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Quality and antibiotic residues in raw milk sold informally in Manabí and Santo Domingo de los Tsáchilas, Ecuador

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Abstract
This study aims to evaluate the quality and presence of antibiotic residues in raw cow's milk marketed through informal channels in the provinces of Santo Domingo de los Tsáchilas and Manabí, Ecuador. A total of 406 samples were collected between April 2023 and August 2024. Only 12.32% (50/406) met the requirements established by the Ecuadorian Technical Standard INEN 9, while 87.68% (356/406) failed to comply with at least one quality parameter. The most frequent non-conformities were observed when analyzing pH (72.41%), titratable acidity (68.72%), and protein stability (55.67%), likely reflecting deficient hygienic practices during production and handling. Furthermore, 6.40% (26/406) of the samples tested positive for antibiotic residues. These findings underscore significant shortcomings in milk quality within informal markets and underscore the potential public health risks associated with consuming improperly handled raw milk in these Ecuadorian provinces.

Introduction
Bovine milk is widely regarded as a highly nutritious food with high biological value due to its composition, which includes mineral salts, water-soluble and fat-soluble vitamins, enzymes, and essential nutrients such as water, proteins, lactose, and fat. However, these same characteristics make it susceptible to adulteration and contamination, like the addition of water that causes changes in density, decrease nutritional quality, and increases the risk of proliferation of microorganisms, resulting in a milk that is not suitable for human consumption (Piston *et al*., 2014; Renhe *et al.*, 2019; Magan *et al*., 2021; Techane, 2023).

In Ecuador, the production of bovine milk is a fundamental axis of the economy, especially for small and medium dairy producers, as it contributes approximately 10% of total employment at the national level. In 2023, it was estimated the total milk production of 5.6 million liters per day nationwide, mainly contributed by the highland provinces (79.5% of total production), followed by the provinces from the coastal region (16.3% of the total production) and provinces from the Amazonian region (4.2% of the total production). In the Coastal region, the province of Manabí is the largest bovine milk producer, accounting for approximately 11.31% of total production; whereas Santo Domingo de los Tsáchilas contributes 4.67% (INEC-ESPAC, 2023).

One of the main issues in Ecuador is that only 16% of the milk produced is industrialized, and more than 80% of the national milk production is sold in the informal markets. The milk sold informally lacks quality control, and there is no guarantee that it is safe or suitable for human consumption or for processing. The distribution of this milk occurs in the absence of a cold chain and under substandard hygiene conditions, resulting in the contamination of the milk with zoonotic pathogens (Artursson *et al*., 2018). Additionally, this milk may also be contaminated with drugs such as antibiotic, posing a health risk to consumers (Pahlavanzadeh *et al*., 2021; Zhang *et al*., 2022; Santacroce *et al*., 2023).

Previous studies carried out in the Pichincha province, Ecuador, showed that 94.31% of raw milk samples collected in informal markets do not fulfill at least one of the quality parameters established by the Ecuadorian Technical Standard (NTE) INEN 9:2012 (INEN, 2012; Puga-Torres *et al*., 2024). A large part of the population buys milk in informal markets because it is cheaper, easy to access, and there is widespread cultural consumption of this milk (Terán, 2019). Therefore, informal markets pose a significant risk to public health and present a substantial challenge to local regulatory agencies. In order to ensure the quality of milk sold at these sale points, it is essential that these agencies implement effective monitoring programs. In this context, this study aimed to determine the quality and presence of antibiotic residues in raw bovine milk sold informally in the provinces of Manabí and Santo Domingo de los Tsáchilas, as these provinces contribute 16% of the national milk production, where informal markets happen, and the quality of the milk sold to local consumers is unidentified.

Materials and Methods

Study design and sampling

This cross-sectional study was conducted between April 2023 and August 2024. Samples of raw bovine milk were collected in informal markets located in the 22 cantons of the province of Manabí (24 de Mayo, Bolívar, Chone, El Carmen, Flavio Alfaro, Jama, Jaramijó, Jipijapa, Junín, Manta, Montecristi, Olmedo, Paján, Pedernales, Pichincha, Portoviejo, Puerto López, Rocafuerte, San Vicente, Santa Ana, Sucre, and Tosagua), and the 2 cantons of Santo Domingo de los Tsáchilas (Santo Domingo and La Concordia) in Ecuador.

The number of informal markets in both provinces is unknown; therefore, walking routes were established in specific areas of each canton based on information from locals. All informal markets and sale points found were considered sampling points; thus, a convenience sampling method was used. At each sampling point, 1 liter of raw milk was collected 4 times, with an interval of 15 days between each collection. In total, 406 raw milk samples were collected, 72 from Santo Domingo de los Tsáchilas and 334 from Manabí. Milk samples were not collected from the same place or seller, as informal milk sales are not constant in one location. We purchased milk samples that were sold in cafeterias, artisanal dairies, open markets, bakeries, butcher shops, stores, motorized vehicles (pickups and motorcycles), homes, as well as in public areas (street vendors).

Laboratory analysis

The 406 samples were obtained according to the NTE INEN ISO 707:2014 guidelines (INEN, 2014). Milk samples were placed in two sterile 50 mL containers, one containing Bronopol and the other without this preservative. The containers were filled with 50 mL, ensuring that no air was left inside. Each sample was assigned an alphanumeric code considering the province initials, sampling canton, and chronological order of sampling. Milk samples were transported in coolers (temperature ranging 4±2°C) to the Laboratory of Milk Quality Control at Centro Experimental Uyumbicho of the Facultad de Medicina Veterinaria y Zootecnia of the Universidad Central del Ecuador. The processing of samples was conducted within 72 hours post-sampling.

Milk quality parameters were determined using the LACTOSCAN analyzer (Boeckel+Co GmbH+Co KG, model SAP-CC-028608, Hamburg - Germany). This rapid-analysis device employs ultrasound technology to simultaneously assess key physicochemical parameters of raw milk, including relative density, percentage of added water, protein, lactose, fat, total solids, and non-fat solids. The device operates by emitting and detecting ultrasonic waves at a specific frequency. After turning on and calibrating the equipment for cow's milk, the homogenized sample is placed into the intake tube and the "Enter" key is pressed. The analyzer draws in and processes the sample in approximately 60 seconds, after which the results are displayed on the screen.

The somatic cell count (SCC) was performed using the EKOMILK SCAN analyzer (EON Trading INC, 6006, Stara Zagora Industrial Area, Promishlena Str. 18, Bulgary). This equipment estimates SCC using ultrasonic technology in combination with a surfactant agent added to the milk. The surfactant forms a gel whose density increases proportionally with the number of somatic cells. The time it takes for the sample to pass through a calibrated capillary is measured and automatically converted to the SCC (cells/mL). To ensure accuracy, both the milk sample and the surfactant solution must be maintained at 20-22°C. The equipment is turned on 5 minutes before testing. A volume of 5 mL of surfactant solution is poured into the reaction flask, followed by 10 mL of raw milk (previously agitated). After pressing "OK," the analyzer automatically mixes the sample. Upon completion of the analysis, the SCC result is immediately displayed.

Titratable acidity (expressed in Dornic degrees) and pH were measured using a potentiometer (EON TRADING, Model PHT 004TA, Serial SN H00601659, Promishlena Str. 18, Bulgary). For analysis, the electrode is immersed in the homogenized milk sample, and the pH value is recorded once the reading stabilizes on the display.

Protein stability was assessed using the 68% alcohol test. Two milliliters of milk were mixed with 2 ml of 68% ethanol using a graduated pipette. The sample was gently agitated in a circular motion,

and results were interpreted visually. A positive result was indicated by the presence of coagulation or clumping, whereas a negative result showed no visible clots or aggregates.
Finally, antibiotic residues (β-lactams, tetracyclines, and sulfonamides) were detected using TriTest BTS rapid test strips (Ring Biotechnology Co. Ltd., Beijing, China). This test is based on lateral flow immunochromatography, designed to detect antibiotic residues above maximum residue limits. The milk sample is brought to room temperature, and 200 µL is transferred to a microwell using a pipette. The sample is aspirated and expelled at least five times to mix. After a 3-minute incubation at 20-25°C, the test strip is fully immersed in the mixture. Following a second 3-minute incubation at room temperature, the results are interpreted. Each strip displays four lines: C (control), B (β-lactams), T (tetracyclines), and S (sulfonamides). For the test to be valid, the control line must appear colored. The result is negative when the T, B, or S lines are darker than the control line. A positive result is indicated when one or more of the T, B, or S lines are lighter than or absent compared to the control line.

Data analysis
Microsoft Excel (version 2024, Microsoft Corporation, Redmond, United States) was used to collect the laboratory results and construct the database. All statistical analysis was performed using RCRAN version 1.2.5019 (RStudio Inc., Boston, MA, USA) and RStudio version 2024.04.2+764.
Descriptive statistical analysis was used to determine the proportion of samples that fulfill the quality parameters for raw milk, according to the Ecuadorian regulations. Frequency tables were used to compare the reference values of the NTE INEN 9 and the observed values according to provinces. In addition, the mean, minimum, and maximum values were calculated for the numeric variables such as SCC, relative density, titratable acidity, percentage of added water, protein, lactose, fat, total solids, and non-fat solids: for the qualitative variables, such as protein stability, and presence of antibiotic residues, results were expressed in frequency and percentages.
To determine whether there was a significant difference in quality requirements fulfillment between provinces, the chi-square test was used for the qualitative variables, and for the numeric variables, the non-parametric Mann-Whitney U test was used, as observed results did not follow a normal distribution. A significance level of 5% was considered.

Results and Discussion
Overall analysis and according-to-province analysis
Of the 406 raw milk samples collected from Manabí and Santo Domingo de los Tsáchilas, 87.68% (356/406) failed to meet one or more quality parameters established by NTE INEN 9, rendering them unsuitable for human consumption or processing. Among these, 97.75% (348/356) originated from Manabí and 2.25% (8/356) from Santo Domingo. No significant difference in overall compliance between the provinces was observed ($p=0.08$). Similar studies have reported variability in compliance across Ecuadorian regions, particularly in formal markets (Puga-Torres *et al*., 2022). Compared to previous studies in Pichincha, where non-compliance ranged from 74.0% to 94.3%, our findings are relatively consistent (Guamán & Puga-Torres, 2022; Puga-Torres *et al*., 2024).
The most frequently non-compliant parameters were pH (72.41%), titratable acidity (68.72%), protein stability (55.67%), and lactose content (47.04%), all suggesting poor hygienic conditions. In contrast, total solids (28.08%), protein concentration (23.65%), and antibiotic residues (6.40%) had lower non-compliance rates (Table 1).
The results obtained by each province are detailed in Table 2.

Relative density and water adulteration
Relative density was out of range (1.028-1.033 g/mL) in 43.35% (176/406) of samples, with 28.98% (51/176) occurring outside the permitted values (mean: 1.029; range: 1.009-1.038). Added water was detected in 28.82% (117/406) of the samples. No significant differences in these parameters were found between provinces ($p>0.9$). Compared to Pichincha province (22.0-26.0% non-compliance),

our results indicate a higher incidence (Guamán & Puga-Torres, 2022; Puga-Torres *et al*., 2024). Low density typically reflects dilution, while elevated density may indicate fat removal (Alichanidis *et al*., 2016; De Souza Gondim *et al*., 2017; Liu *et al*., 2015). Water addition is a common adulteration method aimed at increasing milk volume, but it reduces the nutritional value by diluting components such as solids-not-fat, and it promotes microbial growth (Condé *et al*., 2020; Techane, 2023).

Titratable acidity and pH
Titratable acidity exceeded the legal range (13-17ºD) in 68.72% (279/406) of samples, with 76.70% (214/279) above the upper limit. Average acidity was 17.65 ºD (range: 5.3-44ºD). Significant differences were noted between both provinces ($p<0.0001$), with 80.29% of non-compliant samples coming from Manabí. These results likely indicate microbial contamination or subclinical mastitis (Fusco *et al*., 2020). Comparable non-compliance was observed in Pichincha (72.7%), but it was lower in Rumiñahui (46%) (Guamán & Puga-Torres, 2022; Puga-Torres *et al*., 2024). Analysis of pH was out of range (6.6-6.8) in 72.41% (294/406) of samples; 27.21% (80/294) were above the upper limit. The majority of these cases (77.55%) were from Manabí, with significant interprovincial differences ($p<0.0001$). Low pH and high acidity are attributed to lactose fermentation into lactic acid (Hanrahan *et al*., 2018; Khaldi *et al*., 2022; McHugh *et al*., 2020), driven by microbial activity due to poor hygiene and temperature management during milking, transport (Bettera *et al*., 2023; Ichimura, 2024) and commercialization (Obladen, 2014; Wang *et al*., 2024). These changes compromise casein micelle stability, impairing pasteurization and leading to coagulation (Eshpari *et al*., 2017; Hanuš *et al*., 2021). Conversely, elevated pH may indicate mastitis, as inflammation increases mammary epithelial permeability, allowing influx of ions like sodium and bicarbonate, altering milk pH (Sumon *et al*., 2020; Hossain *et al*., 2021; Costa *et al*., 2025).

Nutritional parameters
Fat content was below the 3.0% minimum in 32.76% (133/406) of samples (mean: 3.47%; range: 0.10-7.57%). Crude protein levels were under 2.9% in 23.65% (96/406), and lactose was below 4.8% in 47.04% (191/406). Total solids and non-fat-solids were also below their minimum reference values (11.2% and 8.0%, respectively), depicted in 28.08% (114/406) and 29.06% (118/406) of samples (Table 1). The proportions of non-compliance were similar across provinces with no significant differences ($p>0.05$), but the majority of non-compliant samples came from Manabí. These findings align with prior research in Pichincha (Guamán & Puga-Torres, 2022; Puga-Torres *et al*., 2024) and with broader studies reporting general compliance with nutritional parameters in Ecuador (Arias-Sandoval & Bonifaz-García, 2025). International comparisons, such as data from Pakistan, also show high compliance levels (Nawaz *et al*., 2022).

Somatic cell count
SCC exceeded permissible levels in 32.51% (132/406) of samples, with a mean of 679,485 cells/mL (range: 13,600-3,678,000). Significant interprovincial differences were observed ($p=0.0184$), with 84.91% of non-compliant samples from Manabí. These rates are higher than those previously reported for Pichincha (22.77%) and Rumiñahui (21%) (Guamán & Puga-Torres, 2022; Puga-Torres *et al*., 2024). Milk from healthy udders typically contains <200,000 cells/mL (Alhussien & Dang, 2018). Elevated SCC is indicative of mastitis, as inflammatory cells—primarily leukocytes—migrate into the mammary gland (Alhussien & Dang, 2018; Hossain *et al*., 2021; Wei *et al*., 2021). These findings suggest a high prevalence of subclinical mastitis in cows supplying informal markets in both provinces. While Ecuador permits SCC values up to 700,000 cells/mL, many countries (*e.g*., Argentina, New Zealand, EU) enforce stricter thresholds of 400,000 cells/mL (Sharma *et al*., 2011). SCC also correlates with reductions in protein, fat, and lactose content (Riveros-Galán & Obando-Chaves, 2020; Sumon *et al*., 2020), leading to economic losses for producers due to treatment costs and diminished milk quality (Sharun *et al*., 2021).

Protein stability
Protein instability was observed in 55.67% (226/406) of samples, with significant differences between provinces ($p<0.0001$). Manabí accounted for 78.32% of the unstable samples, highlighting the potential impact of poor hygiene and high microbial load on milk protein integrity. Protein instability often results from high bacterial activity or mastitis, leading to alterations in casein structure and increased risk of coagulation during pasteurization; these factors reflect inadequate udder health management and post-milking handling (Silva *et al*., 2020; Hanuš *et al*., 2021).

Antibiotic residues
Antibiotic residues above Codex Alimentarius limits were detected in 6.40% (26/406) of samples, predominantly from Manabí (76.92%, $p<0.0001$). Among positive cases, tetracyclines were found in 65.38% of samples, β-lactams in 26.92%, and sulfonamides in 7.69%. Similar results were reported by Guamán & Puga-Torres (2022) in Rumiñahui canton (7%). Milk is a common excretion route for antibiotic residues following therapeutic treatments for mastitis, pneumonia, or laminitis. The presence of such residues poses health risks, including allergic reactions, toxicity, disruption of gut microbiota, antimicrobial resistance (AMR), and congenital abnormalities (Abbring *et al*., 2019; Almashhadany, 2021; Rahman *et al*., 2021). AMR is exacerbated by the improper use of antibiotics in animal production and is recognized as a major global threat to public health and food safety (Mohamed *et al*., 2020; Alves *et al*., 2024; Liu *et al*., 2020).

Conclusions
87.68% of raw milk samples collected in informal markets located in the provinces of Santo Domingo de los Tsáchilas and Manabí, Ecuador, did not fulfill at least one of the quality parameters established by the Ecuadorian legislation. There was a high percentage of non-compliance with the reference values for pH (72.41%), titratable acidity (68.72%), and protein stability (55.67%) parameters, which may be attributed to the substandard hygienic quality of the milk, a consequence of excessive bacterial proliferation and subsequent udder infections, such as mastitis. Moreover, the 6.40% of the raw milk samples tested positive to antibiotic residues. The informal market for commercialized milk, without any quality control prior to consumption or processing, constitutes a health threat for local consumers. It is imperative that local regulatory authorities implement measures to regulate the unauthorized sale of raw milk..

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Table 1. Analysis of compliance and non-compliance of quality milk parameters according to the NTE INEN 9 of raw bovine milk samples collected in informal markets located in the Provinces of Manabí and Santo Domingo de los Tsáchilas in Ecuador, n=406 (Total results).

Parameters	Reference value	Minimun	Maximum	Mean	Compliance n (%)	Non-complieance n (%)
Relative density (g/mL)	1.028-1.033	1.009	1.038	1.029	230 (56.65)	176 (43.35)
Titratable acidity (ºD)	13-17	5.30	44.00	17.65	127 (31.28)	279 (68.72)
pH	6.6-6.8	4.47	7.23	6.52	112 (27.59)	294 (72.41)
Fat (%)	Min. 3.2	0.10	7.57	3.47	273 (67.24)	133 (32.76)
Crude protein (%)	Min. 2.9	2.00	3.92	3.11	310 (76.35)	96 (23.65)
Total solids (%)	Min. 11.2	6.42	16.23	11.99	242 (71.92)	114 (28.08)
Non-fat solids (%)	Min. 8.2	5.49	10.72	8.52	288 (70.94)	118 (29.06)
Lactose (%)*	Min 4.8	3.01	8.81	4.69	215 (52.96)	191 (47.04)
Water added (%)	0	0.00	35.96	3.16	289 (71.18)	117 (28.82)
Count of somatic cells (CS/mL)	Max. 700000	13600	3678000	679485	274 (67.49)	132 (32.51)
Protein stability	Negative	-	-	-	180 (44.33)	226 (55.67)
Antibiotic residues	MRL	-	-	-	380 (93.69)	26 (6.40)

MRL, maximum residue limit (established by the *Codex Alimentarius*).

Table 2. Comparative analysis of compliance and non-compliance of quality milk parameters according to the NTE INEN 9 of raw bovine milk samples collected in informal markets according to provinces, Manabí and Santo Domingo de los Tsáchilas in Ecuador (Results by province).

Parameters	Reference NTE INEN 9	Province	Minimun	Maximum	Mean	Compliance, n (%)	Non complieance, n (%)	p
Relative density (g/ml)	1.028-1.033	Santo Domingo	1.018	1.032	1.028	19 (26.39)	53 (73.61)	0.91
		Manabí	1.009	1.038	1.029	205 (61.38)	129 (38.62)	
Titratable acidity (ºD)	13-17	Santo Domingo	16.10	34.00	19.93	18 (25.00)	54 (75.00)	<0.0001
		Manabí	5.30	44.00	17.16	125 (37.43)	209 (62.57)	
pH	6.6-6.8	Santo Domingo	4.56	6.71	6.39	18 (25.00)	54 (75.00)	<0.0001
		Manabí	4.47	7.23	6.55	106 (31.74)	228 (68.26)	
Fat (%)	Min. 3.2	Santo Domingo	1.93	6.89	3.44	46 (63.89)	26 (36.11)	0.15
		Manabí	0.10	7.57	3.48	231 (69.16)	103 (30.84)	
Crude protein (%)	Min. 2.9	Santo Domingo	2.00	3.59	3.06	53 (73.61)	19 (26.39)	0.19
		Manabí	2.21	3.92	3.13	255 (76.35)	79 (23.65)	
Total solids (%)	Min. 11.2	Santo Domingo	6.42	15.75	11.77	53 (73.61)	19 (26.39)	0.24
		Manabí	7.59	16.23	12.04	244 (73.05)	90 (26.95)	
Non-fat solids (%)	Min. 8.2	Santo Domingo	5.49	9.82	8.36	53 (73.61)	19 (26.39)	0.16
		Manabí	6.18	10.72	8.56	235 (70.36)	99 (29.64)	
Lactose (%)	Min 4.8	Santo Domingo	3.01	8.81	4.65	48 (66.67)	24 (33.33)	0.27
		Manabí	3.32	5.89	4.69	167 (50.00)	167 (50.00)	
Water added (%)	0	Santo Domingo	0.00	35.96	4.55	54 (75.00)	18 (25.00)	0.93
		Manabí	0.00	26.73	2.85	235 (70.36)	99 (29.64)	
Count of somatic cells (CS/ml)	Max. 700000	Santo Domingo	45.10	3,250.000	488.629	54 (75.00)	18 (25.00)	0.018
		Manabí	13.60	3,678.000	720.628	220 (65.87)	114 (34.13)	
Protein stability	Negative	Santo Domingo	-	-	-	23 (31.94)	49 (68.56)	<0.0001
		Manabí				157 (47.01)	177 (52.99)	
Antibiotic residues	MRL	Santo Domingo	-	-	-	66 (91.67)	6 (8.33)	<0.0001
		Manabí				314 (94.01)	20 (5.99)	

MRL, maximum residue limit (established by the *Codex Alimentarius*).