

Bacterial contamination of raw milk in smallholder dairy farms in northwestern Algeria

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Article information

Article history:

Received May 3, 2026

Accepted June 25, 2026

Published July 5, 2026

Keywords:

Bacterial contamination

Dairy hygiene

Escherichia coli

Klebsiella spp

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Abstract

Raw milk production in smallholder dairy systems remains a major challenge for food safety due to inconsistent hygiene practices that can easily lead to bacterial contamination. This study investigated the distribution of important pathogenic bacteria *Klebsiella spp.* and *Escherichia coli* in small dairy farms located in northwestern Algeria. The main objective was to understand whether contamination levels are mainly influenced by differences between farms or by the biological characteristics of the bacteria themselves. Aliquots of bulk tank milk were collected aseptically over a 20-day period and analysed using selective culture media for bacterial isolation and enumeration. The effect of the farm of origin, the bacterial species and their interaction on the bacterial load was evaluated using a two-way ANOVA with data $\log_{10}$ transformed. Comparisons between the farms were conducted using the Kruskal–Wallis test. The results showed that bacterial counts were very similar across the three farms, with no significant differences detected between them for any of the studied species. Statistical analyses, confirmed that the “farm effect” was not significant. In contrast, a clear and significant difference was observed between bacterial species, indicating that the type of microorganism plays a more important role in determining contamination levels than farm-specific management practices. Overall, the findings suggest that contamination in this context is not strongly dependent on individual farm conditions, but is more closely related to the ecological behavior of each bacterial species. This highlights the need for broad, system-wide hygiene strategies rather than farm-by-farm interventions to improve raw milk safety in smallholder dairy systems.

DOI: [10.33899/ijvs.2026.171419.4721](https://doi.org/10.33899/ijvs.2026.171419.4721), ©Authors, 2026, College of Veterinary Medicine, University of Mosul.

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Introduction

Milk is among the most nutritionally complete foods available to human populations, but milk is also one of the most prone to microbial contamination along the farm-to-consumer chain. The potential for this contaminant to reach humans via human food is increased in smallholder dairy systems, which make up a large portion of the milk produced across North Africa and sub-Saharan Africa, where there are limited refrigeration methods available, infrequent washing of milking equipment, and a high dependence on informal

marketing channels (1). Globally, dairy products are estimated to account for about 4% of the foodborne disease burden (1), while animal-source foods, including milk, cause approximately 12% (2) of food borne illness caused by pathogenic *E. coli* and other enteric pathogens (2).

In communities that regularly consume raw milk, the economic impact of outbreaks is direct and immediate (3).

Among the bacterial groups commonly used to assess raw milk hygiene and safety, *Escherichia coli* and *Klebsiella spp.* are particularly relevant indicators in smallholder dairy systems. *Escherichia coli* is widely known as a faecal

indicator. Its presence in milk indicates contamination that happens during milking or handling (2). In contrast, *Klebsiella spp.* are environmental opportunists most often found in bedding, soil, and water materials that are a constant presence in livestock environments and hard to eradicate without changes to farm structures (4). It is important to understand which of these organisms is most common in a particular farming situation and whether contamination patterns vary between farms to prioritise hygiene interventions effectively.

In Algeria, research on the quality of raw milk has mainly focused on contamination during processing and in pasteurised products (5), leaving an information gap regarding the primary production stage, especially in the smallholder sector, where the bulk of milk is collected informally. If variability in pathogen load is associated with specific farms, targeted interventions may be warranted. However, if contamination is evenly distributed among farms, a broader, systemic public health strategy is needed. The Chlef region is one of the main dairy-producing areas in north-western Algeria. Research carried out in western Algeria has shown that raw cow's milk is heavily contaminated with microbes. Seddaoui and colleagues (6) found that raw milk samples collected from dairies in western Algeria had an average fecal coliform count of 1.89×10^4 CFU/mL, suggesting that milking, handling, and storage practices were not sufficiently hygienic. This contamination indicates the possible presence of enteric bacteria and underscores the importance of ongoing surveillance of raw milk quality.

Thus, the aim of the present study was to test this precise distinction and evaluate the level of bacterial contamination from three smallholder farms located in the Chlef wilaya of northwestern Algeria, using statistical modelling to assess whether the variability was more strongly correlated with farm identity or with bacterial species characteristics.

Materials and methods

Ethical statement

All procedures were carried out in accordance with Algerian legislation on the protection of animals used for experimental purposes (Law No. 88-08 relating to veterinary activities) and with internationally accepted principles for the care of animals. The study design was observational, with no experimental manipulations. Before collecting data, we obtained written permission from the owner of each farm.

Study area and sample collection

Three semi-intensive smallholder dairy farms located in the wilaya of Chlef, in north-western Algeria, were selected as study sites. A geographical map of the study area is presented in Figure 1, showing the location of the three farms: Ferme Pilote Cheikh Belkhedim, Ferme Pilote Si

Yahi M'hamed, and Ferme Belhirach. These farms are representative of the local smallholder dairy production system in terms of herd size, feeding practices, housing conditions, and farm infrastructure. A total of 60 raw milk samples were initially targeted for collection; specifically, an individual milk sample was collected aseptically from each farm's bulk tank during the morning milking over a 20-day consecutive period ($n=20$ samples planned per farm). Due to occasional temporary unavailability of specific laboratory reagents during the analysis periods, some samples could not be processed for all bacterial groups, resulting in the final varying sample sizes (n) reported in the results. The sample tubes were immediately placed in a cool box at 4 °C and transported to the laboratory, where analyses were initiated within 24 hours of collection.

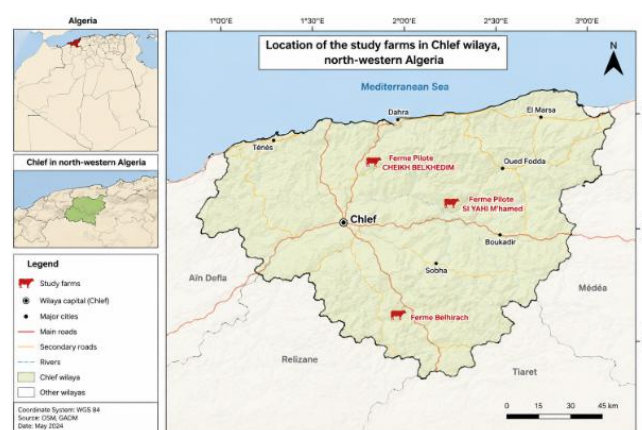


Figure 1: Location of study farms in Chlef wilaya.

Bacterial isolation and enumeration

Selective culture media appropriate for each target organism were used to isolate bacteria (Table 1). Fecal coliforms, including *Escherichia coli*, were quantified using deoxycholate lactose agar (DLA) (Biokar Diagnostics, France) at 37 °C for 24 h. The identification of *E. coli* was confirmed on EMB Levine Agar (Laboratoire Humeau, France). *Klebsiella spp.* were isolated and identified on MacConkey agar (Condalab, Cat. No. 1052) following 24 h of incubation. Presumptive isolates were subsequently confirmed biochemically using Simmons citrate agar (HiMedia Laboratories, India). Colony counts were performed on the 10^{-4} dilution plates and multiplied by the corresponding dilution factor to obtain CFU/mL values.

Statistical analysis

Since the distributions of bacterial counts were not normal, the raw counts were log₁₀-transformed [$\log_{10}(\text{CFU}+1)$] before any statistical analysis was performed. Bacterial counts were compared among the three farms, separately for each species, using the Kruskal–Wallis

nonparametric test. A two-way ANOVA was performed afterwards to analyse the effects of farm, bacterial species and their interaction on log₁₀-transformed counts. The

significance level of all tests was set at $\alpha = 0.05$. XLSTAT (Addinsoft, Paris, France; version 2023) was used for all calculations.

Table 1: Selective media used for the isolation and identification of target bacteria

Microorganism	Selective medium	Manufacturer	Incubation conditions
Faecal coliforms	Lactose Deoxycholate Agar	Biokar Diagnostics, France	37°C, 24 h
<i>Escherichia coli</i>	EMB Levine Agar	Humeau Laboratory, France	37°C, 24 h / 32.5±2.5°C, 24 h
<i>Klebsiella spp.</i>	MacConkey Agar+ Simmons Citrate	Condalab (Cat. 1052); HiMedia, India	37°C, 24 h (biochemical confirmation)

EMB: eosin methylene blue.

Results

Descriptive statistics

Both quantified species displayed considerable variation in bacterial counts both within and between farms (Table 2). Mean concentrations of *Klebsiella spp.* ranged from (85.4 to 89.7) × 10⁴ CFU/mL whereas those of *Escherichia coli* ranged from 81.5 × 10⁴ to 96.3 × 10⁴ CFU/mL. The high standard deviations observed across bacterial taxa and farms

indicate substantial heterogeneity in contamination levels, suggesting that contamination events were sporadic rather than constant within the studied dairy production systems.

Following log₁₀ transformation (Table 3), the mean values showed a significant convergence, with a range of 5.91±0.24 to 6.01±0.18 log₁₀ (CFU/mL +1). This suggests that most of the variability observed on the raw scale is due to a few samples with high counts.

Table 2: Descriptive statistics of bacterial contamination by farm

Bacterium	Farm	n	Min (CFU× 10 ⁴ /mL)	Max (CFU× 10 ⁴ /mL)	Mean ± SD (CFU× 10 ⁴ /mL)
<i>Klebsiella spp.</i>	1	17	2	130	85.4 ± 37.2
<i>Klebsiella spp.</i>	2	15	25	137	88.9 ± 36.8
<i>Klebsiella spp.</i>	3	15	8	151	89.7 ± 42.1
<i>Escherichia coli</i>	1	4	13	130	81.5 ± 44.0
<i>Escherichia coli</i>	2	7	19	149	83.7 ± 47.2
<i>Escherichia coli</i>	3	6	59	165	96.3 ± 35.8

CFU: colony-forming units; SD: standard deviation.

Table 3: Log₁₀-transformed bacterial counts [log₁₀(CFU/mL +1)] by farm and species

Bacterium	Farm	Mean log ₁₀ (CFU/mL +1) ± SD
<i>Klebsiella spp.</i>	1	5.93 ± 0.21
<i>Klebsiella spp.</i>	2	5.95 ± 0.19
<i>Klebsiella spp.</i>	3	5.94 ± 0.23
<i>Escherichia coli</i>	1	5.91 ± 0.24
<i>Escherichia coli</i>	2	5.92 ± 0.26
<i>Escherichia coli</i>	3	6.01 ± 0.18

SD: standard deviation.

Between-farm comparisons

The Kruskal–Wallis test indicated no significant differences in bacterial counts among farms for either *Klebsiella spp.* (H = 0.24, df = 2, p = 0.88) or *Escherichia coli* (H = 0.97, df = 2, p = 0.59) (Table 4). The lack of statistically detectable differences between farms suggests

that contamination is broadly evenly distributed across the three production units.

Table 4: Kruskal–Wallis test results for between-farm comparison of bacterial counts

Bacterium	H statistic	df	p-value	Significance
<i>Klebsiella spp.</i>	0.24	2	0.88	NS
<i>Escherichia coli</i>	0.97	2	0.59	NS

df: degrees of freedom; NS: not significant (P>0.05).

Two-way ANOVA

Two-way ANOVA showed no significant farm effect (F=0.82, P>0.05) and no significant farm × bacterial species interaction (F=0.63, P>0.05) (Table 5). By contrast, bacterial species had a statistically significant effect on contamination levels (F=4.91, P<0.05), suggesting that the identity of the bacterial taxon, rather than the farm from which samples

were collected, was the main factor driving the observed differences in counts.

Table 5: Two-way ANOVA results for the effects of farm origin, bacterial species, and their interaction on log₁₀-transformed bacterial counts

Source of variation	F value	P-value	Significance
Farm	0.82	>0.05	NS
Bacterial species	4.91	<0.05	Significant
Farm×Bacterial species	0.63	>0.05	NS

NS: not significant (P>0.05).

Discussion

Statistical analyses revealed no significant differences in bacterial contamination among the three farms studied. Neither the Kruskal–Wallis test (Table 4) nor the two-way ANOVA (Table 5) detected a significant farm effect on *Klebsiella* spp. Or *Escherichia coli* counts (P> 0.05). This does not indicate the absence of contamination; rather, it suggests that contamination levels were broadly comparable among the three farms studied. In other words, the risk of contamination appears relatively uniform among the different production units. This finding is consistent with previous reports from other smallholder livestock systems operating under similar production conditions.

In Zimbabwe, Washaya et al. (7) observed that the microbiological quality of milk was largely similar across smallholder farms. This was mainly explained by generally poor hygiene practices consistent across farms, rather than by differences in management between individual holdings.

Similarly, in Kenya, Nyokabi et al. (1) showed that even when some farms reported adopting food safety measures, this did not necessarily lead to improved milk bacteriological quality. This suggests that the main constraints stem from the overall production environment (lack of infrastructure, equipment, and resources) rather than from individual farmers' practices.

The major species effect observed in the two-way ANOVA for *Klebsiella* spp. and *E. coli* can be understood biologically and aligns with known ecological differences between these bacterial groups. *E. coli* is mainly of faecal origin and may contaminate milk through the teats during milking or by way of dirty hands and equipment (2,8). In contrast, *Klebsiella* spp. are mainly environmental organisms whose reservoirs, bedding material, soil, and water troughs, are found everywhere in livestock environments and cannot be easily eliminated without specific structural improvements (4).

Thus, the variation in average counts across taxa, even with no differences between farms, is due to these inherent ecological distinctions, not any factor related to management practices (5). The high standard deviations observed for

Klebsiella spp. and *E. coli* across the farms (Table 2) are meaningful in themselves. They suggest irregular fluctuations in contamination levels, more likely the result of sporadic, short-term events than of a constant or stable level of contamination. Such events may include inadequate teat preparation before milking, incomplete cleaning of milking equipment, or temporary failures in cooling systems.

This interpretation is consistent with the findings of Tarrah et al. (3), who showed that when microbial counts exceed critical thresholds, this is generally associated with persistent high-contamination episodes. These are often linked to biofilm formation in milking equipment, rapid bacterial regrowth on contaminated surfaces, and insufficient or irregular cleaning practices. In such situations, even a single lapse in hygiene can lead to a sudden spike in bacterial counts, which may strongly influence average values, particularly in farms where hygiene management is not strictly controlled. Furthermore, the observed bacterial counts, after application of the dilution factor (10⁴), ranged from 8.0 × 10⁵ to 1.65 × 10⁶ CFU/mL. According to Codex Alimentarius guidelines, raw milk intended for further processing should not exceed 10⁵ CFU/mL. (9) All observed values therefore exceeded this acceptable limit, indicating that the raw milk produced by the studied farms did not comply with international microbiological standards and reflecting inadequate hygienic conditions at farm level. To address this source of variability, what is needed is reliable, systematic hygiene practices, rather than reactive measures applied separately to each farm.

Overall, the results lead to a clear practical message: since contamination levels do not vary significantly between farms, intervention strategies should focus on system-wide solutions that can be applied consistently across the smallholder sector. These include standardised teat cleaning and post-milking dipping procedures; regular cleaning and disinfection of milking equipment and storage containers; improving bedding quality and drainage to reduce environmental *Klebsiella* levels; and ensuring reliable access to cold storage.

In a Kenyan smallholder context similar to ours, Mogotu et al. (10) demonstrated that structured hygiene training focused on these practices led to clear, measurable reductions in indicator bacterial counts.

This study has some limitations that should be acknowledged. The sampling was restricted to three farms within a single wilaya, which limits the generalisability of the findings. In addition, the number of samples per bacterium was uneven and relatively low for *E. coli* (n = 4–7), potentially reducing statistical power. We also lacked data on key farm factors such as hygiene practices, bedding type, and equipment age, preventing a mechanistic explanation of the observed contamination patterns. Future studies should expand the geographic scope, include more farms, and integrate antimicrobial resistance profiling,

particularly for *Klebsiella* spp. and *E. coli* (5), along with systematic assessments of farm hygiene to better guide evidence-based interventions.

Conclusion

The raw milk samples collected from the three dairy farms showed comparable contamination patterns for *Klebsiella* spp. and *Escherichia coli*. The significant bacterial species effect, together with the absence of a farm effect, indicates that contamination levels were mainly influenced by the biological characteristics of the bacterial species rather than by differences among farms. Furthermore, all bacterial counts exceeded the microbiological limits recommended for raw milk intended for processing, reflecting inadequate hygienic conditions during milk production and handling. These findings suggest that the implementation of simple, standardised hygiene measures across smallholder dairy farms could substantially improve raw milk quality and safety in the region.

Authors' contributions

Bouhroum N: study design, field sampling, statistical analysis, manuscript writing and revision. Lankri E: microbiological analyses, data validation, manuscript revision. Boudour K: field coordination, data collection, critical review of manuscript. All authors read and approved the final version.

Acknowledgements

The authors sincerely thank the owners and staff of the three participating farms in the Chlef wilaya for their cooperation and for granting access to their premises and animals throughout the sampling period.

Funding

This research received no specific external funding.

Conflict of interest

The author(s) declare no conflicts of interest.

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التلوث البكتيري للحليب الخام في مزارع الألبان الصغيرة في شمال غرب الجزائر

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الخلاصة

يمثل إنتاج الحليب الخام في أنظمة الألبان الصغيرة تحديًا كبيرًا لسلامة الأغذية بسبب عدم ثبات ممارسات النظافة، مما قد يؤدي بسهولة إلى تلوث بكتيري. تناولت هذه الدراسة توزيع بكتيريا ممرضة مهمة هي: الإشريكية القولونية وأنواع الكلبسيلا في مزارع ألبان صغيرة تقع في شمال غرب الجزائر. وكان الهدف الرئيسي هو تحديد ما إذا كانت مستويات التلوث تتأثر أساسًا بالاختلافات بين المزارع أو بالخصائص

البيولوجية للبكتيريا نفسها. تم جمع عينات جزئية من حليب خزانات التجميع بطريقة معقمة على مدى عشرون يومًا، ثم جرى تحليلها باستخدام أوساط زرع انتقائية لعزل البكتيريا وعدّها. وتم تقييم تأثير المزرعة الأصلية، والنوع البكتيري، والتفاعل بينهما على الحمل البكتيري باستخدام تحليل التباين ثنائي العوامل بعد تحويل البيانات لوغاريتميًا كما أجريت المقارنات بين المزارع باستخدام اختبار كروسكال-واليس. أظهرت النتائج أن أعداد البكتيريا كانت متشابهة جدًا بين المزارع الثلاث، دون وجود فروق معنوية بين أي من الأنواع المدروسة. وأكدت التحليلات الإحصائية، أن تأثير "المزرعة" غير معنوي. في المقابل، تم تسجيل فرق واضح ومعنوي بين الأنواع البكتيرية، مما يشير إلى أن نوع الكائن المجهر يلعب دورًا أكبر في تحديد مستويات التلوث مقارنة بممارسات الإدارة الخاصة بكل مزرعة. بشكل عام، تشير النتائج إلى أن التلوث في هذا السياق لا يعتمد بشكل كبير على ظروف كل مزرعة على حدة، بل يرتبط بشكل أكبر بالسلوك البيئي لكل نوع بكتيري. وهذا يبرز الحاجة إلى استراتيجيات نظافة شاملة ومنهجية على مستوى النظام بدلًا من تدخلات فردية لكل مزرعة، من أجل تحسين سلامة الحليب الخام في أنظمة الألبان الصغيرة.