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Presence of *Salmonella* spp., *Campylobacter* spp., and *Listeria* spp. in Different Animal Species Raw Milk in Diyarbakır Province

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Abstract

Contamination of raw or unpasteurized milk with zoonotic pathogens can seriously affect public health. In this study, milk samples were collected from sheep/goat and bovine farms in Diyarbakır province engaged in home breeding to investigate the prevalence of pathogens in raw milk. A total of 253 raw milk samples from bulk milk tanks belonging to 58 cows, 72 buffaloes, 48 sheep, and 75 goats were analyzed by conventional and molecular methods. All milk samples were negative for *Salmonella* spp. through both methods. *Campylobacter* spp. could not be isolated, but the polymerase chain reaction analysis found that 26 (10.28%) of the milk samples were contaminated with *Campylobacter* spp. However, *Campylobacter jejuni* was not detected in any of the samples. The prevalence of *Listeria* spp. was 2.77%. *Listeria* spp. was detected in raw milk from cows, buffaloes, and goats, but not in milk from sheep. While *Listeria monocytogenes* was not detected in any of the milk samples analyzed, the isolated strains were defined as *Listeria innocua*, *Listeria welshimeri*, and *Listeria grayi*. It was concluded that compared to reports from other countries, the prevalence of pathogens was low. Still, raw milk consumption poses a potential risk to public health, more so, as some of the pathogenic bacteria can survive and multiply even at low temperatures.

Keywords: Bovine, foodborne pathogen, PCR, sheep/goat, unpasteurized milk

Introduction

Although scientific evidences are rather weak, there is an increasing consumer interest in raw milk consumption because of the assumptions raised about its higher nutritional value, its effects on beneficial microbiota, and its low risk for persons with asthma, allergies, and lactose intolerance (Claeys et al., 2013; Lucey, 2015). On the other hand, *Campylobacter jejuni* (*C. jejuni*), *Listeria monocytogenes* (*L. monocytogenes*), *Salmonella* spp., *Yersinia enterocolitica*, Shiga toxin-producing *Escherichia coli* (*E. coli*) (or verotoxigenic *E. coli*), and *Staphylococcus aureus* in raw milk can pose a serious risk to public health (Claeys et al., 2013; Oliver et al., 2009). Many of these pathogens can be found in healthy dairy animals' skin or gastrointestinal tract flora or udders and may contaminate milk during or after milking (Oliver et al., 2005; Ricci et al., 2013). Pasteurization is a safe way to destroy pathogens that may be present in milk. However, improper pasteurization process or contamination post-pasteurization may lead to foodborne outbreaks (Giacometti et al., 2012; Oliver et al., 2005, 2009).

The European Food Safety Authority (EFSA) 2015 reported a clear link between raw milk consumption and human diseases caused by foodborne pathogens (EFSA BIOHAZ Panel, 2015). Outbreaks or sporadic cases associated with the consumption of raw milk or dairy products have increasingly been reported (Costard et al., 2017; Lahti et al., 2017; Mylius et al., 2018; Robinson et al., 2014, 2020). A study conducted in Minnesota from 2001 to 2010 revealed that the role of raw milk consumption in foodborne disease outbreaks is largely underestimated (Robinson et al., 2014).

Many studies investigate the prevalence of foodborne pathogens in raw or unpasteurized milk (Bianchi et al., 2013; Bogdanovičová et al., 2016; Chao et al., 2007; Jayarao et al., 2006; Kalmus et al., 2015; McAuley et al., 2014). When these studies are concerned, it is seen that the prevalence of pathogens in raw milk differs between studies. However, Oliver et al. (2005) reported the prevalences of *L. monocytogenes*, *Salmonella* spp., and *C. jejuni* as 2.8–12.6%, 0–66%, and 2–9.2%, respectively, in bulk milk tanks.

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The present study aimed to determine the presence of three foodborne pathogens in raw milk collected from home-breeding farms in Diyarbakir by conventional and molecular methods.

Method

Material

Milk samples were collected at irregular intervals between 2019 and 2021 from home-breeding farms (the number of animals in each farm was 4–10) in the Diyarbakır province. A total of 253 milk samples from cows' (58), buffalos' (72), sheep' (48), and goats' (75) bulk milk tanks were collected into sterile containers and transferred to Dicle University Faculty of Veterinary Medicine Laboratory of Microbiology in cold chain immediately. The milk samples were immediately analyzed.

Isolation and Identification of Bacteria

The ISO 6579-1:2017 procedure was applied for *Salmonella* spp. isolation (EN ISO 6579-1: 2017). After isolation, the slide agglutination test using O antiserum poly A-I & Vi (BD Difco, Sparks, MD, USA) was performed with the suspected *Salmonella* spp. isolates. The ISO 11290-2:2017 procedure was applied for *Listeria* spp. isolation (EN ISO 11290-2:2017). Suspicious isolates were identified at the genus level by biochemical tests (Markey et al., 2013). According to the manufacturer's instructions, the *Listeria* spp. isolates were identified with the Microgen Listeria-ID system (Microgen Bioproducts Limited, Camberley, UK). *Campylobacter* spp. isolation was done according to the ISO 10272-1:2017 procedure (EN ISO 10272-1: 2017). Suspicious isolates were evaluated according to their colony and microscopic morphology (Markey et al., 2013).

DNA Extraction and Polymerase Chain Reaction

Genomic DNA of 253 milk samples collected from cows' (58), buffalos' (72), sheep' (48), and goats' (75) bulk milk tanks was extracted using a commercial kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific). All samples were analyzed at the genus and species level by conventional polymerase chain reaction (PCR) with the primers shown in Table 1 (Sambrook & Russell, 2001). All PCR amplifications were performed containing 1 µL forward primer (10 pmol), 1 µL reverse primer (10 pmol), 0.5 µL dNTPs (10 mM dNTP mix), 3 µL $MgCl_2$ (25 mM), 2.5 µL 10× PCR buffer solution, 0.2 µL Taq DNA polymerase (ThermoScientific, Carlsbad, CA, USA), 2 µL of DNA template, and nuclease-free water to a final volume of 25 µL. Standard strains (*S. enteritidis* ATCC 13076, *L. monocytogenes* ATCC 19111, *C. jejuni* NCTC 11168) and negative control (nuclease-free water) were used for each amplification. A DNA thermal cycler (T100TM, Bio-Rad, Singapore) was used for amplification, which was carried out as an initial denaturation step at 94°C for 3 minutes followed by 30 cycles, denaturation at 94°C for 60 seconds, bonding at 54°C for 60 seconds, extension at 72°C for 60 seconds, and final extension at 72°C for 7 minutes (Sambrook & Russell, 2001). The PCR products were analyzed by electrophoresis in 1.5% agarose gel containing SafeView™ Classic (Applied Biological Materials, Richmond, Canada) at 100 V for 60 minutes. The amplified PCR products were visualized using a gel imaging system (Vilber Lourmat, France).

Results

All bulk milk samples analyzed by molecular and conventional methods were negative for *Salmonella* spp. *Campylobacter* spp. was not isolated from any of the samples, but it was determined that 26 (10.28%) of the 253 bulk milk samples were *Campylobacter* spp. positive. *Campylobacter* spp. was determined in twelve (16%) of the sheep', six (12.5%) of the goats', two (3.45%) of the cows', and six (8.33%) of the buffalos' milk samples. However, *C. jejuni* was not detected in any of the bulk milk samples. The presence of *Listeria* spp. was 2.77% for all bulk milk samples. Only sheep milk was *Listeria* spp. negative, but *Listeria* spp. was determined in four (5.56%) of the buffalos', two (2.67%) of the goats', and one (1.72%) of the cows' milk samples. *Listeria monocytogenes* was not detected in any of the samples analyzed. The strains isolated from buffalos were identified as *Listeria innocua* (one), *Listeria grayi* (one), and *Listeria welshimeri* (two). The strains isolated from cow and goat bulk milk samples were identified as *L. grayi* (Table 2).

Discussion and Conclusion and Recommendations

This study reports the presence/absence of three foodborne pathogens in raw milk samples collected from buffalos', cows', sheep', and goats' bulk milk tanks in the Diyarbakir province over a 3-year period (2019–2021). Similar to the studies in Sweden (Artursson et al., 2018), Romania (Aurelia et al., 2008), Finland (Ruusunen et al.,

Table 1.
Genus and Species-Specific Primers Used for PCR

Target Pathogen	Target Gene	(5′-3′) Primer Sequences	Amplicon Size (bp)	References
Salmonella spp.	*inv*A	GTGAAATTATCGCCACGTTCGGGCAA	284	Rahn et al. (1992)
		TCATCGCACCGTCAAAGGAACC		
Campylobacter spp.	16S rDNA	GGAGGCAGCAGTAGGGAATA	1,062	Persson & Olsen (2005)
		TGACGGGCGGTGAGTACAAG		
C. jejuni	*map*A	CTATTTTATTTTTGAGTGCTTGTG	589	Denis et al. (1999)
		GCTTTATTTGCCATTTGTTTTATTA		
Listeria spp.	16SrRNA	GCCTGTAAGTTGGGGATAA	300	Taliç (2015)
		CCGAAAACCTTCTTCATACA		
L. monocytogenes	*inl*B	GATGGCGATTATGAAAAACC	175	
		CCGTTCCATCAACATCATAACT		

Table 2.

Number and Prevalence of Bacterial Strains Isolated by Cultivation from Bulk Milk Tanks and Detected by Direct Molecular Analysis

	Bacteriological Isolation					Molecular Detection				
	Buffaloes' Milk (*n* = 72)	**Cows' Milk (*n* = 58)**	**Sheep' Milk (*n* = 48)**	**Goats' Milk (*n* = 75)**	**Total (*n* = 253)**	**Buffalos' Milk (*n* = 72)**	**Cows' Milk (*n* = 58)**	**Sheep' Milk (*n* = 48)**	**Goats' Milk (*n* = 75)**	**Total (*n* = 253)**
Salmonella spp.	0	0	0	0	0	0	0	0	0	0
Listeria spp.	4[a] (5.56%)	1[b] (1.72%)	0	2[c] (2.67%)	7 (2.77%)	4 (5.56%)	1 (1.72%)	0	2 (2.67%)	7 (2.77%)
L. monocytogenes	0	0	0	0	0	0	0	0	0	0
Campylobacter spp.	0	0	0	0	0	6 (8.33%)	2 (3.45%)	6 (12.5%)	12 (16%)	26 (10.28%)
C. jejuni	0	0	0	0	0	0	0	0	0	0

Note: [a]One *Listeria innocua,* two *Listeria welshimeri*, and three *Listeria grayi.*
[b]*Listeria grayi.*
[c]Two *Listeria grayi.*

2013), the Czech Republic (Bogdanovičová et al., 2016), and Türkiye (Issa et al., 2010), *Salmonella* spp. was not detected in the present study. Additionally, studies in Malaysia (Chye et al., 2004), China (Chao et al., 2007), USA (Jayarao et al., 2006), Pakistan (Samad et al., 2018), Italy (Giacometti et al., 2012), and Türkiye (Acaröz et al., 2018) have reported *Salmonella* spp. prevalence to be 0.12–28% (Table 3). The prevalence of *Salmonella* spp. in bulk milk tanks may be affected by factors such as geographical region, number of animals on the farm, subclinical shedding, or presence of the microorganism in the environment (Oliver et al., 2005; Ruzante et al., 2010). Huston et al. (2002), reported that subclinical shedding of *Salmonella* spp. is common in the state of Ohio, USA, and the prevalence of the herds varies between 1% and 97%. *Salmonella* sp. can infect humans in severe forms ranging from gastroenteritis to septicemia and life-threatening typhoid fever (Chye et al., 2004). In this respect, the fact that this pathogen was not detected by both methods (bacteriological isolation and molecular detection) in the raw milk analyzed was evaluated as a positive finding in terms of public health.

Unlike the present study results (10.28%), studies conducted in Italy (Amagliani et al., 2012), the Czech Republic (Bogdanovičová et al., 2016), Finland (Ruusunen et al., 2013), and Australia (McAuley et al., 2014) stated that they did not detect *Campylobacter* spp. in the raw milk of cows, sheep, and goats (Table 3). The prevalence of *C. jejuni* was reported as 0.35–22% in Sweden (Artursson et al., 2018), Pakistan (Samad et al., 2018), the USA (Jayarao et al., 2006), and Italy (Giacometti et al., 2012). *C. jejuni* was not detected in the raw milk of different animal species in this study is similar to a study in Iran (Haghi et al., 2015). *Campylobacter* is the leading cause of bacterial foodborne diseases in Europe (EFSA & ECDC 2021, 2022). So, the fact that *C. jejuni*, one of the thermophilic *Campylobacter*s, was not detected in the analyzed raw milk was evaluated as a positive finding regarding public health. Additionally, it is necessary to determine whether *Campylobacter*s detected in raw milk are from thermotolerants other than *C. jejuni*. *Campylobacter* sp. can enter a viable but non-culturable state after exposure to various stresses such as low temperature, oxygen, acidic, or salt-containing environment (Murphy et al., 2006; Silva et al., 2011). This might explain why we could detect *Campylobacter*-DNA but was not successful in isolating viable bacteria.

Similar to the present study results (2.77%), raw milk samples were *Listeria* spp. positive in Iran (Akrami-Mohajeri et al., 2018; Jamali et al., 2013), Türkiye (Acaröz et al., 2017; Issa et al., 2010), Egypt (Osman et al., 2016), the United States (Jackson et al., 2012), and New Zealand (Hill et al., 2012). In contrast, a Romanian study (Aurelia et al., 2008) reported not to have detected *Listeria* spp. in the milk samples they analyzed. The prevalence of *L. monocytogenes* obtained from several studies varies considerably between 0.56% and50% in Italy (Giacometti et al., 2012), the Czech Republic (Bogdanovičová et al., 2016), the USA (Jackson et al., 2012), Malaysia (Chye et al., 2004), Iran (Mansouri-Najand et al., 2015; Jamali et al., 2013), and Türkiye (Issa et al., 2010) (Table 3). However, the fact that *L. monocytogenes* was not detected in the raw bulk milk analyzed in this study is similar to findings reported from Brazil (Nero et al., 2008), China (Chao et al., 2007), Türkiye (Acaröz et al., 2017), Iran (Haghi et al., 2015), and the United States (D'Amico et al., 2008). Poor quality silage can be a source of *L. monocytogenes* infection, which can also cause mastitis in ruminants. In addition, the (intermittent) shedding of subclinically infected ruminants may have also contributed to the great differences in the prevalences of *L. monoctyogenes* (Konosonoka et al., 2012; Oliver et al., 2005). Furthermore, in this study, the detection of *L. innocua* and *L. welshimeri* in the bulk milk of other animal species, except sheep milk, is similar to studies conducted in Iran (Jamali et al., 2013, Akrami-Mohajeri et., 2018), and the determination of *L. grayi* is similar to the study reported in Egypt (Abd El Tawab et al., 2015). While the absence of *L. monocytogenes*, one of the most important foodborne pathogens, in the analyzed raw milk is considered a positive finding, it should also be considered that other *Listeria* spp. may pose a risk for pregnant women, newborns, and people with compromised immune systems.

In conclusion, the present study provided information on the presence of some foodborne pathogens in sheep/goats and bovine farms engaged in home breeding. *Salmonella* spp., *C. jejuni*, and *L. monocytogenes* were not detected in any bulk milk analyzed in this study. Although the presence of *Campylobacter* spp. and *Listeria* spp. is low, the storage of raw milk in inappropriate conditions may cause bacterial growth. Raw milk may significantly increase the potential risk to public health; therefore, it is important to inform consumers about possible pathogens in raw milk. In addition, important points such as improper time/temperature application, etc. and/or contamination at the post-pasteurization steps (recontamination) should be carefully followed for safe processing of raw milk.

Table 3.
Surveys on the Detection of Some Foodborne Pathogens from Bulk Milk Tanks

			Prevalence %				
References	**Animal Species**	**Number of Samples**	***Salmonella* spp.**	***Campylobacter* spp.**	***C. jejuni***	***Listeria* spp.**	***L. monocytogenes***
Artursson et al. (2018)	Cattle (milk filter)	79	0		9		
	Goat-sheep (milk filter)	24			0		
Aurelia et al. (2008)	Buffalo	22	0			0	
Ruusunen et al. (2013)	Cattle	183	0	0			
Bogdanovičová et al. (2016)	Cattle	175	0	0			0.6
	Goat	32					3.1
	Sheep	23					4.4
Issa et al. (2010)	Cattle	350	0			6.57	0.57
Chye et al. (2004)	Cattle	930	1.4				1.9
Chao et al. (2007)	Cattle	209	1.91				0
Jayarao et al. (2006)	—	248	6		2.2		
Samad et al. (2018)	—	200	14		22		
Giacometti et al. (2012)	—	15.282			0.35		
		15.064					0.56
		15.420	0.12				
Acaröz et al. (2018)	Buffalo	100	2				
Amagliani et al. (2012)	Cattle	27		0			
McAuley et al. (2014)	Cattle, sheep, goat	15		0			
Haghi et al. (2015)	Cattle	38			0		0
	Sheep	22					
Akrami-Mohajeri et al. (2018)	—	140				29.2	
Jamali et al. (2013)	Cattle	240				22.5	5.4
	Sheep	165				16.4	2.4
	Goat	41				4.9	2.4
Acaröz et al. (2017)	Buffalo	100				1	0
	Cattle	100				4	
Osman et al. (2016)	Buffalo	100				8	
	Cattle	103				7.77	
Jackson et al. (2012)	—	214				58.3–60.8	50
Hill et al. (2012)	—	295				5.42	
Mansouri-Najand et al. (2015)	—	100					5
Nero et al. (2008)	—	210				0	
D'Amico et al. (2008)	Cattle	62					4.8
	Sheep	22					0
	Goat	49					0

Ethics Committee Approval: The materials used in our study were collected from home-breeding farms in Diyarbakir, and in accordance with the regulation on the working procedures and principles of animal experimentation ethics committees in the official gazette published on February 15, 'milking' are not subject to HADYEK permission.

Informed Consent: There is no 'informed consent' because study materials were not collected from human.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – N.K.S.; Design – N.K.S; Supervision – N.K.S; Resources – ; Materials – S.Y.A; Data Collection and/or Processing – N.K.S; Analysis and/or Interpretation – N.K.S., S.Y.A.; Literature Search – N.K.S.; Writing Manuscript – N.K.S.; Critical Review – N.K.S., S.Y.A.; Other– .

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