5

Discussion

Prevalence

The presence of Salmonella infection in dairy farms was evident in Al-Dhulial Valley, with 12 % farm-level prevalence of infection among calves and 12 % among cows. The herd-level Salmonella shedding in calves and cows in Jordan (12 %) was similar to other findings (Davison et al. 2006). However, higher or lower prevalences were reported in calf and cow dairy herds in other countries (Fossler et al. 2005; Nielsen et al. 2011). The variations in both herd- and individual-level prevalences reported in different countries reflect the effects of a wide range of different management risk factors (Vanselow et al. 2007; Alam et al. 2009; Jones 2011). Cattle herds with Salmonella infection may represent the main animal source of Salmonella infection in human. Salmonella can be spread from cattle to human via direct contact, contamination of the environment (water sources), and contamination of meat and milk products (Schlundt et al. 2004).

In Al-Dhulail Valley, all Holstein–Friesian animals are reared under an intensive closed system, and farms are located beside each other under poor hygienic conditions in a limited area. Animals can become passive carriers for weeks, months, or even years of some serovars by constantly reacquiring Salmonella from the environment (Anon, 2009, 2011).

The current results showed *S. montevideo* infection in one farm involving both calves and cows (Table 2). The rest of the Salmonella-infected farms had either infected calves or infected cows but not both. This finding may reflect the successful separation between cows and calves in these farms.

Diarrhea and Salmonella infection

In this study, 46 % of the examined herds had calf diarrhea, and *Salmonella* spp. was isolated from 17 % of them compared with 4 % from calf herds without diarrhea. At the individual level, the prevalence of *Salmonella* spp. was 7 % among diarrheic calves and 1 % in non-diarrheic ones. These findings are in agreement with other reports (DEFRA 2005; Berge et al. 2006; Cho and Yoon 2014). Similarly, the presence of diarrhea increased the risk of Salmonella shedding (Cummings et al. 2010). However, diarrhea in calves is multifactorial caused by different pathogens (Achá et al. 2004). By contrast, cows shed *Salmonella* spp. without showing diarrhea. Salmonella is carried asymptomatically “passive carriers” in the intestine or gall bladder of many animals and is continuously or intermittently shed in the feces and contaminate the environment (Fossler et al. 2005).

Salmonella serotypes

In the current investigation, four *Salmonella* serovars were identified from calves and cows for the first time in Jordan (Table 2). *S. typhimurium* was the most prevalent. In several studies, *S. typhimurium* was reported as the most common cause of salmonellosis in calves (DEFRA 2005). Moreover, *S. typhimurium* has been found to transmit from animals to man and cause a variety of clinical symptoms (Cherry et al. 2004; Okoro et al. 2015). Other serovars, such as *S. enteritidis*, *S. montevideo*, and *S. anatum*, are shed in feces and associated with calf diarrhea (Izzo et al. 2011b).

Table 2 Distribution and serotypes of 33 *Salmonella* spp. isolates among 91 dairy farms from January to September 2009 in Al-Dhulail Valley, Jordan

Salmonella spp.	Herd level			Individual level		
	Calves	Cows	Dairy farms	Calves (n=455)	Cows (n=455)	Dairy farms (n=910)
S. typhimurium	3	3	6	6	6	12
S. anatum	2	2	4	2	4	6
S. enteritidis	2	0	2	2	0	2
S. montevideo	2	2	4	4	2	6
Salmonella spp.	1	4	5	2	5	7
Total Salmonella infections	10	11	21	16	17	33

Table 3 Antibiotic resistance patterns of 33 Salmonella isolates from fecal shedding in dairy farms in Al-Dhulail Valley, Jordan, using agar gel diffusion test

Antibiotics/concentration	Resistance profile no/%			Inhibition zone (mm)		
	R	I	S	R	I	S
Penicillin 10 U	33/100	00	00	≤11	12–21	≥22
Amoxicillin 25 mcg	8/24	2/6	23/70	≤22	23–30	≥31
Gentamicin 10 mcg	4/12	1/3	28/85	≤12	13–14	≥15
Erythromycin 15 mcg	33/100	00	0	≤13	14–17	≥18
Neomycin 30 mcg	5/11	00	29/89	≤12	13–16	≥17
Trimethoprim-sulphamethoxazole 1.25mcg/23.75 mcg	3/9	00	30/91	≤11	12–16	≥17
Ciprofloxacin 5 mcg	00	00	33/100	≤15	16–20	≥21
Tetracycline 30 mcg	9/27	4/12	20/61	≤14	15–18	≥19
Doxycycline 30 mcg	12/36	5/15	16/49	≤12	13–15	≥16
Colisitin 10 mcg	5/15	2/6	26/79	≤8	9–10	≥11

no number of the isolates

S. enteritidis and *S. montevideo* infect man through the food chain (Ekdahl et al. 2005; Centers for Disease Control and Prevention CDC 2013), and *S. anatum* was also associated with food poisoning outbreak (Ebuchi et al. 2006).

Antibiotic sensitivity

In this study, through a univariable analysis, the use of gentamycin was associated with the reduction of Salmonella shedding in calves and dairy farms in Jordan and

Table 4 Univariable analysis associations between some production and health management risk factors and herd-level *Salmonella* spp. infection in dairy cattle from January to September 2009 in Al-Dhulail Valley, Jordan

Variable	Category	Coding	No.	No. of (Salmonella) positive (%)		
				Calves 11	Cows 11	Farms 21
Type of calf housing	Individual	0	11	0 (0)[a]	1 (9)	1 (9)
	Group	1	80	11 (14)	10 (13)	20 (25)
Barn disinfectant	No	0	33	3 (9)	7 (21)	10 (30)
	Yes	1	58	8 (14)	4 (7)[a,b]	11 (19)
Frequency of cleaning	Less than 2 months	0	83	8 (10)	8 (10)	16 (19)
	2 months or more	1	8	3 (38)[a,b]	3 (38)	5 (63)[a,b]
Type of milk	Replacer	0	10	1 (10)	2 (20)	3 (30)
	Dam	1	81	10 (12)	9 (11)	18 (22)
Milk drinker type	Nipple	0	9	1 (10)	2 (9)	3 (18)
	Bucket	1	82	7 (11)	6 (12)	8 (29)
Water source	Pipe	0	61	7 (12)	2 (10)	12 (20)
	Ground	1	30	4 (13)	5 (17)	9 (30)
Type of water tank	Others	0	66	9 (14)	9 (14)	17 (26)
	Cement	1	25	2 (8)	2 (8)[a]	4 (16)[a]
Diarrhea*	No	0	49	4 (8)	5 (10)	9 (18)
	Yes	1	42	7 (17)[a]	6 (14)	12 (29)[a]
Use of gentamycin**	No	0	9	3 (33)	1 (11)	4 (44)
	Yes	1	82	8 (10)[a]	10 (12)	17 (21)[a]
Use of trimethoprim–sulphamethoxazole **	No	0	11	2 (18)	0 (0)	2 (18)
	Yes	1	80	9 (11)[a]	11 (14)[a]	19 (24)
Rodent control	No	0	56	5 (9)	5 (9)	10 (18)
	Yes	1	35	4 (11)	5 (14)	8 (23)

[a] Simple logistic regression, significant: $P<0.2$, [b] Multivariable model, significant: $P<0.05$, *at least one case of diarrhea on the day of the visit, **used during the last month

Table 5 Final logistic models for herd-level salmonellosis among calves, cows, and dairy farms from January to September 2009 in Al-Dhulail Valley, Jordan (n=91 farms)

Variable/categories	Category	P	OR	95 % CI
Calves[a]				
Frequency of cleaning	Every 2 months or more	0.035	5.6	1.1, 28.0
Cows[b]				
Barn disinfection	Yes	0.021	0.15	0.03, 0.8
Dairy farms[c]				
Frequency of cleaning	Every 2 months or more	0.013	7.0	1.5, 32.3

[a] Likelihood ratio of chi-squared ($LR_X{}^2$): 3.9 on one degree of freedom (df), [b] $LR_X{}^2$: 9.2 on two df, [c] $LR_X{}^2$: 6.4 on one df

trimethoprim–sulphamethoxazole in calves and cows (Table 4). Variable effects of antimicrobial agents on the shedding of Salmonella were reported. Cattle treated systemically with antimicrobials within 14 days of sampling were more likely to be Salmonella-negative than non-treated cattle (Fossler et al. 2005). In pre-weaned calves, the significant factor associated with decreased fecal shedding of *S. enterica* was the use of antimicrobial-supplemented milk replacer (Berge et al. 2006). By contrast, the exposure to and the recent treatment with antimicrobial agents of heifers and cows were considered risk factors that increase the probability of Salmonella isolation (Cho and Yoon 2014). Izzo et al. 2011a demonstrated that in adult cattle, antibiotics could improve recovery and lessen the severity of diarrhea, but they would likely extend the duration of *S. enterica* shedding. However, in claves and young stock, the subtherapeutic use of antibiotics in milk replacer could actually make the diarrhea worse and results in emerging of resistant strains. Therefore, antimicrobial-resistant profiles were created.

It is worth a mention that *S. typhimurium* and *S. enteritidis* which were isolated from dairy farms are considered as food-borne pathogens that cause serious illness in man (DuPont 2007). These two serotypes were found by many investigators to be multiresistant to antimicrobial agents which increase their risk to human and animal safety (Brichta-Harhay et al. 2011; Seyfarth et al. 2014).

Risk factors

Barn disinfection was found to be a reducing factor for Salmonella shedding in cows in Jordan (Table 5). The studied farms used formalin, iodophors, and hydrated lime disinfectants, which have known killing effects on Salmonellae (McLaren et al. 2011). They use these disinfectants as spray after cleaning and at the entrance of the farms for vehicles and personnel. Less frequent cleaning (every 2 months or more) was associated with high Salmonella shedding in dairy farms and calves. Inadequacy of cleaning was reported to be associated with Salmonella shedding. Improved cleaning and disinfection routines for cattle transport vehicles and animal markets are necessary to control salmonellosis in calves (Dee et al. 2005). In Jordan, water shortage forces farmers to decrease the frequency of cleaning, and only a few farms have high water pressure, and none use hot water for cleaning.

Cleaning frequency of less than 2-month period and ordinary disinfection routines for dairy farms, cattle transportation vehicles, and the workers boot and proper use of antimicrobials are expected to reduce calf diarrhea and salmonella infection. The adaptation of these findings by dairy farm owners and workers is expected to improve the poor hygienic condition that leads to reduction of calf diarrhea and salmonella prevalence, thus results in increase dairy farm production and upgrade the milk and meat quality. The countries located in the Mediterranean agro-ecological zone are characterized by similar climatic and environmental properties; therefore, the finding of the current study in Jordan could be adopted in the countries of this zone.

Conclusions and recommendations

Dairy cattle farms in Al-Dhulail Valley had high herd-level prevalence of diarrhea in calves, cows, and cattle farms. *S. typhimurium*, *S. enteritidis*, *S. montevidio*, and *S. anatum* contributed to calf diarrhea. Isolated *Salmonella* spp. was most susceptible to ciprofloxacin, trimethoprim–sulphamethoxazole, and gentamycin. Barn disinfection was associated with the reduction of Salmonella shedding. Cleaning frequency in less than 2 months is expected to reduce Salmonella shedding. Veterinary extension should educate farmers about the frequency of cleaning, proper use of disinfectants, and proper use of antimicrobials which is expected to reduce calf diarrhea and salmonella infection. The control of Salmonella infections in dairy farms will certainly impact on disease control efforts in human.

Acknowledgments This work received financial assistance from the Deanship of Research, Jordan University of Science and Technology.

Conflicts of interest The authors declare that they have no conflict of interest.

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