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PUBLIC HEALTH HAZARDS OF *YERSINIA ENTEROCOLITICA* ISOLATED FROM SHEEP AND GOAT MILK

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ABSTRACT

Introduction: The gastrointestinal disease Yersiniosis, is mainly caused by *Yersinia enterocolitica* along with *Y. pseudotuberculosis,* which is less common. The present study examines the prevalence of raw goat and sheep milk contamination with *Y. enterocolitica* collected from Erbil Governorate, Iraq while analyzing the antibacterial resistance profiles of the isolated strains and monitoring seasonal variations in contamination and resistance patterns. **Methods:** Raw milk samples were collected from a total of 300 animals (150 each from sheep and goat), the samples were tested using culture-based detection methods, yielding a contamination rate of 9.7%. Statistical analysis revealed no significant differences in contamination rates between sheep and goat milk ($p = 0.559$), or between village and sale point sources ($p = 0.715$). Seasonal trends showed a gradual decline in contamination rates from mid-winter to early summer, although these variations were not statistically significant ($p \geq 0.561$). **Conclusion:** The antimicrobial resistance testing revealed alarming resistance patterns to commonly used antibiotics, including cefotaxime, fluoroquinolones, and aminoglycosides. These findings highlight the public health risks posed by *Y. enterocolitica* found in raw milk and underscore the need for improved food safety practices and antibiotic stewardship in the region.

Keywords: Antibiotic resistance, *Yersinia enterocolitica*, Raw milk

INTRODUCTION

Yersinia is one of the bacterial genera that shaped human history through pandemics, most notably the Black Death in the 14th century, which killed millions across Europe and Asia (Bramanti, Stenseth, Walløe, & Lei, 2016). The genus Yersinia belongs to the Yersiniaceae family, which was founded in 2016 after the order Enterobacteriales was divided into seven families: Enterobacteriaceae, Hafniaceae, Morganellaceae, Erwiniaceae, Thorselliaceae, Pectobacteriaceae, and Yersiniaceae. The Yersiniaceae family consists of 8 genera, 67 species, and 3 subspecies, which are validly recognized and correctly named (Adeolu, Alnajar, Naushad, & S. Gupta, 2016; Janda & Abbott, 2021; Moxley, 2022). One of the well-known species in that genus is *Y. pestis*, it was the cause of plague (also known as black death) which was one of the most deadly diseases in human history.

Yersiniosis is a disease of the gastrointestinal tract frequently caused by the bacterium *Y. enterocolitica*, and less commonly by *Y. pseudotuberculosis* (Hordofa, 2022; Mekonnen, 2024). As a food-borne disease, Human yersiniosis is transmitted via contaminated milk, and meat along with their respective products.

Yersinia enterocolitica (*Y. enterocolitica*) is a small, rod-shaped, Gram-negative, psychrotrophic (grows well at low temperatures) bacterium (Murros et al., 2016). The incubation period is 4–6 days (range 1–14 days), and the development of the symptoms may be slower compared to infections caused by other intestinal pathogens. The common symptoms often include fever, abdominal pain, diarrhea, and it may include mesenteric lymphadenitis that mimics appendicitis (Mead, 2015). On rare occasions, *Y. enterocolitica*-related complications such as joint discomfort, skin rashes or bacterial bloodstream infection can also develop (Disease Outbreak Control Division [DOCD], 2024; Triantafillidis, Thomaidis, & Papalois, 2020).

Yersiniosis is the third most prevalent notifiable bacterial zoonoses following campylobacteriosis and salmonellosis in the European Union (European Food Safety Authority [EFSA] & European Centre for Disease Prevention and Control [ECDC], Prevention, & Control, 2023; Duan et al., 2017; Prevention, 2021; Trček, Fuchs, & Trülzsch, 2010). The significant prevalence of gastrointestinal illness including fatal cases of yersiniosis is also reported in many developed and developing countries like New Zealand (Rivas, Strydom, Paine, Wang, & Wright, 2021), England (Šumilo et al., 2023), Bangladesh (Butler, Islam, Azad, Islam, & Speelman, 1987), Iraq (Kanan & Abdulla, 2009), Iran (Soltan-Dallal & Moezardalan, 2004), and Nigeria (Okwori, Martínez, Fredriksson-Ahomaa, Agina, & Korkeala, 2009), which highlights significant underlying issues with food safety in different regions since *Y. enterocolitica* can multiply at refrigerators. However, incidences of yersiniosis and foodborne outbreaks seemed to be lower in developed countries (European Food Safety Authority [EFSA] & European Centre for Disease Prevention and Control [ECDC], 2023; Fredriksson-

Ahomaa, Lindström, & Korkeala, 2009; Rahman, Bonny, Stonsaovapak, & Ananchaipattana, 2011; Riahi, Ahmadi, & Zeinali, 2021).

Outbreaks of yersiniosis have been associated with unpasteurized milk, oysters, and more commonly with the consumption of undercooked food. Most often, people get this infection when they eat raw or undercooked pork or other meat and/or dairy products from cows, Buffaloes, sheep, goats, horses, and rabbits (Chlebicz & Śliżewska, 2018; Espenhain et al., 2019; Le Guern, Martin, Savin & Carniel, 2016). Less commonly, dogs and cats can spread the bacteria, however, direct transmission from infected animals and humans is uncommon. It can also be transmitted through the fecal-oral route (Augustin et al., 2020; Uçar, Yilmaz, & Çakiroglu, 2016). *Y. enterocolitica* can also be found in wild animals (Bari, Hossain, Isshiki, & Ukuku, 2011; Libera et al., 2022; Tsokana et al., 2020). The vast majority of the *Y. enterocolitica* isolates recovered from environmental samples, including those from slaughterhouses, fodder, soil, and water, have not proven to be pathogenic (Joutsen, Johansson, Laukkanen-Ninios, Björkroth, & Fredriksson-Ahomaa, 2020; Rahman et al., 2011).

Y. enterocolitica is prevalent in the environment, enabling it to contaminate water and food at every step from farm to fork, including production, processing, distribution, and consumption (Fredriksson-Ahomaa et al., 2009; Shoaib et al., 2019). *Y. enterocolitica* has been isolated from raw milk and pasteurized milk (Ahmed, Tahoun, Abou Elez, Abd El-Hamid, & Abd Ellatif, 2019; Jamali, Paydar, Radmehr, & Ismail, 2015; Sharifi Yazdi et al., 2023; Wang et al., 2009; Yucel & Ulusoy, 2006). It may have resulted from a malfunction in the process of pasteurization leading to insufficient treatment or postprocess contamination via food handlers. Therefore, the recovery of this pathogenic bacteria in pasteurized milk should be a cause for concern (Martin, Boor, & Wiedmann, 2018; Primavilla et al., 2023). The primary goal of the present study was to assess the prevalence of *Y. enterocolitica* contamination in raw sheep and goat milk. Additionally, to analyze the antibiotic resistance patterns of the isolated *Y. enterocolitica* strains against the most used antibiotics in our region. Lastly, monitor the seasonal variations in contamination and resistance patterns throughout the study period.

METHODS

Study design

This study was conducted from January to June 2023. A total of 300 samples of raw milk were obtained from various locations within Erbil Governorate. Specifically, 150 samples were taken from sheep milk (85 from villages (non-urban countryside) and 65 from sale points in the city). The other 150 samples were from goat milk (90 from villages and 60 from sale points). Approximately 200 ml of milk samples were transferred into labeled sterile plastic containers with screw lids under sanitary conditions. They were promptly transported

on the same day of collection to the Department of Medical Laboratory Sciences at the College of Science, Knowledge University, Erbil, Iraq, under cool conditions (within an icebox ~ 5° C) (Almashhadany & Osman, 2019). In the lab, specimens were examined immediately after being delivered or frozen at -18° C and analyzed later, as the freezing/thawing process has no destructive effect on *Y. enterocolitica* (Azizoglu & Kathariou, 2010; Mancini et al., 2022).

Isolation and Identification of Y. enterocolitica

Isolation of *Y. enterocolitica* was done based on the procedure reported by (International Organization for Standardization [ISO], 2017). Briefly, one ml was added to nine mL of BPW and thoroughly mixed. 9 mL of enrichment broth tube containing phosphate-buffered saline, 0.15% bile salts, and 1% sorbitol were combined with 1 mL of the prior mixture, and the mixture was incubated for three days at 25 °C. After enrichment, 0.5 mL of each tube was added to 4.5 ml of 0.5% KOH, then a loop-full of the mixture was streaked onto *Yersinia* selective agar base with *Yersinia* selective supplement and incubated at 25 °C for 24 to 48 h. All media and reagents were purchased from Himedia Laboratories Pvt. Ltd., India. Suspected colonies appeared in the distinctive bull's eye appearance. The pure suspected colonies were morphologically and biochemically identified according to (Younis, Elkenany & Dowidar, 2020).

Antimicrobial sensitivity testing profile

Antibiogram profiles of the isolates were obtained by screening of ten common antibiotics by utilizing the disc diffusion assay conducted on Mueller-Hinton agar. The Modified Kirby-Bauer technique was used and the inhibition zone diameters were interpreted according to Clinical & Laboratory Standards Institute (CLSI) guidelines (Wayne, 2020).

Ethical Considerations

The current study was conducted according to the ethical standards for research involving biological samples. The sample collection was done after the verbal consent from farmers and vendors, no animal was harmed during the sample collection process, since the raw milk was obtained post-milking. All procedures were performed following local and institutional guidelines for biosafety and sample handling. Data were anonymized to ensure the confidentiality of suppliers and locations.

Statistical analysis

The data analysis was done using the Statistical Package for the Social Sciences (SPSS, version 21) program (IBM, Armonk, NY, USA). The normal approximation process was used to convert estimates into confidence intervals. The Chi-square test was used to investigate the potential differences among groups and the degree of contamination. The statistical significance level was set at 0.05.

RESULTS

Out of 300 tested samples, 29 (9.70%) were contaminated with *Y. enterocolitica* based on the culture-based detection, and statistically, the contamination level is estimated to reach 14% (95% CI: 0.07 - 0.14). There was no significant difference between sheep and goat milk in terms of total contamination (p = 0.559). Similarly, villages and sale points showed roughly similar contamination proportions for both sheep and goat milk types (Table 1). It should be noted that it is unclear whether the detected isolates are genuinely pathogenic.

Table 1. Frequency of *Y. enterocolitica* in milk samples according to location

Source of milk	No. examined	Positive No. (%)	95% Confidence Interval	P value
Sheep milk				
Villages	85	8 (9.40)	0.05 - 0.17	0.715
Sale points	65	5 (7.70)	0.04 - 0.17	
Total	150	13 (8.70)	0.05 - 0.15	
Goat milk				
Villages	90	9 (10.00)	0.05 - 0.18	0.742
Sale points	60	7 (11.70)	0.06 - 0.23	
Total	150	16 (10.70)	0.07 - 0.17	

Regarding the seasonality of *Y. enterocolitica* detection in milk samples, no significant difference was detected between months for sheep milk (0.785) and goat milk (0.561) (Figure 1). Based on this data, a decline in the general occurrence of *Y. enterocolitica* in both types of milk was noticed with correlation coefficients (R^2) = 0.61 and 0.66 for goat and sheep milk samples, respectively.

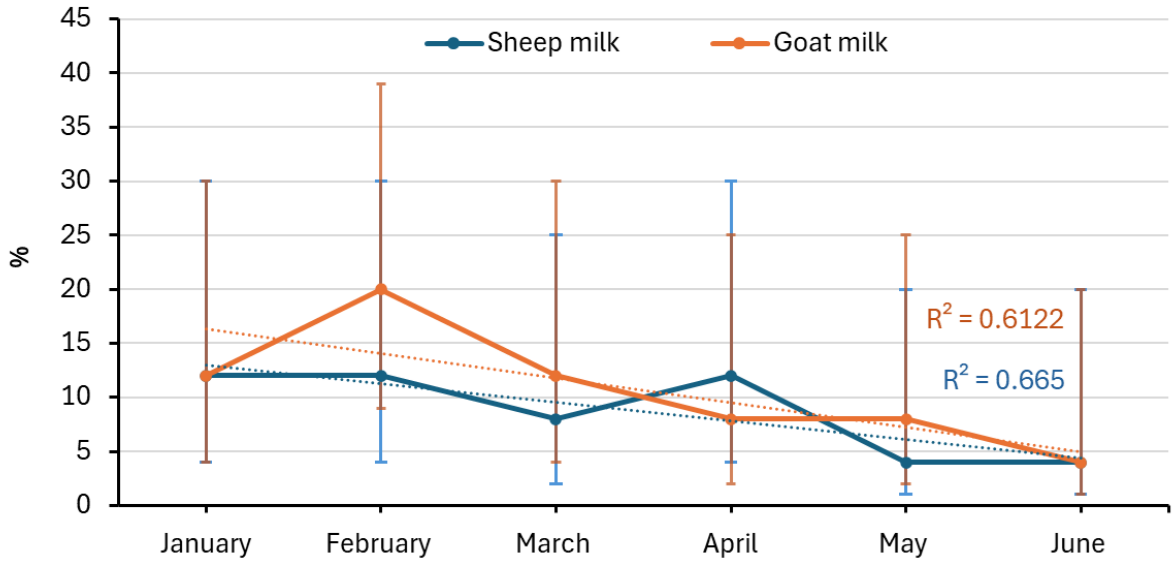

Figure 1: Detection of *Y. enterocolitica* in milk samples over the study period. Error bars represent 95% confidence intervals.

Regarding the antimicrobial resistance profile of the isolates, the intermediate phenotype was also alarming, especially for cefotaxime, streptomycin, and fluoroquinolones (Figure 2A and Table 2). Likewise, an alarming resistance was seen to all antibiotics tested including those considered as the drug of choice to treat yersiniosis (trimethoprim-sulfamethoxazole and fluoroquinolones) (Figure 2B).

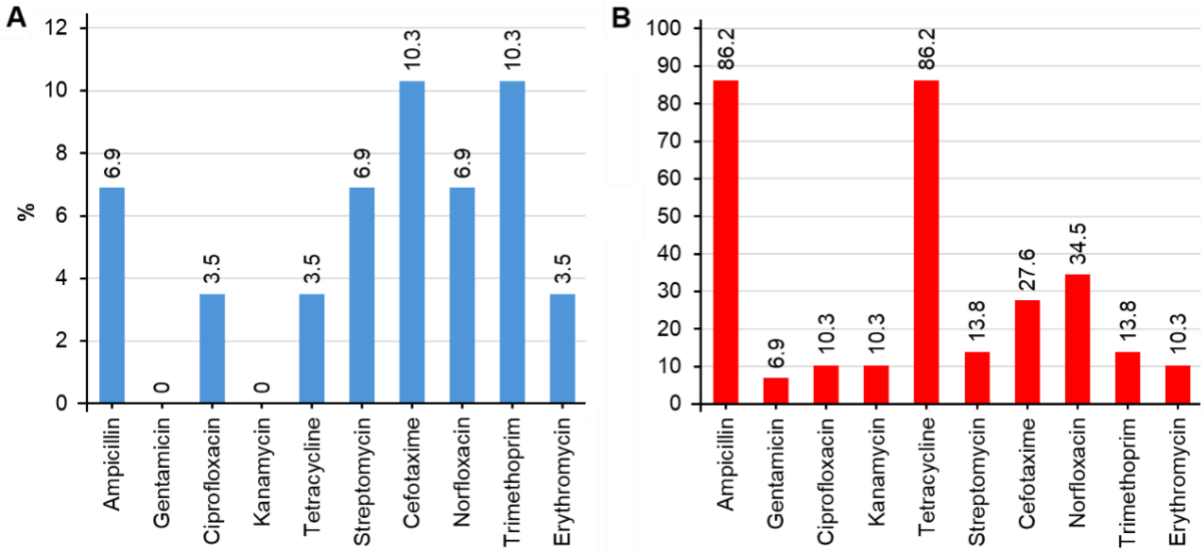

Figure 2: Antibiogram of *Y. enterocolitica* isolates (n=29) to different antibiotics. A) represents intermediate phenotypes while B) represents resistant phenotypes.

Table 2: Analyzing isolates antimicrobial susceptibility to a panel of antibiotics

Antibiotic (Disk conc. µg)	Examined No.	Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)
Ampicillin (10)	29	2 (6.9)	2 (6.9)	25 (86.2)
Gentamicin (10)	29	27 (93.1)	0 (0)	2 (6.9)
Ciprofloxacin (5)	29	25 (86.2)	1 (3.5)	3 (10.3)
Kanamycin (30)	29	26 (89.7)	0 (0)	3 (10.3)
Tetracycline (30)	29	3 (10.3)	1 (3.5)	25 (86.2)
Streptomycin (10)	29	23 (79.3)	2 (6.9)	4 (13.8)
Cefotaxime (30)	29	18 (62.1)	3 (10.3)	8 (27.6)
Norfloxacin (10)	29	17 (58.6)	2 (6.9)	10 (34.5)
Trimethoprim (5)	29	22 (75.9)	3 (10.3)	4 (13.8)
Erythromycin (15)	29	25 (86.2)	1 (3.5)	3 (10.3)

DISCUSSION

In Iraq, the frequency of *Y. enterocolitica* in foods, particularly milk, and its significance as a foodborne pathogen in public health remains unknown. While several studies (Ali & Al-Samarai, 2020a; Ali & Al-Samai, 2020b; Khalid & Abbas, 2021; Yousif & Sorchee, 2022), from Erbil and Iraq, have focused on detecting *Y. enterocolitica* in various food types, level of contamination in raw milk of sheep and goat remains unexplored. A recent molecular study in Erbil examined samples such as raw milk, soft cheese, ice cream, and meat, finding *Y. enterocolitica* in 5.1% of all samples (Yousif & Sorchee, 2022). However, the study did not specify the distribution of positive samples across different food types, though it did indicate that meat had the highest contamination levels.

The prevalence found in this study is in good agreement with the findings reported by a previous study in central and southern Iraq in which 168 cow and 72 buffalo raw milk samples from markets in five provinces (Baghdad, Karbala, Babylon, Al Najaf, and Al Qadysia) showed a 12% prevalence using the automated VITEK2 system and PCR detection (Ali & Al-Samarai, 2020). Moreover, a similar prevalence rate was also reported from different animal milk samples in Egypt (Sotohy, Diab, Ewida, Aballah, & Eldin, 2024). In contrast, other studies have reported a lower prevalence of *Y. enterocolitica* compared to the findings in this study. For instance, a complete set of 50 samples from cows, buffaloes, and sheep were collected from various markets in Basrah province, revealing a 4% prevalence of *Y. enterocolitica* through PCR detection (Khalid & Abbas, 2021). Additionally, locally produced soft cheese samples (167 cow cheese and 73 sheep cheese) from these regions exhibited a 4.5%

prevalence according to PCR detection (Ali & Al-Samari, 2020). In a study conducted by Rahimi and associates in Iran, the prevalence of *Y. enterocolitica* was examined in traditional and commercial dairy products in Isfahan Province. Out of 552 milk and dairy product samples, 28 (5.07%) were confirmed to be positive for *Y. enterocolitica* through culture methods. Additionally, 24 of these 28 isolates (4.59%) were confirmed positive using PCR tests. *Y. enterocolitica* was most prevalently detected in untreated cow milk and traditional cheese. Interestingly, no positive results were recorded in pasteurized cow milk, untreated camel milk, yogurt, commercial ice cream, Doogh, commercial cheese, butter, and curd (Rahimi, Sepehri, Dehkordi, Shaygan, & Momtaz, 2014). Such variations in detection rates can be attributed to different factors including the detection method, sample size, geographical location, and local climate and environmental conditions (Gupta, Gulati, Bhagat, Dhar, & Virdi, 2015; Shoaib et al., 2019).

In terms of seasonality, the gradual decrease in prevalence of *Y. enterocolitica* as time progresses from mid-winter to early summer is consistent with the findings reported previously (Bernardino-Varo, Quiñones-Ramírez, Fernández, & Vázquez-Salinas, 2013). However, a recent study indicated that the prevalence of *Y. enterocolitica* found in food samples, including dairy products, does not consistently correlate with seasons (Primavilla et al., 2023). The mentioned study conducted in the Umbria region of Italy found no significant seasonal variation in the prevalence level of *Y. enterocolitica* in food samples over several years.

Y. enterocolitica is known to produce a pair of distinct β-lactamases. One of them is a class A constitutive enzyme, while the other is an inducible class C enzyme that is not affected by β-lactamase inhibitors (Pham, Bell, Martin, & Carniel, 2000). The β-lactamase confers resistance to different penicillin, which has been observed in this study and elsewhere (Sotohy et al., 2024). Despite this resistance, the organism is believed to remain susceptible to extended-spectrum cephalosporins. However, we noticed an alarming resistance or intermediate susceptibility to cefotaxime, fluoroquinolones, and aminoglycosides. Resistance to aminoglycosides and cefotaxime has also been reported in isolates from milk samples (Sotohy et al., 2024). The resistance to these classes of antibiotics can be mediated by one or more different mechanisms including efflux pumps, antibiotic-modifying enzymes, or mutation in the target protein/RNA (Capilla et al., 2004; Cornelis, 2018).

CONCLUSION

This study provides critical insights into the prevalence, antibiotic resistance patterns, also seasonal trends of *Y. enterocolitica* in raw sheep and goat milk from Erbil Governorate. The detection of *Y. enterocolitica* in 9.7% of tested samples underscores the potential food safety risks associated with raw milk consumption in the region. While no significant seasonal or locational differences were observed, the declining trend in contamination from winter to summer warrants further exploration. The alarming resistance patterns to key antibiotics emphasize the urgent need for enhanced monitoring, regulation, and public health interventions to mitigate the risks posed by this pathogen. Future research should focus on exploring the mechanisms of antibiotic resistance and evaluating effective strategies to control *Y. enterocolitica* contamination in dairy products.

Conflicts of Interest

The authors declare no conflicts of interest.

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