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# *Listeria* Contamination of Raw Bovine Milk and the Factors Influencing Its Occurrence in Greater Luweero District, Uganda

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***Authors' contributions***

*Author DM designed the study, collected data and prepared the first draft of the manuscript. Author CK carried out statistical data analysis. Author JLN supervised data collection, managed the literature searches and prepared the subsequent drafts of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** To establish the occurrence of *Listeria* contamination of raw bovine milk and the contributory factors in Greater Luweero.

**Methods:** A cross-sectional study was carried out during 2013; in Luweero, Nakaseke and Nakasongola districts, in Central Uganda. All the 16 milk collecting centers in the study area were sampled and the supplying farms identified for trace back. A total of 100 bulk raw milk samples, each representing a farm, were analysed using the VIDAS® *Listeria monocytogenes* II (LMO2) enzyme-linked fluorescent immunoassay (ELFA) kit (BioMérieux, Durham NC, USA). The supplying farms were systematically random sampled; and the managers together with those of the milk collecting centers were interviewed to establish the management practices and environmental risk

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factors associated with *Listeria* contamination of the milk.

**Results:** High level of *Listeria* contamination of raw milk from farms (72%); knowledge gap and significant non-adherence to milk trade guidelines (50% and 31.25%; and 88% and 39% among milk collecting center and farm managers, respectively) were encountered. Among the factors influencing occurrence of *Listeria* in raw milk, the significant ones ($P$=.05) included improper hygienic practices; such as poor faecal disposal, improper cleaning of milking utensils and of hands before milking; and non-adherence to Dairy Development Authority (DDA) guidelines; lack of access to dairy extension services; and absence of farm entry restriction and biosecurity measures.

**Conclusions:** There is a high occurrence of *Listeria* contaminated raw bovine milk from farms in Greater Luweero district. The risk factors that were significantly associated with the contamination can be minimized through sensitization and training of farmers and center managers. Listeriosis is of great public health significance, hence effective inspections to assess compliance to guidelines for quality and safety is recommended. Establishing *Listeria monocytogenes* carrier status of cattle; and microbial levels in milk will inform on policies for prevention of contamination.

*Keywords: Listeria monocytogenes; milk contamination; predisposing practices; VIDAS®immunoassay.*

## 1. INTRODUCTION

*Listeria monocytogenes* is a psychrotolerant food-borne zoonotic bacterial pathogen, which survives heating, and drying, thus creating safety hazards to the food industry [1,2]. The organism causes Listeriosis, which is associated with consumption of various contaminated food categories, including dairy products [3]. Particular groups of consumers, such as the elderly, children, pregnant women and those with weakened immunity are more at risk [4]. Among the livestock, sheep seem to be more susceptible to *L. monocytogenes* than cattle [5]. Other *Listeria* species exist but are of less significance, although, *L. seeligeri* and *L. innocua* have been reported in humans; while *L. ivanovii* has occasionally been associated with abortions in sheep and cows, or septicemia in sheep and some affect humans too [6]. Infected animals have been reported to persistently excrete the microorganisms in their milk [7,8]. Hence, the sources of *Listeria* include the production (farm) and processing environments of various foods, especially those of animal origin [9].

Livestock production is one of the major activities in Greater Luweero, which currently comprises of Luweero, Nakaseke and Nakasongola districts, in Uganda. In Luweero, milk is mainly consumed in unpasteurised forms, while the rest is sold to the collecting centers; and eventually to processing plants, often located in other districts. Outbreaks of Listeriosis, more so due to *L. monocytogenes*, have been reported in developed countries, however limited information exist for most African countries [9-11]. Listeriosis among humans could be one of those febrile illnesses often clinically mis-diagnosed as malaria, which is very prevalent and endemic in Uganda. Previously, *Listeria* species and *Listeria monocytogenes* contamination of raw milk was reported, at 60% and 13% respectively, in Kampala [12]. The milk sold in Kampala is sourced from various districts of Uganda including Luweero, Nakaseke and Nakasongola, but no information on *Listeria* contamination exists in the areas of origin. Hence, this study was undertaken to establish the occurrence of *Listeria* contaminated milk and the contributory factors, in the greater Luweero district.

## 2. MATERIALS AND METHODS

A cross-sectional study was carried out during 2013; in Luweero, Nakaseke and Nakasongola districts, in Central Uganda. All the 16 milk collecting centers in the study area were sampled and the supplying farms identified for trace back.

Basing on previous *Listeria* prevalence in raw milk of 60% in Kampala Uganda [12]; and at 90% level of confidence with a desired absolute precision of 10%; a sample size estimate of 92 was obtained, using the formula $n=1.96^2\, P_{exp}\,(1-P_{exp})/d^2$ by Thrushfield [13]. However, a total of 100 bulk milk samples, each representing a farm, proportionately distributed for each district, were obtained. Therefore, the number of farms from each district was 24, 48 and 28 for Luweero, Nakaseke and Nakasongola districts, respectively; however, 11, 64 and 25 milk samples were obtained, respectively.

The supplying farms were systematically random sampled and assessed for the management practices and environmental risk factors associated with the contamination. In addition, both the farmers and the milk collecting center managers were interviewed. The checklist for the interview comprised of anticipated risk factors including sanitation, interaction of different animals on farm, disease control strategies, housing, feeding, milk containers and transportation means, knowledge of and challenges faced in adherence to Dairy Development Authority (DDA) guidelines and regulations. The latter include quality, safety, hygiene practices for milk and dairy products, dairy facilities and premises (www.dda.or.ug/ visited *19/7/2014*).

Raw milk samples were aseptically collected on delivery at the collection centers and transported on ice to the laboratory within three hours. The samples were analysed in duplicate using the VIDAS® *Listeria monocytogenes* II (LMO2) enzyme-linked fluorescent immunoassay (ELFA) kit (BioMérieux, Durham NC, USA) following the manufacturer's instructions. A test value was generated for each of the samples and compared to internal references (thresholds) allowing interpretation as positive or negative where it was ≥ 0.05 or <0.05 respectively.

The Epi Info statistical package version 3.4.3 and SPSS version 16.0 allowed establishment of the statistical associations using Odds Ratio and Chi-square and significance was defined as a *P*-value <.05, at 95% confidence interval.

## 3. RESULTS

Table 1 present data from the milk collecting center in relation to the managers' education level, knowledge of the DDA guidelines and practices; and access to the extension services. The level of education of milk collecting center managers ranged from no formal education to tertiary level; with 50% having gone beyond the primary level. Fifty per cent (50%) of the center managers had knowledge and copies of the guidelines issued by the DDA, however, five (62.5%) did not follow them. The challenges faced by center managers in following the DDA guidelines, included use of inappropriate milk containers (37.5%) by the farmers and traders; and adulteration by farmers (18.8%). A statistical association between knowledge of DDA guidelines among center managers and access to dairy extension services existed; with those not accessing the services being about 12 times unlikely to know the guidelines compared to those who did ($\chi^2$=4.65, *P*=.04 and Odds Ratio=11.68).

The licensing authorities included the Town Councils and DDA, each licensing 50% of the milk collecting centers. For those centers licensed by the Town Councils, none was inspected compared to those by DDA that had varying frequencies of inspection as indicated in Table 1. There was a relationship between the licensing body and inspection ($\chi^2$=21.26, *P*=.00); and of inspection and access to extension services ($\chi^2$=7.71, *P*=.01).

Out of the 68 milk samples from farms supplying the DDA licensed collection centers, 47 (69.1%) were contaminated with *Listeria* whereas of the 32 samples delivered to those centers not licensed by DDA, 25 (78.1%) were contaminated. Farms that supplied non-DDA licensed milk collection centers were 1.13 times more likely to have *Listeria* contamination.

Table 2 presents the occurrence of *Listeria* in raw milk and the association with various practices on the farms. From the laboratory analysis, 72% of the farms (n=100) had *Listeria* spp detected in their milk. Majority (64%) of the farm managers/attendants did not go beyond the primary level of education. Majority (69%) of the milking personnel used disinfectants and soap for cleaning their hands before milking; and not water only. Un-boiled water was commonly (65%) used for cleaning utensils and hands and such farms were 3.6 times more likely to have *Listeria* contaminated milk than those that used disinfectants and soap ($\chi^2$=5.67, *P*=.02; Odds Ratio=3.6). Most of the farms (86%) used water from communal dams, followed by boreholes (11%) while some (3%) used piped water. Farms that used water from communal dams were twice more likely to have milk contamination than those that used boreholes ($\chi^2$=11.66, *P*=.00 and Odds Ratio=2.07). There was a significant relationship between occurrence of *Listeria* and milking personnel hygienic practices (hand washing) ($\chi^2$=61.72, *P*=.00); and with water source ($\chi^2$=10.06, *P*=.01). On majority of farms (97%) cows grazed and fed on pastures within paddocks while 3% reported use of silage, hay and concentrates in addition to pastures. All the three farms that offered silage had *Listeria* contaminated milk. On majority of the farms (61%) faecal matter was left within the housing environment compared to 39% that had manure

**Table 1. Educational level, knowledge of DDA guidelines, practices and access to extension services by the milk collecting center managers**

| Particulars | Response | Frequency (%) n=16 |
|---|---|---|
| Education level of milk collection center managers | No formal education | 2 (12.5) |
| | Primary level | 6 (37.5) |
| | Secondary level | 5 (31.3) |
| | Tertiary level | 3 (18.7) |
| Having knowledge about DDA guidelines | Yes | 8 (50) |
| | No | 8 (50) |
| Challenges to following the DDA guidelines | Use of improper containers | 6 (37.5) |
| | Milk adulteration | 3 (18.8) |
| | None | 7 (43.75) |
| Licensing authority | DDA* | 10 (62.5) |
| | Town Council | 6 (37.5) |
| Milk collecting center inspection | Yes | 10 (62.5) |
| | No | 6 (37.5) |
| Frequency of Inspection | 0 | 6 (37.5) |
| | 1 | 2 (12.5) |
| | 2 | 5 (31.3) |
| | 3 | 1 (6.3) |
| | 4-5 | 2 (12.5) |
| Access to extension services | Yes | 6 (37.5) |
| | No | 10 (62.5) |

** DDA - Dairy Development Authority*

pits; and farms with no designated area for manure disposal were 5.6 times more likely to have contaminated milk compared to the latter ($\chi^2$=13.51, *P*=.00 and Odds Ratio=5.57). Majority of the farms (69%) had no paddocks designated for lactating cows; while 31% had such. On most farms (70%) cows were milked from outside in the kraal, 22% on the paddocks, 7% in milking parlour and 1% in crush. A significant relationship between milking place and *Listeria* contamination existed ($\chi^2$=12.75, *P*=.01 at 95% CI) and milk from animals milked from the kraal was more than twice likely to be contaminated ($\chi^2$=11.99 and *P*=.00 and Odds Ratio=2.5). Only 38.5% of the farms reported cleaning the milking area daily, while 39.7% and 21.8% of farms did so after a week and more than two weeks respectively. The frequency of cleaning the milking area was associated with *Listeria* contamination ($\chi^2$=4.66 and *P*=.05 at 95% CI) especially those that cleaned after two weeks (37.2%). Although many of the farms (56%) had no farm entry restriction and biosecurity measures, where they existed, it included fencing off the area with specific entrances, prohibited entry of non-farm workers/strangers and changing of attire and footwear on re-entry; and cleaning of hands and boots with disinfectants before entry. There was a relationship between farm entry restriction/biosecurity measures and prevalence of *Listeria* where farms without such were 3.14 times more likely to have contaminated milk ($\chi^2$=6.51, *P*=.01 and Odds Ratio=3.14). Interaction between wildlife and domestic livestock was reported on 83% of the farms. The wildlife species were reported to originate from forest foci around the farm areas and a gazetted wildlife (rhino) ranch in Nakasongola district.

Considering the type of milk containers and milk handling practices during transportation from farms to the collection centers, majority of the farms (52%) used plastic jerry cans, 42% used Aluminium cans, while 6% used both; and none of the farms had refrigeration facilities. There was a strong relationship between use of plastic jerry cans as milk containers and contamination by *Listeria* spp (Odds Ratio=2.72, $\chi^2$=10.2 and *P*=.00). Farms that used plastic jerry cans were 2.72 times more likely to have *Listeria* contamination compared to those that used Aluminium cans. Majority of the farms (63%) had no access to dairy extension services. Those farms that accessed dairy extension services, were provided by NGOs (62.2%); DDA (21.6%), area government veterinarians (13.5%); and private farm veterinarians (2.7%). There was a significant relationship between lack of access to extension services and prevalence of *Listeria*

($\chi^2$=12.42, *P*=.00 and Odds Ratio=5.02) as farms that had no access to extension services were five times more likely to have *Listeria* contaminated raw milk than those that did.

Majority of the managers/farmers (88%) knew the milk trade guidelines as set by DDA while 12% were ignorant. Out of the 88 managers/farmers that knew the guidelines, 69.3% had *Listeria* contamination in their milk compared to 91.7% of 12 that did not know the guidelines. Among the farm managers who knew DDA guidelines, 39 (44.3%) followed them, 18 (20.5%) partially did while 31 (35.2%) did not. The major challenges faced by the farmers/managers in adhering to DDA guidelines included high costs of white coats/overall (13%); and of aluminium cans, which were also claimed to be very heavy for transportation by bicycles and motorcycles (63%). Another challenge included adulteration of milk by herdsmen, which leads to poor quality of milk that is eventually rejected by milk collecting centers (1%). However, 23% of the managers/farmers did not report any challenges to adhering to the DDA guidelines. There was a relationship between farm non-adherence to DDA guidelines and *Listeria* contamination (Odds Ratio=6.81, $\chi^2$=14.62, *P*=.00), where farms that were not following guidelines were 6.81 times more likely to have contamination of raw milk than those that followed them.

As presented in Table 2, of the 100 farms, 72% had their milk contaminated by *Listeria*. By district of origin, Luweero had the highest with all the milk from the 11 farms contaminated, followed by Nakaseke with 70% (n=64) and lastly Nakasongola with 64% (n=25). There was an association between prevalence of *Listeria* contamination and origin of the samples which was more statistically significant for samples that originated from Nakaseke district ($\chi^2$=4.8 and *P*=.03).

**Table 2. Education level of managers/attendants; management practices; challenges and occurrence of *Listeria monocytogen*es at the farms (n=100)**

| Factor | Response | *Listeria* status | | Total |
|---|---|---|---|---|
| | | + (n=72) | -(n=28) | (n=100) |
| Education level of the farm managers | No formal | 29 | 9 | 38 |
| | Primary | 19 | 7 | 26 |
| | Secondary | 20 | 12 | 32 |
| | Tertiary | 4 | 0 | 4 |
| Milking personnel hygiene | Adequate/With disinfectants | 45 | 24 | 69 |
| | Inadequate/Water only | 27 | 3 | 31 |
| Washing of utensils | Hot water | 9 | 26 | 35 |
| | Un-boiled water | 63 | 2 | 65 |
| Source of water at the farm | Communal dams | 58 | 28 | 86 |
| | Boreholes | 0 | 11 | 11 |
| | Piped water | 0 | 3 | 3 |
| Faecal matter disposal | Manure collecting pits | 20 | 19 | 39 |
| | No established site | 53 | 9 | 61 |
| Management of lactating cows | Separated | 19 | 12 | 31 |
| | Mixed with others | 53 | 16 | 69 |
| Milking place | Crush | 0 | 1 | 1 |
| | Grazing paddock | 14 | 8 | 22 |
| | Milking parlour | 7 | 0 | 7 |
| | Outdoor kraal | 51 | 19 | 70 |
| Frequency of cleaning milking area | Daily | 26 | 7 | 33 |
| | Weekly | 22 | 7 | 29 |
| | More than a week | 8 | 8 | 16 |
| Farm entry restriction and biosecurity | Yes | 26 | 18 | 44 |
| | No | 46 | 10 | 56 |
| Interaction with wildlife | Yes | 60 | 23 | 83 |
| | No | 12 | 5 | 17 |
| Milk containers Used | Plastic cans | 38 | 14 | 52 |
| | Aluminium | 29 | 13 | 42 |
| | Both | 5 | 1 | 6 |

| **Factor** | **Response** | ***Listeria* status** | | **Total** |
|---|---|---|---|---|
| | | **+ (n=72)** | **-(n=28)** | **(n=100)** |
| Knowledge of guidelines | Yes | 61 | 27 | 88 |
| | No | 11 | 1 | 12 |
| Following DDA* guidelines | Yes | 20 | 19 | 39 |
| | Partially | 15 | 3 | 18 |
| | No | 27 | 4 | 31 |
| Challenges | Expensive and heavy Aluminium cans/Long distances | 42 | 21 | 63 |
| | Expensive Aluminium cans and overcoats | 12 | 1 | 13 |
| | Milk adulteration | 1 | 0 | 1 |
| | None | 17 | 6 | 23 |
| Feeding | Pastures only | 69 | 28 | 97 |
| | Silage and hay | 3 | 0 | 3 |
| Access to extension | Yes | 19 | 18 | 37 |
| | No | 53 | 10 | 63 |
| Source of extension services | DDA* | 6 | 2 | 8 |
| | NGO's** | 12 | 11 | 23 |
| | Government | 2 | 4 | 6 |
| *Listeria* contamination (positive farms and %) | Nakaseke | 45 (70%) | 19 | 64 |
| | Nakasongola | 16 (64%) | 9 | 25 |
| | Luweero | 11 (100%) | 0 | 11 |
| | Total | 72 (72%) | 28 | 100 |

** DDA – Dairy Development Authority; ** NGO's – Non-Governmental Organisations*

## 4. DISCUSSION

This study reported a prevalence of *Listeria* contaminated raw milk at 72%, which was higher than 60% in bulked raw milk that was reported earlier in Kampala by Mugampoza et al. [12], 50% in USA by Jackson et al. [14]; but slightly lower than 75% in Portugal by Pintado et al. [15]. The differences in contamination compared to the study by Jackson et al. [14]; could be attributed to the test methods employed; since the latter employed, a combination of pre-enrichment; VIDAS test and sub-culture on selective agars. VIDAS®LMO2 used in this study was approved as AOAC Official Method 2004.02 for detection of *L. monocytogenes* in dairy products; and is also an acceptable alternative method for detection of *L. monocytogenes* in various foods [16]. However, it is recommended to confirm and validate the results by isolation on selective media, that is Palcam and Oxford medium; and confirm the isolates using biochemical tests. Comparable contamination levels were detected although cultural methods were employed by Mugampoza et al. [12] and Pintado et al. [15]. During this study, further analyses to confirm *L. monocytogenes* were not done. Reports from previous research, indicate that *Listeria monocytogenes* contamination rates of raw milk at 8.3% in Morocco; 6.5% in Ethiopia and 13% in Kampala, Uganda; 12.5% in USA; 46% in Portugal are lower than that for *Listeria* spp in general. Hence, it is likely that *Listeria monocytogenes* contamination of milk obtained from farms from greater Luweero are also lower [12,14,15,17,18]. According to Meyer et al. [19], the VIDAS® LMO2 test is suitable for screening *Listeria* negative samples and samples strongly positive (<2) for *Listeria* by this test are positive for *L. monocytogenes*. Although the test is advocated for screening for *Listeria monocytogenes* in various foods; false negative samples are encountered due to presence of non-monocytogenes *Listeria* spp which have been reported to complicate its recovery during selective enrichment [20,21]. Hence, there is need to confirm VIDAS ® LMO2 positive samples using cultural methods for confirming the *Listeria* species.

*Listeria* contamination of the raw milk is likely to have originated from the farm due to management practices such as improper faecal disposal systems, since infected animals usually shed the organisms in their faeces [5,22,23]. In addition, the poor hygienic practices such as use of water from communal dams; and lack of use of disinfectants, are likely to have contributed to contamination of the milk handling equipment as reported by some previous researchers [24]. The significant association between milking hygienic practices and prevalence of *Listeria monocytogenes* has also been reported [25]. In addition, the variation in prevalence of *Listeria* contamination of milk, especially in USA are most likely to be due to differences in hygienic practices, knowledge of prescribed sanitary, use of treated piped water in production process, knowledge status of guidelines directed towards prevention of contamination; and difference in education levels.

In general, variability in frequency of contamination reflects difference in farm management practices, geographical locations, yearly seasons, sampling or analytical methods [17,26]. In addition, the education status of the farmers and lack of access to dairy extension services by both the farmers and milk collecting centers could have contributed to the high frequency of contamination as reported by previous researchers [17,27]. The absence of farm entry restriction and biosecurity measures; as encountered in this research has been reported to be a risk factor for farm environmental contamination with *Listeria monocytogenes* elsewhere [28-31].

Silage has been reported as a major risk factor to Listeriosis; however only 3% of the farms used it to feed the animals; and all milk samples were contaminated with *Listeria*. This is in agreement with what was reported earlier, that ensiling and stored forage is a risk factor for presence of *L. monocytogenes* on farms [32-34]. However, for this study, it was not possible to establish whether silage feeding was significantly associated with *Listeria* contamination of the milk because of the rarity of the practice among sampled farms.

Considering the knowledge of DDA guidelines, there was a difference between milk collection center managers and farm managers; and also prevalence of *Listeria* contaminated raw milk. Lack of knowledge about clean milk production and unclean milk equipment as a factor for *Listeria monocytogenes* contamination of raw milk has been reported elsewhere [27,35]. Lack of refrigeration facilities coupled by the practice of transportation of milk in non-recommended containers, like plastic jerry cans, are a contributory factor since the latter are difficult to clean, are conducive for the formation of biofilms; and *L. monocytogenes* has been reported to adhere to polymeric materials, especially at temperatures greater than 30℃ and low pH (4 to 7) [36-38]. Apart from the costly milking utensils, the low rate of inspection may have contributed to the high level of non-adherence to guidelines among milk collection centers and farms.

## 5. CONCLUSION

A high proportion (72%) of raw milk supplied to the collecting centers was contaminated with *Listeria*; and therefore consumption of raw milk from this area is of health risk. The factors that contributed to the high frequency of *Listeria* contaminated milk included absence of proper faecal disposal methods on farms; poor unhygienic practices like cleaning of utensils and hands without disinfectants and soap; lack of farm entry restriction and biosecurity measures; use of untreated water from communal dams; absence of established milking areas; infrequent cleaning of milking area; and lack of access to dairy extension services, sensitization and subsequent non-adherence to DDA guidelines.

We therefore recommend that the farmers and milk collecting center managers should be sensitized and trained as far as the DDA guidelines for quality, safety, hygiene practices for milk and dairy products, dairy facilities and premises; and to carry out effective inspections to assess compliance. In addition, confirming the *Listeria* species contaminating milk from various farms; assessing *Listeria monocytogenes* carrier status of cattle; the microbial levels in milk; as well as the influence of silage feeding will inform on policies for prevention of contamination. Food processing environs are of particular importance as far as *L. monocytogenes* contamination is concerned; hence the status of the milk collecting centers needs to be explored.

## STATEMENT OF ANIMAL RIGHTS

“All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”. However, this research did not involve direct manipulation of the animals.

## ETHICAL APPROVAL

The research was approved by the College of Veterinary Medicine, Animal Resources and

Biosecurity Higher Degrees Committee and the College Institutional Review Board.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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