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Microbiological quality of raw milk at the Sokode district in Togo: prevalence of *Mycobacterium spp, Staphylococcus aureus, Salmonella spp* and coliforms

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ABSTRACT

This study aimed at assessing the quality of raw milk from the local traditional dairy farm and outlet market in Togo. The samples were collected around the Sokode district in a random dairy farms (n=10) and the local milk markets (n=10). Milk samples were withdrawn from 10 cows per farm and one retailer per market. The samples were screened for total germs, coliforms, *Escherichia coli* (*E. coli*), *Mycobacterium spp*., *Salmonella spp*., and *Staphylococcus aureus* using microbiological culture methods. The three pathogenic bacteria evidenced were *E. coli* (farm: 89% *vs* market: 40%; $\chi^2 = 52.43$; $p = 0.001$), *Mycobacterium spp.* (farm : 2% *vs* market: 00%; $\chi^2 = 2.02$, $p = 0.25$) and *Staphylococcus spp.* (farm : 7% *vs* market: 10%; $\chi^2 = 4.69$, $p = 0.032$). The global contaminations levels in CFU/mL by *E. coli*, *Mycobacterium spp.* and *Staphylococcus spp*. were $5.80\ 10^3 \pm 1.87\ 10^3$, 3.45 ± 2.45, and $5.34\ 10^1 \pm 1.14$, respectively. All milk samples were not compliant with quality rules based on total germs and coliforms. It could be concluded that milking routine and raw milk hygiene remain a challenge in Togo and regular control from National Health Laboratory should be set up in that area.

Keywords: Bacteria, traditional dairy farm, milk hygiene, health.

INTRODUCTION

Milk is a nutritious food per excellence whose consumption could contribute to the reduction of malnutrition in rural areas and thus to food security in sub-Saharan Africa (Faye, 2016; Sultana et al., 2015) and in Togo in particular. In Togo, cattle farming is the main source of local milk production, providing an average of 4,062 tons of fresh milk per year (Talaki, 2017). This local milk production is

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supplied to consumers in the form of various products: raw milk, curdled milk and local cheese. The most common is local cheese, also called "Wangashi" (Mattiello et al., 2018) whose production technology involves heating. However, the consumption of raw milk is still widespread in many localities, especially in rural areas, influenced by people traditions and cultures (Chengat Prakashbabu et al., 2020).

In general, although very rich in nutrients (Kalandi et al., 2015), local raw milk can be subject to various sources of contamination by pathogens that can be derived from the farming environment through contact with feces and soiled fodder, air and production equipment (Fusco et al., 2020). Contamination can also occur a lack of compliance with hygienic practices in the collection, transport and processing of milk (Millogo et al., 2018). Study conducted in Mali reported contamination of milk with the organochlorine pesticides (Maïga et al., 2019). The healthy status of herds is often implicated in specific zoonotic diseases where germs carried by infected cows can contaminate milk. Studies have also highlighted a route of milk contamination before extraction through teat ports (Taponen et al., 2019) or by an endogenous route of bacterial transmission through the mesenteric lymph nodes (Garrido et al., 2020). Thus, produced under unsanitary or poorly treated environmental conditions, raw milk and milk-derived products have generally been the cause of many cases of zoonoses reported worldwide (van den Brom et al., 2020). In Togo, the presence of pathogenic germs in cattle farms has been shown in previous studies (Vikou et al., 2018). Among pathogens found in cattle farms, there were *Mycobacterium spp.* which has also been found in raw cow's milk from neighbouring Benin (Parsons et al., 2019; Farougou et al., 2011).

The objective of the present study was to assess the prevalence of pathogenic bacteria, such as *Escherichia coli* (*E. coli*), *Mycobacterium spp.*, *Salmonella spp*., and *Staphylococcus aureus* (*S. aureus*), in raw cows' milk that may cause health problems for cattle herds and consumers of raw milk.

MATERIALS AND METHODS

Study area

Togo is a country of 600 km long and about 50 km wide. The study was conducted in the Central Region (Figure 1), one of the five economic regions of the country. The climate in this region is Sudano-Guinean, with a rainy season from April to October and a dry season from November to March. In the area, July, August and September are the rainiest months with peak rainfall varying between 1074 and 1649 mm of water. Cattle breeding in the area is peri-urban. The herds are of the breeding type, consisting mainly of Taurin-Zebus crossbred cattle (Boma et al., 2018). Animals are fed exclusively on natural pasture. However, during the dry season, when grasses become scarce, natural grazing is combined with branches and crop residues, with a supplement of cooking salt in some herds.

Sampling

Sample collection was carried out in 2017, in the months of July and August, in the rural site of Tchaoudjo. The ten cattle herd villages were selected around Tchaoudjo prefecture to represent the peri-urban area of the Sokode district. The list of cattle herds was obtained from the district veterinarian office, and the village was selected based on the representation of the respective herd's accessibility in the relationship with the representative village animal health personal. Herds grazing in the same grazing area were considered as one village herd. Per herd, raw milk samples were withdrawn from 10 randomly selected dairy cows taking into account individual and collective past health status of cows. For the sampling of raw milk from markets, 10 milk sale points were selected

based on their relationship with the herds selected for the study. Then, by raw milk market point of survey, samples were collected from only one raw milk seller randomly selected.

Raw milk collection

To avoid contamination of the samples during milking, the teats were disinfected with a 70% alcohol solution and rinsed with sterile distilled water. Hands were also disinfected and protected with sterile latex gloves. A volume of 10 mL of milk was withdrawn from each cow and a final volume of 1,000 mL was available for each farm. The same volume of the milk samples from each retail outlets and the other collection points were collected using a sterile syringe.

Microbiological analysis and bacteria count

Samples were stored at 4°C and transported to two different laboratories for specific analyses. One (01) mL of milk sample was suspended in 9 mL of sterile diluent and mixed thoroughly. The suspension was serially diluted 10-fold up to 10^{-8}. 0.1 mL aliquots of appropriate dilutions (10^{1} to 10^{-8}) were spread onto duplicate corresponding agar plates in sterile conditions.

Analyses were carried out in the local private laboratory for results approval. Analyses were performed for: i) total viable aerobic plat count (EN ISO 6222) with Plate Count Agar (PCA) at 37°C for 24 h to 48 h, ii) total coliforms and thermotolerant coliforms (NF EN ISO 9308-1) with Violet Red Bile Lactose agar (VRBL) at 30°C and 44°C, respectively, for 24 h, and iii) *E. coli* with Eosin Methylene (EMB) agar at 37°C for 24 h (NF EN ISO 9308-1). *E. coli* colonies were known by their blue-black colour with green metallic sheen.

For *S. aureus*, screening was performant by culturing the prepared sample on Baird-Parker Agar and then counting positive coagulase colonies on Chapman agar after 24 and 48 h of incubation at 37°C.

The culture of *Salmonella spp*. was carried out according to ISO 6579 guidance by incubating samples in brain heart infusion broth at 37°C for 24 and 48 h.

For the culture of *Mycobacterium spp.*, samples were centrifuged at 2500 rpm for 15 min and 200 µl of the pellet was inoculated onto slants of Lowenstein Jensen medium. The sloops were incubated at 37°C for 28 days. Positive cases were confirmed by staining microscopic analysis. Each milk sample was additionally inoculated in peptone broth and incubated at 37°C for 24 h and bacterial cultures on blood agar plates (brain heart infusion agar: BHIA; Difco) was performed as described by Donkor et al. (2007) method.

An automatic colony counter was used for counting the number of colonies from each culture and was expressed as a colony-forming unit (CFU/mL).

Statistical analysis

The statistical analysis was carried out using SPSS 20 software. The mean values of the bacterial contamination levels were calculated with the standard error specified, and were compared with quality criteria of French Standardization Association (AFNOR) (AFSSA, 2001). For this purpose, the values were Logarithm-transformed by replacing zero values (negative results) with "1". Fisher's exact test was used to compare the frequency of pathogenic bacteria found at a precision level of 5%. The Tukey test was performed to compare the mean values per type of contamination between the two sampling points.

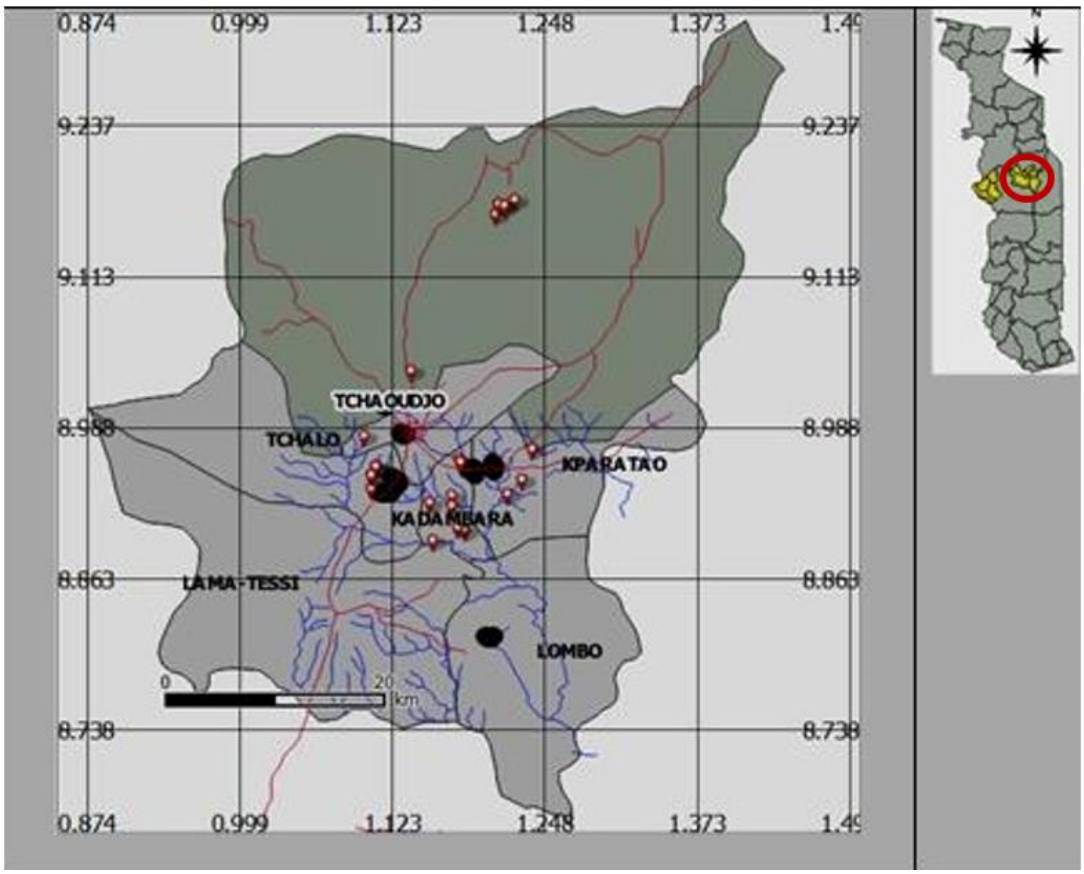

Figure 1: Study area (Legend: Cattle herds and milk sampling point are indicated in red points).

RESULTS

Prevalence of bacterial contaminations

The analysis of the milk samples was planned to include 100 samples from farms and another 100 from markets. Analyzing the samples separately, make it possible to determine whether or not (detection) the targeted germs are present in each sample of milk, and therefore give an idea of the prevalence in the sampled dairy farms. Nevertheless, the final dataset used for the analysis consisted of 110 samples (100 from dairy farms, 10 from retailers) because most retailers packaged milk in one container. In order to accommodate this field reality, a single composite sample per farm was constituted for the bacteria culture and count.

The analysis of potential pathogenic bacteria retrieved in milk samples showed the prevalence of *E. coli* at 89% (89/100) in farms *vs* 40% (4/10) in markets (df = 109, $\chi^2 = 52.43$, $p < 0.001$, Table 1), *S. aureus* at 7% (7/100) in farms vs 10% (1/10) in markets (df = 109, $\chi^2 = 4.69$, $p = 0.032$, Table 1) and *Mycobacterium ssp.* at 2% (2/100) in farms vs 00% (n= 0/10) in markets ($\chi^2 = 2.0$, $p = 1$, Table 1). In addition, all samples were positive to total coliforms, thermotolerant coliforms and total germs. *Salmonella spp.* was not detected in all milk samples (Figure 2).

Quantitative findings

Comparisons using Tukey test indicated high level of contamination with similar values for total germs (farm: $7.57\ 10^5 \pm 3.72\ 10^5$ CFU/mL vs market: $8.31\ 10^6 \pm 4.26\ 10^6$ CFU/mL, $df = 19$, $F = 3.11$, $p = 0.095$, Table 1), total coliforms (farm: $2.28\ 10^4 \pm 1.02\ 10^4$ CFU/mL vs market: $2.28\ 10^4 \pm 9.03\ 10^3$ CFU/mL, $df = 19$, $F = 0.0$, $p = 0.99$, Table 2)

and thermotolerant coliforms (farm: $1.02\ 10^3 \pm 3.72\ 10^2$ CFU/mL vs market = $1.15\ 10^4 \pm 3.19\ 10^3$ CFU/mL, *df* = 19, *F* = 10.53, *p* = 0.04, Table 2). For all milk samples, the average contamination levels of the pathogenic bacteria, *E. coli*, *Staphylococcus spp*., and *Mycobacterium spp*. were, respectively: $5.80\ 10^3 \pm 1.87\ 10^3$ CFU/mL, $5.34\ 10^1 \pm 1.14\ 10^1$ CFU/mL and 3.45 ± 2.45 CFU/mL (Table 2). Contamination levels from farm milk samples were $1.02\ 10^3 \pm 3.28\ 10^2$ CFU/mL (*E. coli*), $5.20\ 10^1 \pm 1.61\ 10^1$ CFU/mL (*Staphylococcus spp*.) and 5.90 ± 4.9 CFU/mL (*Mycobacterium spp*.). That figure was $1.06\ 10^4 \pm 3.10\ 10^3$ CFU/mL (*E. coli*), $5.47\ 10^1 \pm 1.69\ 10^1$ CFU/mL (*Staphylococcus spp*.) and 0.0 CFU/mL (*Mycobacterium spp.)* in the market samples (Table 2). Comparing the level of contamination of the samples from farms to those from local retail markets, the difference was established by the type of bacterial contamination (Table 3).

Table 1: Presumptive test results of the raw milk samples from herds and retail outlets.

Bacteria	Raw milk from herds	Raw milk from the retail outlet	Study area	Exact Sig. (1-sided) df = 109
Escherichia coli	89% (89/100)[a]	40% (04/10)[b]	84.54%	$\chi 2 = 52.43$, $p < 0.001$
Mycobacterium spp.	2% (02/100)[a]	0% (0/10)[a]	2%	$\chi^2 = 2$, $p = 1$*
Staphylococcus spp.	7% (07/100)[a]	10% (01/10)[a]	1.81%	$\chi^2 = 4.69$, $p = 0.032$,

Th.: Thermotolerant. Coliform count, Thermotolerant coliform, and Total germs count were evidenced in all samples analyzed. *Salmonella* spp. has not been detected. Values followed by the same letter (s) are not significantly different at $p \leq 0.05$ according to the Fisher's Exact Test. Exact Sig. (1-sided): Fisher's Exact Test. *: 2-sample test for equality of proportions with continuity correction.

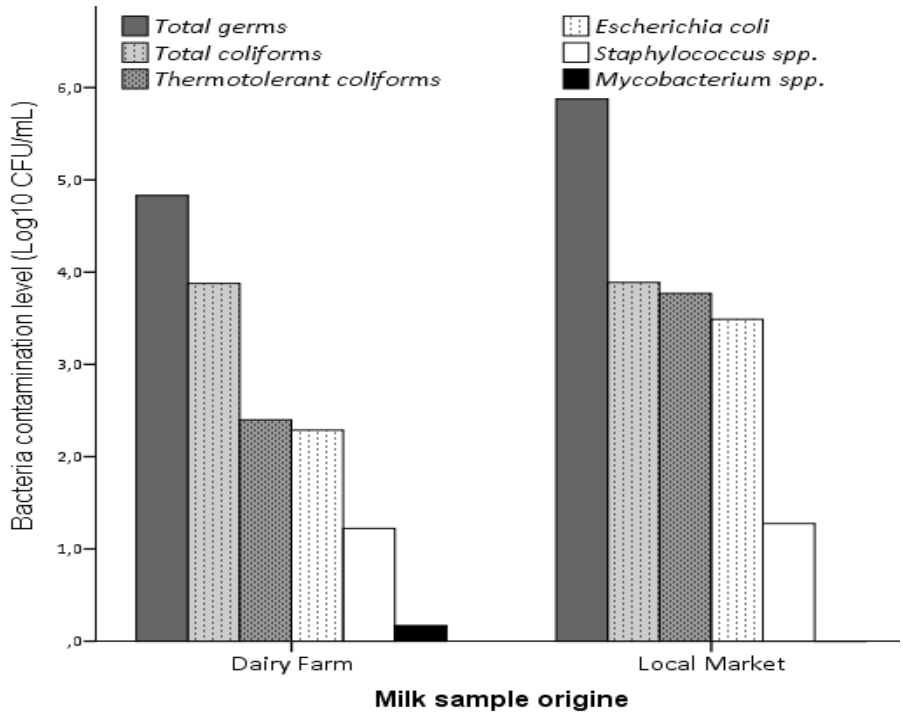

Figure 2: Level of contamination of bacteria evidenced in milk samples collected from dairy farms and local markets.

Table 2: Number of bacteria colonies evidenced in milk samples collected from the farm and the market.

Statistical parameters		*T. germs*	*T. colif*	*Th. colif*	*E. coli*	*Staph.*	*Myco.*
		m = 5 10^5	m = 100	m = 100	m = 100	m = 100	m = 0
		CFU/mL					
Farm	Mean	7.57 10^5	2.28 10^4	1.02 10^3	1.02 10^3	5.20 10^1	5.90
	SE	3.72 10^5	1.02 10^4	3.72 10^2	3.28 10^2	1.61 10^1	4.90
Market	Mean	8.31 10^6	2.28 10^4	1.15 10^4	1.06 10^4	5.47 10^1	1.00
	SE	4.26 10^6	9.34 10^3	3.19 10^3	3.10 10^3	1.69 10^1	0.00
Total	Mean	4.53 10^6	2.28 10^4	6.24 10^3	5.80 10^3	5.34 10^1	3.45
	SE	2.25 10^6	6.75 10^3	1.97 10^3	1.87 10^3	1.14 10^1	2.45

Legend: SE: Standard error, CFU: unit form colony, *T. germs*: Total germs, *T. colif*: Total coliforms. *Th. colif*: Thermotolerant coliforms. *E. coli*: *Escherichia coli*, *Staph.*: *Staphylococcus spp.*; *Myco.*: *Mycobacterium spp.*; m: standard value according to AFSSA (2001)

Table 3: Comparison of the mean values of bacteria evidenced in milk samples collected from milking and markets.

Bacteria	Statistic	S of Squares	df	M. Square	*F*	*p*
Total germs	Between Groups	2.85 10^{14}	1	2.85 10^{14}	3.12	0.09
	Within Groups	1.65 10^{15}	18	9.15 10^{13}		
	Total	1.93 10^{15}	19			
Total coliforms	Between Groups	7.22 10^3	1	7.22 10^3	0.00	0.99
	Within Groups	1.73 10^{10}	18	9.61 10^8		
	Total	1.73 10^{10}	19			
Thermotolerant coliforms	Between Groups	5.45 10^8	1	5.45 10^8	10.53	0.00
	Within Groups	9.31 10^8	18	5.17 10^7		
	Total	1.48 10^9	19			
Escherichia coli	Between Groups	4.58 10^8	1	4.58 10^8	9.41	0.00
	Within Groups	8.76 10^8	18	4.87 10^7		
	Total	1.33 10^9	19			
Staphylococcus aureus	Between Groups	3.65 10^1	1	3.65 10^1	0.01	0.91
	Within Groups	4.91 10^4	18	2.73 10^3		
	Total	4.91 10^4	19			
Mycobacterium spp.*	Between Groups	1.20 10^2	1	1.20 10^2	1.00	> 0.05
	Within Groups	2.16 10^3	18	1.20 10^2		
	Total	2.28 10^3	19			

Legend: *F:* Fischer value; *p*: probability value (p-value) according to the Tukey test at the threshold of 0.05., S.: sum, M.: mean, df: degree of freedom, ***** Only two positive samples.

DISCUSSION

The present study explored some bacterial contamination of raw milk composite samples including 100 from ten traditional cattle herds and 100 from ten raw milk retail surrounding the Sokode district (Togo). Among specific pathogenic bacteria, samples were analyzed for *S. aureus, Salmonella spp. E. coli* and *Mycobacterium spp*. For specific pathogen bacteria found in raw milk samples of the study, microbiological analyses have confirmed the presence of *S. aureus* and *Mycobacterium spp.* in herds raw milk samples. The results give this opportunity to explore the contamination of raw milk with these bacteria and their distribution pattern between cattle herds and raw milk retail in the district of Sokode.

According to the quality criteria of the AFNOR standard, the level of microbial contamination of dairy products is 5 10^5 CFU/mL for total germs (AFSSA, 2001). All samples collected at retail outlets as well as in herds showed a level of contamination higher than the standard value (5 10^5 CFU/mL). Also, all samples showed a level of *E. coli* and Coliform's contamination above the recommended value of 10^2 CFU/mL.

E. coli known commonly as *Coli bacillus* is a facultative aerobe-anaerobic, gram-negative bacillus (*Enterobacteriaceae* family) belonging to the normal resident flora of rumen (Munns et al., 2015). In the present study, despite belonging to different herds and health precautions taken by sanitizing cow's teats and hands with alcohol before milking, all raw milk samples were preeminently contaminated by this bacterium. This finding is in line with previously published studies of bovine raw milk in West Africa (Donkor et al., 2007; Fusco et al., 2020) in traditional herds. Although most *E. coli* strains of cattle rumen are commensals, some are known to be pathogenic, whereby causing enteritis for humans (Denny et al., 2008). In addition, numerous cases of calves's diarrhea have been reported to be related to *E. coli* (Mohammed et al., 2019; Tarekegn, 2017). However, the epidemiological implication of *E. coli* evidenced in the framework of this study is still unknown and suggests thus further research for possible pathogenic strains as well as their sensitivity to antimicrobial drugs.

As a bacterial pathogen found in raw milk samples, *Staphylococcus aureus* was frequently found in bovine milk (Donkor et al., 2007). Samples of milk were with similar management. However, results showed some specific bacterial diversity that was the case of the unique raw milk sample from one site in which *S. aureus* was detected. On the other hand, the study showed that the point of raw milk collection (herd) is the most important factor influencing differences in *S. aureus* contamination. Thus, the frequency of *S. aureus* in the samples varies widely between herds emphasizing that the role of some factors notably, global herds health management, soiling of the cow, and grazing practices. However, the data collected in our study do not allow us to confirm this correlation. Numerous studies reported that the *S. aureus* bacteria cause a significant reduction in milk production in cows (Abebe et al., 2016). Thus, from the results from herds, the high prevalence of *S. aureus* contamination revealed the huge potential milk production loss the traditional cattle breeding suffers in Togo.

In correlation with our results, numerous cases of *Mycobacterium spp.* infections were most reported in Togo, in particular, clinical cases of tuberculosis have been diagnosed in herds by animal health officers (Dagnra et al., 2001; Domingo, 2000). In this study, the prevalence of *Mycobacterium spp.* contamination from raw milk was relatively low, although samples from suspect cows were taken into account. De Jong et al. (2010) reported that, on Lowenstein-Jensen solid agar, *M. africanum* grew more slowly than *M. tuberculosis*, with cultures occasionally yielding growth only after 10 weeks, compared to 3 – 4 weeks in *M. tuberculosis.* The media culture duration in the present study was limited to four weeks and may therefore constitute a limit in the detection of the different strains of *Mycobacterium spp.* possibly present as reported by De Jong et al. (2010).

Conclusion

The pathogenic bacteria found in milk samples could constitute a real health problem for consumers and cattle herds in the Sokode area. Indeed, although a number of milk samples were found to be negative for the investigated disease-causing bacteria, *E. coli*, *Mycobacterium spp*., *S. aureus* and *Salmonella spp*., the contamination was above the threshold levels. Some germs, such as *Mycobacterium spp*. although found exclusively in the milk samples from farm, showed rather high bacterial concentration levels. This highlights the necessity of strengthening health control systems in Togo's dairy milk sector, according to the "One Health" approach. Therefore, it is important for the public authorities to strengthen the capacity of public health laboratories in the area in order to allow better control of animal health and livestock food products.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

RAB and JBS provided substantial scientific support to the data analysis and scientific interpretation of the results. EM, AL and TN contributed to the collection of samples and data processing. MBS contributed to data analysis and language editing. YN provided the scientific supervision of the work.

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