Isolation and Identification of *Listeria* Species along the Milk Value Chain in one region of Tanzania

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Introduction

The genus *Listeria* contains gram positive, non-spore forming, catalase-positive, oxidase-negative and facultative anaerobic bacteria. Two species are pathogenic of which *L. monocytogenes* represents the only pathogen of public and veterinary health significance and *L. ivanovii* is usually restricted to causing disease in ruminants. *L. monocytogenes* causes large outbreaks of Listeriosis in humans, with a mortality rate of 9 – 44%. Most cases are foodborne related and consumption of unpasteurised milk and dairy products is the major means of zoonotic transmission. Currently, there is no information in available literature on the presence of *Listeria* along the milk value chain in Tanzania. In this study, the awareness of milk contamination was evaluated, total plate count (TPC) was quantified and isolation of *Listeria* species was attempted along the milk value chain in two districts of Tanga region in Tanzania.

Methods

Study sites included five villages in Handeni district and five villages in Lushoto district. In total, 114 respondents were interviewed on awareness and strategies for solving the problem of milk contamination. Then, raw milk samples were aseptically collected from each of the 114 respondents for laboratory analyses using the standard ISO procedures for food microbial analyses — Horizontal methods. Four tests i.e. haemolysis on a sheep blood agar, CAMP, oxidase and *Listeria* test kit were carried out to confirm the presence of *L. monocytogenes* and other *Listeria spp.* in test samples against control cultures. Data were analysed for descriptive statistics and Chi-square using the Statistical Package for Social Sciences (SPSS) version 17.0. TPC values were analysed against independent variables using the General Linear Models (GLM) procedures of the Statistical Analysis System (SAS) version 9.1 for Windows.

Results

About 90 percent of all raw milk samples processed showed TPC above standard acceptable in the Eastern Africa countries (EAC) of $2.0x10^5$ CFU/ml (Grade 2). Also, there was more contamination in milk samples obtained from the household farms than those which were collected from the milk suppliers and in the extensive farming system than in the intensive system ($P < 0.05$). Three *Listeria* species namely *L. innocua* (11.4%), *L. ivanovii* (1.7%) *and L. monocytogenes* (42.1%) were identified. *L. monocytogenes* were isolated and identified as the common contaminants in milk along the value chain in the study region (Table 1).

Table 1. Average TPC and distribution of *Listeria* species along the milk value chain

Variable	Total samples collected per sampling point	No. of positive samples for aerobic bacteria	Mean TPC CFU/ml	Positive samples		
				L. innocua n (%)	L. ivanovii n (%)	L. monocytogenes n (%)
Extensive farming system	67	40	$2.2 \pm 1.9x10^6$	10(14.9)	1(1.5)	34(50.7)
Intensive farming system	47	40	$2.3 \pm 2.1x10^6$	3(6.4)	1(2.1)	14(29.8)
Total	114	80	$2.3 \pm 2x10^6$	13(11.4)	2(1.7)	48(42.1)
Households	54	30	$2.9 \pm 2.6x10^6$	6(11.1)	1(1.8)	25(46.3)
Suppliers	25	24	$1.6 \pm 1.4x10^6$	3(12)	0(0.0)	9(36)
Street vendors	13	16	$1.7 \pm 1.7x10^6$	2(15.3)	0(0.0)	6(46.1)
Restaurants	18	10	$2.3 \pm 1.4x10^6$	1(5.5)	0(0.0)	7(38.8)
Collection centres	4	4	*	1(25)	1(25)	1(25)
Raw milk	103	71	$2.3 \pm 2.1x10^6$	12(11.6)	2(1.9)	47(45.6)
Boiled milk	11	9	$2.2 \pm 1.5x10^6$	1(9.1)	0(0.0)	1(9.1)

*Minimum detection of $1x10^1$ CFU/ml or too numerous to count (TNTC)
EAC harmonized standard microbial limit in raw milk for TPC is $2.0x10^5$ CFU/ml
Large standard deviations are due to majority of deviations far from the mean

Survey showed poor hygienic practices by the majority of actors. Most of the farmers were skipping most of the important safe hand milking steps. Few (11.8%) households were keeping cattle in well-designed and clean shelters. Only, 12.9% of households and 11.1% of restaurateurs were using Aluminium vessels/stainless steel containers for milk handling. Furthermore, very few cooling facilities were observed along the milk value chain as presented in Table 2.

Table 2. Evaluation of hygienic practices and awareness of milk contamination

Variable	Households (%) n = 54	Suppliers (%) n = 25	Vendors (%) n = 13	Restaurateurs (%) n = 18	Collection centres (%) n = 4
Washing hands before milking	55.5	-	-	-	-
Cleaning cow teats before milking	38.9	-	-	-	-
Drying cow teats after washing	5.6	-	-	-	-
Fore-stripping on the udder quarters	0.0	-	-	-	-
Use of teat dip	12.9	-	-	-	-
Well-designed and clean shelters	11.8	-	-	-	-
Consumption of fermented raw milk	53.7	-	-	-	-
Consumption of actual raw milk	35.2	-	-	-	-
Milk sick cow and consume at home/sell	27.8	-	-	-	-
Use of Lactometer/alcohol test	-	36.0	23.1	50.0	100
Pooling of milk	-	68.0	69.2	72.2	100
Use of metal milk containers	12.9	0.0	0.0	11.1	0.0
Use of portable water supply	12.9	36.0	53.8	50.0	100
Use of detergents	100	100	100	100	100
Use of boiled water	12.9	88.0	92.3	88.9	100
Cold storage facilities	0.0	0.0	0.0	0.0	75.0

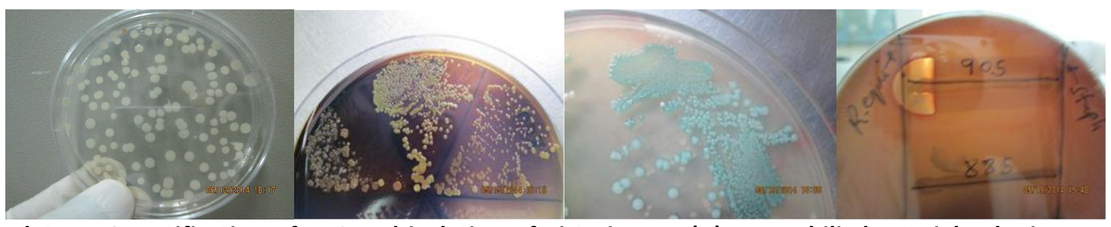

Plate 1: Quantification of TPC and isolation of *Listeria spp*. (A) Mesophilic bacterial colonies on plate count agar (B) *Listeria spp*. on *Listeria* Oxford agar (C) *Listeria spp*. on Colorex *Listeria* agar and (D) Shovel shaped synergistic reaction of *L. ivanovii* against a streak of *Rhodococcus equi*

Plate 2: Containers commonly used by communities for handling and storing milk (A) Dirty plastic milking bucket in one of the surveyed households (B) Calabash and (C) Closed containers, are not easily cleanable and (D) Storage plastic container fitted with plastic bag – poor handling practices that predispose the milk to microbial contamination

Conclusion

Poor bacteriological quality of milk indicates poor animal husbandry practices and poor milk handling practices. Presence of *L. monocytogenes* in milk provides evidence on the microbial status of milk in the Tanzanian milk value chain and insights into the magnitude and public awareness on the health risks associated with consumption of raw milk. To ensure milk safety, the awareness of actors must be improved through training on animal husbandry practices and public education on general hygienic practices in milk. Also, Sector policies, organizational structures and support services must be well concentrated to stimulate dairy development.

Tropical Animal Diseases and Veterinary Public Health: Joining Forces to Meet Future Global Challenges
First Joint AITVM – STVM Conference
Berlin, September 4 – 8, 2016

The research was carried out with the financial support of the Federal Ministry for Economic Cooperation and Development, Germany, and the CGIAR Research Program on Agriculture for Nutrition and Health, led by the International Food Policy Research Institute, through the Safe Food, Fair Food project led by ILRI.