Received: 21 October 2022 | Revised: 28 February 2023 | Accepted: 18 March 2023

DOI: 10.1111/jfs.13055

Journal of Food Safety WILEY

ORIGINAL ARTICLE

# Contamination of beef and beef products by *Listeria* spp. and molecular characterization of *L. monocytogenes* in Mpumalanga, South Africa

Khomotso C. Moabelo[1] | Nomakorinte Gcebe[2] | James Gana[1,3] | Yusuf B. Ngoshe[4] | Abiodun A. Adesiyun[1,5]

[1]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Pretoria, South Africa

[2]Bacteriology Department, Serology section, Onderstepoort Veterinary Research, Agricultural Research Council, Pretoria, South Africa

[3]Department of Agricultural Education, Federal College of Education, Kontagora, Niger State, Nigeria

[4]Department of Production Animal Studies, Epidemiology Section, University of Pretoria, Pretoria, South Africa

[5]Department of Paraclinical Sciences, School of Veterinary Medicine, The University of the West Indies, St. Augustine, Trinidad and Tobago

**Correspondence**
Abiodun A. Adesiyun, Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, Pretoria 0110, South Africa.
Email: abiodun.adesiyun@up.ac.za

**Funding information**
Red Meat Research and Development South Africa

## Abstract

This study determined the prevalence, risk factors, and molecular characteristics of *Listeria* species detected in beef and beef products sampled in Mpumalanga province, South Africa. Four hundred beef and beef products were collected from 30 retail outlets in three districts (Bronkhorstspruit, Emalahleni, and Middelburg) within the province. Standard bacteriological and polymerase chain reaction (PCR) assays were used in the study. The overall prevalence of *L. monocytogenes* and other *Listeria* spp. in the samples was 8.3% (33/400) and 30% (120/400) ($p < .05$), respectively. For the five variables investigated, statistically significant effects were evident only for the region ($p < .001$) and type of product ($p < .0001$) for *L. monocytogenes*, the type of outlet ($p = .011$) and the type of product ($p < .0001$) for *Listeria* spp. Of the 20 types of beef and beef products tested, 15 (75%) and 17 (85%) were contaminated by *L. monocytogenes* and *Listeria* spp., respectively ($p = .429$). Among the four categories of products tested, the prevalence of *L. monocytogenes* was 7.3% (8/109), 10.6% (11/104), 7.5% (8/106), and 7.4% (6/81) for raw beef, ready-to-eat (RTE) products, milled beef, and offal & organs, respectively ($p > .799$). Among the 33 *L. monocytogenes* isolates, PCR genoserogroup IIa (42.4%, 1/2a-3a) was most frequently detected. All (100%) of the isolates carried one or more of the eight virulence-associated genes assessed, with genes *inlC* and *inlJ* detected in all the isolates. The overall prevalence of *L. monocytogenes* (8.3%) and the high frequency of virulent serogroups of *L. monocytogenes* commonly associated with human listeriosis pose a food safety risk to consumers of beef and beef-based products contaminated by *L. monocytogenes*.

## 1 | INTRODUCTION

*L. monocytogenes* is a gram-positive, zoonotic bacterial pathogen with the ability to survive in different environments, including soil, water, animal feed, and fresh and frozen meat (Paduro et al., 2020; Shourav, Hasan, & Ahmed, 2020). It is a causative agent of foodborne listeriosis, both invasive and noninvasive diseases (Zamuz et al., 2021). Listeriosis rarely occurs in humans; however, the disease is associated with severe clinical manifestations such as abortion, preterm birth or stillbirth in pregnant women, meningitis or encephalitis, and death. Other clinical manifestations of the disease include fever, muscle aches, nausea, vomiting, stomach

cramps, and diarrhea (Pinto, Novello, Montemurro, Bonerba, & Tantillo, 2010).

The genus *Listeria* currently includes 27 recognized species, but only two species, *L. monocytogenes*, and *L. ivanovii*, are considered pathogens (El Hag, El Zubeir, & Mustafa, 2021; Ledlod, Bunroddith, Areekit, Santiwatanakul, & Chansiri, 2020). To date, only *L. monocytogenes* (Gana, Gcebe, Pierneef, Moerane, & Adesiyun, 2022; Kayode & Okoh, 2022), *L. innocua* (El-Zowalaty et al., 2019; Makumbe, Tabit, & Dlamini, 2021), and *L. welshimeri* (Manqele, Gcebe, Pierneef, Moerane, & Adesiyun, 2023) have been documented as occurring in humans, foods and environmental sources in South Africa.

Serotyping is used in the epidemiological investigation field for classification, disease transformation monitoring, and identification of the source of pathogens (Li et al., 2021). Based on its flagellar (H) and somatic (O) antigens, 13 *L. monocytogenes* serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) have been identified, which are further characterized into polymerase chain reaction (PCR) genoserogroups IIa (1/2a-3a), IIb (1/2b-3b-7), IIc (1/2c-3c), IVa (4a-4c), and IVb (4ab-4b, 4d-4e) (Bouymajane et al., 2021). However, only four (1/2a, 1/2b, 1/2c, and 4b) of the 13 serotypes account for approximately 95% of human listeriosis cases (Bouymajane et al., 2021). In South Africa, the serotypes of *L. monocytogenes* identified to date include 1/2a, 3a, 4b, 4d, 4e, 1/2c, and 3c in meat and meat products across the nine provinces in the country (Matle et al., 2019), 1/2b, 3b, 4b, 4d, and 4e from meat and food processing facilities (Mafuna, Matle, Magwedere, Pierneef, & Reva, 2021) and 4b from humans (Koopmans, Brouwer, Vázquez-Boland, & van de Beek, 2022). Information on the serotypes of *L. monocytogenes* recovered from beef and beef products according to the isolates' origin (district, size of retail outlets, and type of samples) is lacking.

Virulence genes in *L. monocytogenes* have been associated with the ability of the organism to cause listeriosis in humans and animals (Gray et al., 2021). Some virulence genes are located on the *Listeria* pathogenicity island 1 (LIPI-1), including *PrfA*, *plcA*, *plcB*, *hlyA*, *mpl*, and *actA*. They provide *L. monocytogenes* with the ability to survive and spread in host cells (Sereno et al., 2019). These genes have been identified in *L. monocytogenes* isolates from imported and local meat and meat products in the country (Matle et al., 2019) and food processing facilities (Mafuna et al., 2021).

Between 2017 and 2018, South Africa experienced the largest outbreak of human listeriosis in the world, and "polony," a beef-based ready-to-eat (RTE), was implicated in 2018 (Allam et al., 2018; Boatemaa et al., 2019; Olanya et al., 2019). Since then, although studies have been conducted on *L. monocytogenes* that contaminated meat products and food processing facilities across the country, information is lacking on the distribution of this pathogen according to the districts, the size of retail outlets from where the samples originated as well as the types of beef products in Mpumalanga province. This is important because 4.53% of the cases documented in the outbreak of 2017–2018 in the country originated in Mpumalanga province (Kayode, Igbinosa, & Okoh, 2020). It is, therefore, imperative to identify the possible factors associated with the contamination of beef and beef products by virulent strains of *L. monocytogenes* and the potential health risk posed to consumers.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design

A cross-sectional study was conducted at 30 retail outlets in Mpumalanga province, South Africa, to determine the prevalence of *Listeria* in beef and beef products. Retail outlets were selected based on the information obtained from the Consumer Goods Council of South Africa (CGCSA) regarding their location and relative sizes. Retail outlets were selected and classified based on their size (chain supermarkets, large, medium, and small outlets). Identified retail outlets were randomly selected for participation in the study. A sample size of 401 was determined using a formula by Thrusfield (2007) with a precision value of 3.5% and a prevalence of 14.7% (Matle et al., 2019). A total of 400 samples were collected for the study. The strategy used for classifying retail outlets and the types and number of beef and beef products sampled for the study is shown in Table S1. The number of samples collected was proportional to the size of the outlets as follows: 160, 128, 80, and 32 from the chain, large, medium, and small outlets, respectively. At the selected outlets, convenience sampling of the beef and beef products was done based on availability. A total of 400 beef and beef samples were collected for the study. The samples included raw, chilled, and frozen beef and beef products and dried beef RTE products. All samples were collected from November 2019 to May 2021.

### 2.2 | Descriptions of selected beef and beef products unique to Gauteng province, South Africa

Two of the products sampled in the current study are unique and popularly consumed in South Africa, namely:

*Biltong*: A South African RTE meat product prepared through a process that involves salting and spicing the strips of meat from herbivorous animals of the antelope and bovine families before being dried. Vinegar is also added in salting to inhibit microbial growth and enhance flavor (Jones, Arnaud, Gouws, & Hoffman, 2019). The presence of vinegar makes biltong different from other dried meats.

*Polony*: A commonly consumed deli meat in South Africa known as bologna sausage, which is produced mainly from mechanically recovered meat (Beef/pork/chicken) and processed by food manufacturers in South Africa (Olanya et al., 2019). The cooked meat products are usually stuffed into tubular nylon casings, which is removed before consumption. The extensive use of meat trimmings (beef, pork, or chicken) makes polony an inexpensive and affordable meat product (Cluff, Kobane, Bothma, Hugo, & Hugo, 2017).

### 2.3 | Isolation and identification of *L. monocytogenes* and Listeria spp.

Beef and beef products were analyzed using the *Listeria* Precis method according to a protocol described by Matle et al. (2019) with minor modifications. Beef samples (brisket/rump/steak/T-bone,

boerewors, "biltong," Vienna, and "polony") were aseptically cut into pieces using forceps and scissors. Ten grams of cut meat was transferred aseptically into 90 mL of ONE Broth-*Listeria* (Thermo Fisher, South Africa) in a stomacher bag and homogenized (Stomacher Lab Blender 400, Seward Ltd., West Sussex, UK) at normal speed for 2 min. The homogenate was aerobically incubated at 35°C for 48 h.

Following 48 h incubation, 10 µL of enriched broth samples were inoculated onto chromogenic Brilliance *Listeria* Agar (BLA) (Thermo Fisher, South Africa) plates and incubated at 35°C for 48 h. Suspect colonies on BLA plates with the characteristic appearance of *Listeria* spp. (blue without a halo for *Listeria* spp. and blue with a white/cream halo for *L. monocytogenes*) were initially identified using phenotypical methods followed by molecular methods (Matle et al., 2019; Ryu et al., 2013). Confirmed *L. monocytogenes* isolates were preserved in 50% brain–heart infusion (BHI)/50% glycerol (Thermo Fisher, South Africa). The study design was to identify the *Listeria* isolates as *L. monocytogenes* and other *Listeria* spp. (i.e., non-*L. monocytogenes*).

## 2.4 | Molecular identification and characterization of *Listeria* spp. and *Listeria monocytogenes*

### 2.4.1 | Extraction of DNA from enriched broth cultures

DNA was extracted by the boiling-centrifugation method, as described by Soumet, Ermel, Fach, and Colin (1994). The DNA in the supernatant was used in PCR to characterize *Listeria* to further characterize *Listeria*.

### 2.4.2 | Screening of broth cultures for *Listeria* spp. by multiplex PCR (mPCR)

All enriched broth samples were screened by mPCR for the existence of *Listeria* spp., that is, *Listeria* genus. The screening was performed using an mPCR assay that targets *prs* as well as *orf2110*, *orf2818*, *imo1118*, and *imo0737* genes, as described by Doumith, Buchrieser, Glaser, Jacquet, and Martin (2004), were used to characterize *L. monocytogenes* genoserogroups (Doumith et al., 2004). The primers used in the current study are shown in Table S2. The primers were prepared as follows: 12.5 µL of 2× red Taq master mix, 5 µL nuclease-free water, 5 µL DNA template, and 4 µL primer mix for 1 PCR (Doumith et al., 2004). The PCR conditions used were as follows: initial denaturation step at 94°C for 3 min; 35 cycles of 94°C for .40 min; 53°C for 1.15 min, 72°C for 1.15 min, and 1 final cycle of 72°C for 7 min in a thermocycler (Eppendorf, Germany). DNA extracts were prepared as described above and were used as templates in the mPCR. The PCR products were subjected to electrophoresis on 3% agarose gel (CSL AG500, Cleaver Scientific Ltd., United Kingdom) for 3 h at 120 v. *L. monocytogenes* ATCC 19111 was used as a positive control, and distilled water as a negative control.

### 2.4.3 | Culture for isolation and extraction of DNA from the isolates of *Listeria* spp.

DNA was extracted from individual pure colonies typical of *Listeria* (blue without a halo for *Listeria* spp. and blue colonies with a white/cream halo for *L. monocytogenes* colonies) and confirmed as previously described by Matle et al. (2019). Before DNA extraction, the preserved *L. monocytogenes* isolates were inoculated into BHI broth, followed by overnight aerobic incubation at 35°C. A loopful from incubated BHI broth culture was used to inoculate BLA plates, which were incubated at 35°C for 48 h. For DNA extraction from the colonies, 200 µL of sterile distilled water was aliquoted into 2 mL tubes. Each was inoculated with a colony of bacterial culture harvested from the BLA plates. The bacterial suspension was then vortexed for 10 s, heated at 95°C for 10 min, cooled at room temperature (22 ± 2°C), and centrifuged at 15,493 ×*g* for 5 min. The supernatant was transferred into sterile Eppendorf tubes, and precipitate was discarded. The crude supernatant was stored at −20°C and subsequently used as a DNA template in the PCR to detect the genus *Listeria* for genoserogrouping and virulence profiling.

## 2.5 | Detection of *Listeria* genus and determination of the genoserogroups of *L. monocytogenes*

The mPCR assay that targets the five fragments of *L. monocytogenes* at the following final concentrations: *Imo1118*, *Imo0737*, *orf2110*, *orf2819*, and *prs* (specific for *Listeria* spp.) at the final concentrations of .05 µM was used to confirm the presence of *Listeria* genus and to determine the serogroups of *L. monocytogenes* as previously described by Doumith et al. (2004). The primers and PCR conditions are indicated in Table S2. PCR preparations and conditions described for detecting *Listeria* in broth cultures were used to determine serogroups. *L. monocytogenes* ATCC 19111 was used as a positive control. The products were subjected to electrophoresis in 3% agarose gel (CSL AG500, Cleaver Scientific Ltd., UK), and a gel documentation system (Vacutec, SA) was used to capture the bands. A sample of the PCR gel images obtained in assays to detect the serogroups of *L. monocytogenes* is shown in Figure S1.

## 2.6 | Detection of virulence-associated genes in *L. monocytogenes* isolates

The presence of selected virulence-associated genes in the isolates of *L. monocytogenes* was determined, as described by Rawool, Malik, Shakuntala, Sahare, and Barbuddhe (2007). Multiplex PCR was used to detect eight virulence-associated genes of *L. monocytogenes: plcA*, *hlyA*, *actA*, *inlB*, *iap*, *inlA*, *inlC*, and *inlJ*, in two reactions. Reaction 1 (mPCR 1) contained five primer pairs (*plcA*, *hlyA*, *actA*, *inlB*, and *iap*), while Reaction 2 (mPCR 2) consisted of three primer sets (*inlA*, *inlC*, and *inlJ*) (Table S3) for the virulence genes. The PCR assays and agarose gel electrophoresis of the PCR products were performed using

**TABLE 1** Prevalence of *Listeria monocytogenes* and *Listeria* spp. in beef and beef products by district, size of outlet, presentation to consumers, and temperature of storage.

| Variable | Level | No. of samples tested | *L. monocytogenes* No. (%) of samples positive[a] | *Listeria* spp. No. (%) of samples positive[b] | *p* value |
|---|---|---|---|---|---|
| District | Bronkhorstspruit | 150 | 1 (0.67) | 37 (24.7) | <.001 |
| | Emalahleni | 149 | 21 (14.1) | 52 (34.9) | <.001 |
| | Middleburg | 101 | 11 (10.9) | 31 (30.7) | <.001 |
| | *p* value | | *p* < .001 | .153 | |
| Type of outlet | Chain | 170 | 11 (6.5) | 40 (23.5) | <.001 |
| | Large | 106 | 8 (7.6) | 29 (27.4) | <.001 |
| | Medium | 101 | 13 (12.9) | 41 (40.6) | <.001 |
| | Small | 23 | 1 (4.4) | 10 (43.5) | .002 |
| | *p* value | | .252 | .011 | |
| Presentation for consumption | RTE[c] | 103 | 11 (10.7) | 26 (25.2) | .006 |
| | Raw | 297 | 22 (7.4) | 94 (31.7) | <.001 |
| | *p* value | | .298 | .221 | |
| Temperature of presentation | Nonchilled[d] | 32 | 3 (9.4) | 7 (21.9) | .168 |
| | Chilled | 288 | 23 (8.0) | 84 (29.2) | <.001 |
| | Frozen | 80 | 7 (8.8) | 29 (36.3) | <.001 |
| | *p* value | | .948 | .274 | |

[a]Based on a total of 33 contaminated samples.
[b]Based on a total of 120 contaminated samples.
[c]RTE: Ready-to-eat.
[d]At room temperature.

the procedure described by Rawool et al. (2007). The PCR assay was prepared as follows: 12.5 µL of 2× of red Taq master mix (Lasec, SA, Pty, Cape Town, South Africa), 5 µL nuclease-free water, 5 µL DNA template (prepared by adding one colony forming unit of pure culture of *Listeria* to 200 µL of PCR water), and 3 µL (concentration of .4 µM for each primer) primer mix for mPCR 1 and mPCR 2 primers. The DNA amplification conditions were as follows: 2 min initial denaturation at 94°C; 35 cycles of denaturation at 94°C for 30 s; annealing at 55°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. A sample of the PCR gel images was obtained in assays to detect the virulence-associated genes in *L. monocytogenes* isolates is displayed in Figure S2.

## 2.7 | Data analysis

Results obtained from the laboratory data for the prevalence of *Listeria monocytogenes* and other *Listeria* spp., serogroups, and virulence-associated genes characterized by the survey were entered into Microsoft Excel 2016.

Data were analyzed using the Epi Info software (version 7.0), and the association of variables was determined using Fisher's Exact and chi-square tests. The significant difference was evaluated using Fisher's exact ($p < .05$), and percentages were calculated at a 95% confidence interval.

Epi Info was also used to generate percentages for categorical data on the prevalence of *Listeria* spp. in sample types, sample storage, geographical distribution, and retail groupings. Epi Info also determined the frequency of isolation of genoserogroups and virulence-associated genes.

# 3 | RESULTS

The overall prevalence of *Listeria* spp. from the retail outlets was 38.3% (153/400, 95% CI: 33.62–43.10). The prevalence of *L. monocytogenes* in the beef and beef-based products samples was 8.3% (33/400; 95%CI: 5.93–11.36), while for the other *Listeria* spp. (non-*L. monocytogenes*), it was 30.0% (120/400, 95%CI: 25.72–34.66). The difference was statistically significant ($p < .001$).

The prevalence of *L. monocytogenes* in beef and beef products in the three districts (Bronkhorstspruit, Emalahleni, and Middleburg), which were sampled was .67% (1/150), 14.1% (21/149), and 10.9% (11/101), respectively. The differences were statistically significant ($p < .001$), as shown in Table 1. There were no significant differences in the prevalence of *L. monocytogenes* when comparing the effect of the size of the outlet ($p = .252$), the presentation for consumption (RTE vs. raw) ($p = .298$), and the temperature of the product displayed for sale ($p = .948$).

**TABLE 2** Prevalence of *L. monocytogenes* and other *Listeria* species in beef and beef-based products.

| Types of samples | No. of types Samples | No. of Samples tested | No. (%) of samples positive for | | *p* value |
|---|---|---|---|---|---|
| | | | *L. monocytogenes* | *Listeria* spp. | |
| Raw beef | 6[a] | 109 | 8 (7.3) | 30 (27.5) | <.001 |
| RTE | 4[b] | 104 | 11 (10.6) | 26 (25.0) | .007 |
| Milled beef | 3[c] | 106 | 8 (7.5) | 35 (33.0) | <.001 |
| Offal & Organs | 7[d] | 81 | 6 (7.4) | 29 (35.8) | <.001 |
| *p* value | | | .799 | .346 | |
| Total | 20 | 400 | 33 (8.3) | 120 (30.0) | <.001 |

[a]Consists of beef brisket ($n = 24$), beef chuck ($n = 16$), beef steak ($n = 22$), beef short rib ($n = 11$), beef stew pieces ($n = 31$), and beef stir-fry ($n = 5$).
[b]Comprising "Biltong" ($n = 31$), "Polony" ($n = 29$), "Russian" ($n = 18$), and Vienna ($n = 26$).
[c]Made up of beef burger ($n = 16$), minced beef ($n = 45$), and boerewors ($n = 45$).
[d]Consisted beef heart ($n = 4$), beef kidney ($n = 6$), beef tongue ($n = 3$), beef liver ($n = 31$), beef lung ($n = 8$), spleen ($n = 3$), and beef tripe ($n = 26$).

The prevalence of other *Listeria* spp. was statistically significantly affected by the size/type of outlets only ($p = .011$), where the prevalence ranged from 23.5% in the chain outlets to 43.5% in the small outlets (Table 1). However, no statistically significant effect was exerted by the district ($p = .153$), presentation for consumption ($p = .221$), and the temperature of displayed beef and beef products ($p = .274$).

For the 12 levels at which samples were collected, the prevalence of other *Listeria* spp. in the beef and beef products was statistically significantly ($p < .05$) higher than found for *L. monocytogenes*, except for nonchilled beef and beef products ($p = .168$) (Table 1).

For the four categories of food types (raw beef, RTE, milled beef, and offal & organs) comprising 20 different types of beef and beef products assessed, the prevalence of *L. monocytogenes* ranged from 7.3% (8/109) for raw beef to 10.6% (11/104) for RTE, but the differences were not statistically significant ($p > .799$) (Table 2). Similarly, the prevalence of *Listeria* spp. did not vary significantly ($p = .346$) across beef types ranging from 25% (26/104) for RTE to raw beef to 35.8% (29/81) for offal & organs. However, data analysis on each of the 20 beef and beef products revealed a statistically significant ($p < .01$) higher prevalence of *Listeria* spp. than found for *L. monocytogenes* (Table S4) For the beef and beef products for which 20 or more samples were tested, the prevalence of *L. monocytogenes* in raw beef was 8.3% (2/24), 0% (0/22), and 6.5% (2/31) for beef brisket, beef steak, and beef stew pieces, respectively; for RTE, it was 9.7% (3/31), 6.9% (2/29), and 15.4% (4/26) for "biltong", "polony" and Vienna, respectively; for milled beef, the prevalence was 6.7% (3/45) and 6.7% (3/45) for minced beef and boerewors, respectively; for offal and organ samples, the prevalence was 3.2% (1/31) and 7.7% (2/31) for beef liver and beef tripe, respectively. For the 20 different types of beef and beef products assessed, 15 (75%) and 17 (85%) were positive for *L. monocytogenes* and *Listeria* spp., respectively, but the difference was not statistically significant ($p = .429$) (Table 2). However, the overall prevalence in the 20 types of products varied significantly for both *L. monocytogenes* ($p < .0001$) and *Listeria* spp. ($p < .0001$). For the two RTE products, namely, "Biltong" and "Polony," unique to South Africa, the prevalence of *L. monocytogenes* and *Listeria* spp., respectively, was 9.7%, and 19.4% ($p = .279$), 6.9%, and 34.5% ($p < .001$).

Of the 153 isolates of *Listeria* recovered from the 400 samples of beef and beef products assessed, *L. monocytogenes* and *Listeria* spp. constituted 21.6% (33/153) and 78.4% (120/153), respectively ($p < .0001$).

For the characterization of *L. monocytogenes* isolates by their serogroups and serotypes, the prevalence of serogroups detected was 3.5% (14/400), 1.0% (4/400), 1.3% (5/400), and 2.5% (10/400) for serogroups 1/2a-3a, 1/2b-3b, 1/2c-3c, and 4b-4d-4e, respectively. The differences were statistically significant ($p = .046$). The frequency distributions of the serogroups among the 33 isolates of *L. monocytogenes* were 42.4% (14/33, 95%CI: 25.48–60.78), 12.1% (4/33, 95%CI: 3.40–28.20), 15.12% (5/33, 95%CI: 5.11–31.90), and 30.3% (10/33, 95%CI: 15.59–48.71) for serogroups 1/2a-3a, 1/2b-3b, 1/2c-3c, and 4b-4b-4e, respectively. The differences were statistically significant ($p = .015$).

The frequencies of detection of *L. monocytogenes* serogroups by the district are presented in Table 3. Across the three districts where the samples originated, the frequency of *L. monocytogenes* serogroups varied considerably, but the differences were not statistically significant ($p > .05$). The dominant serogroup by the district was 1/2a-3a (47.6%) in Emalahleni, 1/2b-3b (14.3%) in Emalahleni, 1/2c-3c (27.3%) in Middleburg, and 4b-4d-4e (100%) in Bronkhorstspruit.

According to the size of retail outlets, although the differences in the frequencies of serogroups were not statistically significant ($p > .05$), the predominant serogroup was 1/2a-3a (100%), 1/2b-3b (37.5%), 1/2c-3c (27.3%), and 4b-4d-4e (50%) in the small, large, chain, and large outlets, respectively.

The distribution of the serogroups of *L. monocytogenes* varied considerably by the type of beef and beef products. The highest frequency of detecting the four serogroups by sample type was as follows: Serogroup 1/2a-3a (50%; 4/8) in RTE and milled beef, 1/2b-3b (27.3%, 3/11) in RTE, 1/2c-3c (18.2%, 2/11) in RTE, and 4b-4d-4e (50%, 3/6) in offal & organ isolates. The frequency of distribution of the four serogroups across the types of beef and beef products varied significantly ($p < .05$).

**TABLE 3** Frequency distribution of *Listeria monocytogenes* serogroups by the district, size of outlets, and type of product.

| District | No. of isolates tested[b] | No. (%) positive[a] for serogroup by the district of outlet | | | |
|---|---|---|---|---|---|
| | | 1/2a-3a | 1/2b-3b | 1/2c-3c | 4b-4d-4e |
| Bronkhorstspruit | 1 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) |
| Emalahleni | 21 | 10 (47.6) | 3 (14.3) | 2 (9.5) | 6 (28.6) |
| Middleburg | 11 | 4 (36.7) | 1 (9.1) | 3 (27.3) | 3 (27.3) |
| **Size of outlet** | **No. of isolates Tested** | **No. (%) Positive for serogroup by the size of outlet** | | | |
| | | 1/2a-3a | 1/2b-3b | 1/2c-3c | 4b-4d-4e |
| Chain | 11 | 6 (54.6) | 0 (0.0) | 3 (27.3) | 2 (18.2) |
| Large | 8 | 1 (12.5) | 3 (37.5) | 0 (0.0) | 4 (50.0) |
| Medium | 13 | 6 (46.2) | 1 (7.7) | 2 (15.4) | 4 (30.8) |
| Small | 1 | 1 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| **Type of product** | **No. of isolates Tested** | **No. (%) Positive for serogroup by the type of product** | | | |
| | | 1/2a-3a | 1/2b-3b | 1/2c-3c | 4b-4d-4e |
| Raw beef | 8 | 4 (50.0) | 0 (0.0) | 1 (12.5) | 3 (37.5) |
| RTE | 11 | 4 (36.4) | 3 (27.3) | 2 (18.2) | 2 (18.2) |
| Milled beef | 8 | 4 (50.0) | 1 (12.5) | 1 (12.5) | 2 (25.0) |
| Offal and organs | 6 | 2 (33.3) | 0 (0.0) | 1 (16.7) | 3 (50.0) |

[a]Of a total of 33 isolates of *L. monocytogenes*.
[b]All the 42 samples of the following beef and beef products tested were negative *for L. monocytogenes*, namely, beef heart ($n = 4$), beef lungs ($n = 8$), beef spleen ($n = 3$), beef steak ($n = 22$), and beef stir-fry ($n = 5$).

**TABLE 4** Frequency of selected virulence genes in *Listeria monocytogenes* according to the district, size of outlets and type of beef and beef products.

| District | No. of isolates tested[a] | No. (%)[b] of isolates of *L. monocytogenes* positive for virulence-associated genes by the district of outlet | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *hlyA* | *inlB* | *plcA* | *actA* | *iap* | *inlA* | *inlC* | *inlJ* |
| Bronkhorstspruit | 1 | 1 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) | 1 (100.0) | 1 (100.0) | 1 (100.0) |
| Emalahleni | 21 | 16 (76.2) | 9 (42.9) | 3 (14.3) | 14 (66.7) | 11 (52.4) | 20 (95.2) | 21 (100.0) | 21 (100.0) |
| Middleburg | 11 | 10 (90.9) | 6 (54.5) | 4 (36.4) | 8 (72.7) | 8 (72.7) | 11 (100.0) | 11 (100.0) | 11 (100.0) |
| **Size of outlet** | **No. of isolates tested** | **No. (%) of isolates of *L. monocytogenes* positive for virulence genes by size of outlet** | | | | | | | |
| | | *hlyA* | *inlB* | *plcA* | *actA* | *iap* | *inlA* | *inlC* | *inlJ* |
| Chain | 11 | 10 (90.9) | 1 (9.1) | 2 (18.2) | 5 (45.5) | 5 (45.5) | 11 (100.0) | 11 (100.0) | 11 (100.0) |
| Large | 8 | 4 (50.0) | 5 (62.5) | 0 (0.0) | 6 (75.0) | 4 (50.0) | 8 (100.0) | 8 (100.0) | 8 (100.0) |
| Medium | 13 | 12 (92.3) | 9 (69.2) | 5 (38.5) | 11 (84.62) | 11 (84.6) | 12 (92.3) | 13 (100.0) | 13 (100.0) |
| Small | 1 | 1 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) | 1 (100.0) | 1 (100.0) |
| **Type of meat and meat products** | **No. of isolates tested** | **No. (%) of isolates of *L. monocytogenes* positive for virulence genes by type of beef and beef products** | | | | | | | |
| | | *hlyA* | *inlB* | *plcA* | *actA* | *iap* | *inlA* | *inlC* | *inlJ* |
| Raw beef | 8 | 7 (87.5) | 4 (50.0) | 2 (25.0) | 7 (87.5) | 4 (50.0) | 8 (100.0) | 8 (100.0) | 8 (100.0) |
| RTE | 11 | 7 (63.6) | 6 (54.5) | 3 (27.3) | 7 (63.6) | 8 (72.7) | 10 (90.9) | 11 (100.0) | 11 (100.0) |
| Milled beef | 8 | 7 (87.5) | 3 (37.5) | 0 (0.0) | 4 (50.0) | 4 (50.0) | 8 (100.0) | 8 (100.0) | 8 (100.0) |
| Offal & Organs | 6 | 6 (100.0) | 2 (33.3) | 2 (33.3) | 4 (66.7) | 4 (66.7) | 6 (100.0) | 6 (100.0) | 6 (100.0) |

[a]All the 42 samples of the following beef and beef products tested were negative *for L. monocytogenes*, namely, beef heart ($n = 4$), beef lungs ($n = 8$), beef spleen ($n = 3$), beef steak ($n = 22$), and beef stir-fry ($n = 5$).
[b]Of a total of 33 isolates of *L. monocytogenes*.

**TABLE 5** Frequency of detection of selected virulence genes in *Listeria monocytogenes* according to serogroup.

| | | No. (%) of *L. monocytogenes* isolates positive for virulence gene | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Serogroup | No. of isolates tested | *hlyA* | *inlB* | *plcA* | *actA* | *iap* | *inlA* | *inlC* | *inlJ* |
| 1/2a-3a | 14 | 12 (85.7) | 4 (28.6) | 4 (28.6) | 10 (71.4) | 7 (50.0) | 13 (92.9) | 14 (100.0) | 14 (100.0) |
| 4b-4d-4e | 10 | 9 (90.0) | 5 (50.0) | 1 (10.0) | 7 (70.0) | 7 (70.0) | 10 (100.0) | 10 (100.0) | 10 (100.0) |
| 1/2c-3c | 5 | 5 (100.0) | 3 (60.0) | 2 (40.0) | 3 (60.0) | 4 (80.0) | 5 (100.0) | 5 (100.0) | 5 (100.0) |
| 1/2b-3b | 4 | 1 (25.0) | 3 (75.0) | 0 (0.0) | 2 (50.0) | 2 (50.0) | 4 (100.0) | 4 (100.0) | 4 (100.0) |
| *p* value | | 0.018 | 0.317 | 0.381 | 0.851 | 0.571 | 1 | 1 | 1 |
| Total | 33 | 27 (81.8) | 15 (45.5) | 7 (21.2) | 22 (66.7) | 20 (60.6) | 32 (97.0) | 33 (100.0) | 33 (100.0) |

Regarding the frequency of detection of virulence-associated genes in the isolates of *L. monocytogenes*, the frequency of the eight virulence-associated genes assessed in *L. monocytogenes* recovered from the 400 beef and beef products sampled was *inlC*, 8.3% (33/400), *inlJ*, 8.3% (33/400), *inlA*, 8% (32/400), *hlyA*, 6.8% (27/400), *actA*, 5.5% (22/400), *iap*, 5.0% (20/400), *inlB*, 3.8% (15/400), and *plcA*, 1.8% (7/400). The differences were statistically significant ($p < .001$).

The frequency distribution of the virulence-associated genes among the 33 positive isolates was as follows: *inlC*, 100% (33/33, 95%CI: 89.42–100.00), *inlJ*, 100% (33/33, 95%CI: 89.42–100.00), *inlA*, 96.97% (32/33, 95%CI: 84.24–99.92), *hlyA*, 81.82% (27/33, 95% CI: 64.54–93.02), *actA*, 66.67% (22/33, 95%CI: 48.17–82.04), *iap*, 60.61% (20/33, 95%CI: 42.15–71.09), *inlB*, 45.45%, (15/33, 95%CI: 28.11–63.65), and *plcA*, 21.2% (7/33, 95%CI: 9.28–39.97). The differences were statistically significant ($p < .001$).

The distribution of virulence-associated genes according to the district, size of outlets, and type of beef products revealed that for each of the three districts (Bronkhorstspruit, Emalahleni, and Middleburg), virulence-associated genes *hlyA*, *InlA*, *inlC*, and *inlJ* were predominantly (76.2%–100%) detected. However, the districts did not significantly ($p > .05$) affect the frequency of detection of virulence-associated genes (Table 4). The frequency of detection ranged from (76.2%–100%; $p = .527$) for *hlyA*, (0%–54.5%; $p = .533$) for *inlB*, (0%–36.4%; $p = .335$) for *plcA*, (0%–72.7%; $p = .335$) for *actA* (52.4%–100%; $p = .382$) for *iap*, (95.2%–100%; $p = .744$) for *inlA*, (100%; $p = 1$) for *inlC* and *inlJ*.

The size of the outlets had a significant ($p = .014$) effect only on the detection of virulence genes *inlB* (range: 0.0%–69.2%; $p = .014$). For each of the four types of retail outlets assessed, high frequencies (>75%) of detection of *InlA*, *inlC*, and *inlJ* were observed (Table 4).

For the four categories of beef and beef products, the frequencies of detection of virulence-associated genes were comparatively low for *inlB*, where the range for positive samples was 33.3% (2/6) in offal & organs to 54.5% (6/11) for RTEs, and for *plcA*, the frequency ranged from 0% (0/8) in milled beef to 33.3% (2/6) in offal & organs. However, the detection frequency of *inlC* and *inlJ* was high, where regardless of the type of beef and beef products, all (100%) of the 33 isolates of *L. monocytogenes* were positive for the virulence-associated genes. The frequency of the samples positive for virulence-associated genes varied significantly across the eight genes ($p = .047$).

The frequency distribution of carriage of virulence-associated genes among the four serogroups is shown in Table 5. Of the eight virulence-associated genes assessed, statistically significant differences ($p = .018$) were detected across the four serogroups only for the virulence-associated gene *hlyA*. The gene detection frequency ranged from 25% in serogroup 1/2b-3b to 100% in serogroup 1/2c-3c. The detection frequencies for the other seven virulence-associated genes were generally high across the serogroups, but the differences were not statistically significant ($p > .05$).

## 4 | DISCUSSION

Considering that listeriosis is an important foodborne bacterial zoonosis globally, coupled with the fact that South Africa in 2017–2018 experienced the largest outbreak of human listeriosis, which was associated with consuming a contaminated meat product, makes the findings in the current study of public health are significant. In this study, 8.3% of beef and beef products were contaminated with *L. monocytogenes*, emphasizing the fact that these products still have the potential to cause human listeriosis, particularly as 48 (4.53%) of the 1,060 cases of human listeriosis in the most recent outbreak in the country originated from Mpumalanga province (Kayode et al., 2020).

In the first reported study after the recent outbreak (Thomas et al., 2020) in the country, Matle et al. (2019), using the same methodology applied in the current study, documented a comparable prevalence of 8.6% (13/151) for *L. monocytogenes* in meat and meat products in Mpumalanga province, which originated from poultry, cattle, sheep, pork, and game, unlike this study where only beef and beef products were sampled. It is, however, pertinent to mention that in the earlier study conducted on meat and meat products tested across the nine provinces in South Africa, the prevalence of *L. monocytogenes* was 14.1% (147/1039), ranging from 0% (Western Cape) to 27.9% (Gauteng province), an indication that the risk of meatborne human listeriosis is still present in South Africa, albeit at different frequencies. In other countries, the prevalence of *L. monocytogenes* in beef and beef products using similar methods has been reported to be variable, such as a prevalence of 0.3% in Italy (Latorre et al., 2007), 6.5% in Markurdi, Nigeria (Peter, 2016), 19.4% in Poland (Wieczorek, Dmowska, & Osek, 2012), and 20.0% in China (Wu et al., 2015). It has been documented that beef and beef products could be contaminated to varying degrees by *L. monocytogenes* during processing (Demaître et al., 2021).

The overall prevalence of *Listeria* spp. (other than *L. monocytogenes*) in the beef and beef products sampled in the current study was 30%,

which was statistically significantly higher than the 8.3% for *L. monocytogenes*, as well as being detected at all levels of sampling of the five variables (district, type of outlet, presentation for consumption, temperature of display, and types of products) investigated. Other studies have reported detecting the detection of different species of *Listeria*, such as *L. innocua*, at higher frequencies than *L. monocytogenes* in beef and beef products. For example, the prevalence of Listeria spp. and L. monocytogenes, respectively, was in meat and meat products 4.6% and 1.2% in Iran (Jalali & Abedi, 2008), 45.8% and 0% in Nigeria (Okorie-Kanu et al., 2020), 28.4% and 4% in Egypt (El-Malek, Ali, Hassanein, Mohamed, & Elsayh, 2010), and 50% and 0% in Saudi Arabia (Yehia, Ibraheim, & Hassanein, 2016), respectively. However, a higher prevalence (80.8.%) of *L. monocytogenes* than a combined prevalence of 69.2% for eight other *Listeria* spp. in raw beef in Nigeria (Chuku, Obande, & Eya, 2019). Although *L. monocytogenes* is the most critical *Listeria* species responsible for animal and human listeriosis, it has been demonstrated that other species of *Listeria*, specifically *L. ivanovii*, cause listeriosis and mortalities in animals (Guillet et al., 2010; Sergeant, Love, & McInnes, 1991; Snapir, Vaisbein, & Nassar, 2006; Zamuz et al., 2021), and *L. innocua* has been reported to cause listeriosis in immuno-compromised humans (Perrin, Bemer, & Delamare, 2003) and is known to possess virulence genes (Moura et al., 2019).

The district locations of the outlets and the types of beef and beef products were the only factors significantly associated with the prevalence of *L. monocytogenes*. Others have reported similar findings regarding the significance of the outlets in South Africa (Matle et al., 2019), in China (Liu et al., 2020), and in Bangladesh (Islam, Husna, Islam, & Khatun, 2016). These findings could be accounted for by differences in the sources, practices, and handling of beef at slaughterhouses and abattoirs, as well as during processing at processing plants and retail outlets (Demaître et al., 2020, 2021; Terentjeva et al., 2021).

It was not unexpected that the prevalence of *L. monocytogenes* varied significantly across the types of beef and beef products, considering how they are handled prepresentation for sale at retail outlets. For example, the prevalence of *L. monocytogenes* in raw beef (8.3%: brisket), minced beef (6.7%), and RTE products (15.4%: Vienna) in this study. The differences in the prevalence of *L. monocytogenes* among the individual types of samples processed may be partly due to factors such as the geographical location, as documented by the significant differences according to districts as documented by others (Chapin, Nightingale, Worobo, Wiedmann, & Strawn, 2014; Maktabi, Pourmehdi, Zarei, & Fooladgar, 2016; Strawn et al., 2013), hygienic factors during processing, and the ways the products are prepared (Aalto-Araneda, Lundén, Markkula, Hakola, & Korkeala, 2019). It was evident that RTE products in our study had a higher prevalence of *L. monocytogenes* (10.6%) than either raw beef (7.3%) or offal & organs (7.4%). The fact that RTEs included products such as minced beef, where machines for grinding beef have been known to contribute to the contamination of products (Belias, Sullivan, Wiedmann, & Ivanek, 2022; Ripolles-Avila, Hascoët, Martínez-Suárez, Capita, & Rodríguez-Jerez, 2019), and "polony", which is essentially raw beef spiced and solar dried in the open air, thus, exposed to environmental contamination as reported by others (Mogomotsi & Chinsembu, 2012). The findings in other studies agree with ours; for example, Matle et al. (2019) reported a prevalence of *L. monocytogenes* in raw processed beef and RTEs to be 19.5% and 13.5%, respectively, for packaged minced beef/meat by others: 0.0% in Turkey (Öktem, Bayram, Ceylan, & Yentür, 2006), 1% in Switzerland (Fantelli & Stephan, 2001), 42.1% in Belgium (Van Coillie, Werbrouck, Heyndrickx, Herman, & Rijpens, 2004), 48.7% in Brazil (Ristori et al., 2014). Compared to the overall prevalence of 10.6% for *L. monocytogenes* detected in RTE beef products in this study, significantly lower prevalence has been documented by others such as the 0.0% in the Republic of Ireland (Khen, Lynch, Carroll, McDowell, & Duffy, 2015), .52%–3.09% in the USA (Levine, Rose, Green, Ransom, & Hill, 2001), 1.5% in Jordan (Osaili et al., 2014), 3.2% in China (Liu et al., 2020) but lower than the 17.1% recorded in Jordan (Awaisheh, 2010). The differences in the prevalence of *L. monocytogenes* across the types of beef and beef products among countries can be attributed to many factors, including the geographical locations, carriage of *Listeria* spp. in cattle on farms, and cattle presented for slaughter, hygienic practices during slaughter, processing, and retailing, and the types of beef and beef products as well as the methods used to analyze the samples in the laboratory (Maktabi et al., 2016).

Others have implicated RTE foods as the cause of human listeriosis (Allam et al., 2018; Buchanan, Gorris, Hayman, Jackson, & Whiting, 2017; Lopez-Valladares, Danielsson-Tham, & Tham, 2018). In this study, the prevalence of *L. monocytogenes* in RTE beef products (biltong, Vienna, "polony," etc.) was 10.7% which is much higher than the 4.4% reported for RTE meat products sampled in Mpumalanga province by Matle et al. (2019), and the 8.1% documented in Trinidad and Tobago (Syne, Ramsubhag, & Adesiyun, 2011). The high prevalence of *L. monocytogenes* in RTE products in our study suggests that cross-contamination may have occurred during the handling and processing of the products. Our finding of relevance to South Africa is that two RTE products ("biltong" and "polony"), unique to our country and popularly consumed by the population, were contaminated with *L. monocytogenes*. This is important because these two products are mainly consumed without further heat treatment. "Biltong" is a product that is like the American dried meat product known as Jerky (Matsheka et al., 2014); and is sold in the dried or moist form at retail outlets across the district and is contaminated by *L. monocytogenes* (9.7%) and *Listeria* spp. (19.4%), thus posing a food safety concern to consumers. The source of beef, open-air solar drying, and the level of sanitation during preparation have been reported to have an impact on the product's microbial quality (Cherono, Mwithiga, & Schmidt, 2016). "Biltong" in South Africa has also been contaminated by *Salmonella* spp. (Manqele, 2018), and Shiga-toxin *Escherichia coli* (STEC) (Onyeka et al., 2020). Another beef RTE product assessed in our study is "polony", which is produced by one of the largest companies in South Africa and which was confirmed, through bacteriological and molecular analysis, to be the vehicle for the *L. monocytogenes* strain responsible for the recent large human outbreak of listeriosis in the country (Thomas et al., 2020). The fact that 6.9% and 34.5% of the "polony" samples collected from the retail outlets in Mpumalanga province were contaminated by *L. monocytogenes* and *isteria* spp.,

respectively, is of food safety importance. These findings suggest that "polony" continues to pose a risk of listeriosis in human consumers in the country.

The predominant serogroups of *L. monocytogenes*, comprising serotypes 1/2a, 3a, 1/2c, 3c, 1/2b, 3b, 4b, 4d, and 4e isolated in this study, have been recovered from beef and beef products elsewhere (Althaus et al., 2014; Arslan & Baytur, 2019; Khen et al., 2015) In the earlier study conducted in South Africa, Matle et al. (2019) reported serotype 1/2a to be predominant followed by 4b and 1/2b and 1/2c. This agrees with the current findings. However, in the 2017–2018 outbreak of human listeriosis, the predominant serotype of *L. monocytogenes* detected was 4b (ST6) (Allam et al., 2018). Variability in the predominant serotypes of *L. monocytogenes* have been reported by others, for example, the predominance of serotypes as follows: 1/2a (58%) in the Republic of Ireland (Khen et al., 2015), 1/2a (44.2%) in Switzerland (Fantelli & Stephan, 2001), 1/2a (61.7%) in Poland (Wieczorek et al., 2012), and 1/2a (57.6%) in Turkey (Arslan & Baytur, 2019). The differences in the distribution of serotypes of *L. monocytogenes* in several countries may be attributed, in part, to circulating serotypes of the pathogen as well as to varying laboratory techniques (phenotypic and molecular) used (Borucki & Call, 2003; Morobe, Obi, Nyila, Matsheka, & Gashe, 2012; Nadon, Woodward, Young, Rodgers, & Wiedmann, 2001). Our results confirm the presence of the main serotypes of *L. monocytogenes*, suggesting that these strains may be pathogenic to exposed humans. This emphasizes the importance of improving the control measures, zero-tolerance policy, and surveillance of microorganisms in South Africa. Sporadic cases of listeriosis are associated with serogroups 1/2a-3a and 1/2c-3c (Todd & Notermans, 2011), which were among the serogroups detected in the current study. It has been reported by Lopez-Valladares et al. (2018) that in the mid-1990s and early 2000s, a shift from *L. monocytogenes* serovar 4b to serovar 1/2a causing human listeriosis occurred, and serovar 1/2a is becoming more frequently linked to outbreaks of listeriosis, particularly in Europe and Northern America.

The widespread distribution of virulence genes at high frequencies (81.8%–100.0%), particularly *inlC*, *inlJ*, *inlA*, and *hlyA*, could have food safety and clinical significance if expressed following infection by the strains of *L. monocytogenes*. In a study conducted on *L. monocytogenes* recovered from meat products of domestic sources in South Africa, Matle et al. (2019) reported a relatively low occurrence of virulence-associated genes *plcA* (20.3%) and *inlA* (32.4%) but high frequencies for *iap* (95.6%) and *inlJ* (98.7%). These findings are slightly different from those in this study. The differences between both studies could be accounted for by the geographical locations of the retail outlets sampled, types of samples, natural genetic variation of the strains, and sources of samples. Compared to the reports from other studies, all the isolates of *L. monocytogenes* recovered from beef products sampled at retail outlets in Poland were positive for virulence-associated genes *inlC*, *inlJ*, and *inlA* (Maćkiw et al., 2020). Arslan and Baytur (2019) found that the isolates of *L. monocytogenes* from retail outlets in Turkey were positive for all the eight virulence-associated genes assessed, a finding slightly different from our study, where only isolates from the beef chuck, beef tongue, beef short rib, beef tripe, "biltong," "polony" and Vienna were positive for virulence-associated gene *plcA*. The virulence-associated genes in isolates of *L. monocytogenes* recovered from beef and beef products in our study indicate their virulence potential and food safety concerns. However, considering that not all virulence-associated genes documented in the literature were investigated in our study, coupled with the fact that not all virulence-associated genes are expressed, should be considered in assessing the food safety, and clinical risks posed by the *L. monocytogenes* strains positive for virulence-associated genes. Notwithstanding the limitation associated with the extrapolation of data on virulence-positive *L. monocytogenes* to food safety, several of the virulence-associated genes in our study were detected at high frequencies, particularly, *inlC* and *inlJ*, which were present in all 33 isolates of *L. monocytogenes*, the health risk posed to consumers cannot be ignored. This is because these virulence-associated genes have been detected in strains of *L. monocytogenes* recovered from foods and human listeriosis outbreaks. The eight virulence-associated genes detected in the *L. monocytogenes* isolates in our study have been recovered from foods and human cases of listeriosis by others (Pournajaf et al., 2016). Furthermore, the modes of action of these virulence genes have been documented in the literature (Quereda et al., 2021), and it has been shown that the virulence genes in *L. monocytogenes* are necessary for their pathogenesis (Rawool et al., 2007).

In conclusion, demonstrating the presence and distribution of *L. monocytogenes* (8.3%) and *Listeria* spp. (30.0%) in various sample types collected from retail outlets in Mpumalanga province, South Africa, is indicative of the risk of human exposure to the pathogen following consumption of *L. monocytogenes*-contaminated beef and beef products. Furthermore, the detection of serogroups of *L. monocytogenes* usually associated with human listeriosis and their possession of virulence-associated genes suggests the potential of the isolates being pathogenic for exposed humans. The contamination of beef RTE products by virulent *L. monocytogenes* suggests the need to implement improved sanitary practices at retail outlets and at abattoirs or processing plants from where the raw beef originates.

## ACKNOWLEDGEMENTS

We thank the Red Meat Research and Development, South Africa (RMRD-SA), for funding this project. We are grateful to the ARC-OVR, Onderstepoort, for allowing us to use the laboratory facilities for the research and for the assistance provided by the technical staff. The Research Committee of the Faculty of Veterinary Science Research, University of Pretoria, Onderstepoort, approved the research project (REC146-19) before its commencement. We thank Dr. Francis for assisting with part of the statistical analysis. Finally, we are grateful for the laboratory supervision provided in the initial phase of the study at the Onderstepoort Veterinary Institute (OVI) Laboratories.

## CONFLICT OF INTEREST STATEMENT

Authors have no conflict of interest to declare.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

## INFORMED CONSENT

The consent of the managers/owners of the retail outlets was received before the study was started.

## ORCID

*James Gana* https://orcid.org/0000-0001-9415-5297
*Yusuf B. Ngoshe* https://orcid.org/0000-0002-7559-6617
*Abiodun A. Adesiyun* https://orcid.org/0000-0001-9470-9421

## REFERENCES

Aalto-Araneda, M., Lundén, J., Markkula, A., Hakola, S., & Korkeala, H. (2019). Processing plant and machinery sanitation and hygiene practices associated with *Listeria monocytogenes* occurrence in ready-to-eat fish products. *Food Microbiology*, *82*, 455–464. https://doi.org/10.1016/j.fm.2019.03.017

Allam, M., Tau, N., Shannon, L., Mtshali, P. S., Mnyameni, F., Khumalo, Z. T. H., … Anthony, M. (2018). Whole-genome sequences of *Listeria monocytogenes* sequence type 6 isolates associated with a large foodborne outbreak in South Africa. *Genome Announcements*, *6*(25), e00538–e00518.

Althaus, D., Lehner, A., Brisse, S., Maury, M., Tasara, T., & Stephan, R. (2014). Characterization of *Listeria monocytogenes* strains isolated during 2011–2013 from human infections in Switzerland. *Foodborne Pathogens and Disease*, *11*(10), 753–758. https://doi.org/10.1089/fpd.2014.1747

Arslan, S., & Baytur, S. (2019). Prevalence and antimicrobial resistance of *Listeria* species and subtyping and virulence factors of *Listeria monocytogenes* from retail meat. *Journal of Food Safety*, *39*(1), e12578. https://doi.org/10.1111/jfs.12578

Awaisheh, S. S. (2010). Incidence and contamination level of Listeria monocytogenes and other Listeria spp. in ready-to-eat meat products in Jordan. *Journal of Food Protection*, *73*(3), 535–540. https://doi.org/10.4315/0362-028x-73.3.535

Belias, A., Sullivan, G., Wiedmann, M., & Ivanek, R. (2022). Factors that contribute to persistent *Listeria* in food processing facilities and relevant interventions: A rapid review. *Food Control*, *133*, 108579. https://doi.org/10.1016/j.foodcont.2021.108579

Boatemaa, S., Barney, M., Drimie, S., Harper, J., Korsten, L., & Pereira, L. (2019). Awakening from the listeriosis crisis: Food safety challenges, practices, and governance in the food retail sector in South Africa. *Food Control*, *104*, 333–342. https://doi.org/10.1016/j.foodcont.2019.05.009

Borucki, M. K., & Call, D. R. (2003). *Listeria monocytogenes* serotype identification by PCR. *Journal of Clinical Microbiology*, *41*(12), 5537–5540. https://doi.org/10.1128/JCM.41.12.5537-5540.2003

Bouymajane, A., Rhazi Filali, F., Oulghazi, S., Lafkih, N., Ed-Dra, A., Aboulkacem, A., … Moumni, M. (2021). Occurrence, antimicrobial resistance, serotyping, and virulence genes of *Listeria monocytogenes* isolated from foods. *Heliyon*, *7*(2), e06169 10.1016/j.heliyon.2021.e06169.

Buchanan, R. L., Gorris, L. G., Hayman, M. M., Jackson, T. C., & Whiting, R. C. (2017). A review of *Listeria monocytogenes*: An update on outbreaks, virulence, dose-response, ecology, and risk assessments. *Food Control*, *75*, 1–13. https://doi.org/10.1016/j.foodcont.2016.12.016

Chapin, T. K., Nightingale, K. K., Worobo, R. W., Wiedmann, M., & Strawn, L. K. (2014). Geographical and meteorological factors associated with isolation of *Listeria* species in New York state produce production and natural environments. *Journal of Food Protection*, *77*(11), 1919–1928. https://doi.org/10.4315/0362-028X.JFP-14-132

Cherono, K., Mwithiga, G., & Schmidt, S. (2016). Infrared drying as a potential alternative to convective drying for biltong production. *Italian Journal of Food Safety*, *5*(3), 5625. https://doi.org/10.4081/ijfs.2016.5625

Chuku, A., Obande, G. A., & Eya, S. B. (2019). Listerial contamination of raw beef and chevon in north-central Nigeria. *IMC Journal of Medical Science*, *13*(2), 1–8.

Cluff, M., Kobane, I. A., Bothma, C., Hugo, C. J., & Hugo, A. (2017). Intermediate added salt levels as sodium reduction strategy: Effects on chemical, microbial, textural and sensory quality of polony. *Meat Science*, *133*, 143–150. https://doi.org/10.1016/j.meatsci.2017.06.014

Demaître, N., De Reu, K., Haegeman, A., Schaumont, D., De Zutter, L., Geeraerd, A., & Rasschaert, G. (2021). Study of the transfer of *Listeria monocytogenes* during the slaughter of cattle using molecular typing. *Meat Science*, *175*, 108450. https://doi.org/10.1016/j.meatsci.2021.108450

Demaître, N., Van Damme, I., De Zutter, L., Geeraerd, A. H., Rasschaert, G., & De Reu, K. (2020). Occurrence, distribution, and diversity of *listeria monocytogenes* contamination on beef and pig carcasses after slaughter. *Meat Science*, *169*, 108177. https://doi.org/10.1016/j.meatsci.2020.108177

Doumith, M., Buchrieser, C., Glaser, P., Jacquet, C., & Martin, P. (2004). Differentiation of the major *Listeria monocytogenes* serovars by multiplex PCR. *Journal of Clinical Microbiology*, *42*(8), 3819–3822. https://doi.org/10.1128/JCM.42.8.3819-3822.2004

El Hag, M. M., El Zubeir, I. E. M., & Mustafa, N. E. (2021). Prevalence of *Listeria* species in dairy farms in Khartoum State (Sudan). *Food Control*, *123*, 107699. https://doi.org/10.1016/j.foodcont.2020.107699El-Malek

El-Malek, A. M. A., Ali, S. F. H., Hassanein, R., Mohamed, M. A., & Elsayh, K. I. (2010). Occurrence of *Listeria* species in meat, chicken products, and human stools in Assiut city, Egypt, with PCR used for rapid identification of *Listeria monocytogenes*. *Veterinary World*, *3*(8), 353–359.

El-Zowalaty, M. E., Hickman, R. A., Moura, A., Lecuit, M., Zishiri, O. T., Noyes, N., & Järhult, J. D. (2019). Genome sequence of *Listeria innocua* strain MEZLIS26, isolated from a goat in South Africa. *Microbiology Resource Announcement*, *8*(44), 91–19.

Fantelli, K., & Stephan, R. (2001). Prevalence and characteristics of Shiga-toxin-producing *Escherichia coli* and *Listeria monocytogenes* strain isolated from minced meat in Switzerland. *International Journal of Food Microbiology*, *70*(1–2), 63–69. https://doi.org/10.1016/s0168-1605(01)00515-3

Gana, J., Gcebe, N., Pierneef, R., Moerane, R., & Adesiyun, A. A. (2022). Multiple-locus variable-number tandem repeat analysis genotypes of *Listeria monocytogenes* isolated from farms, abattoirs, and retail in Gauteng Province, South Africa. *Journal of Food Protection*, *85*(9), 1249–1257. https://doi.org/10.4315/JFP-22-081

Gray, J. A., Chandra, P. S., Kaur, M., Kocharunchitt, C., Bowman, J. P., & Fox, E. M. (2021). Characterisation of *Listeria monocytogenes* food-associated isolates to assess environmental fitness and virulence potential. *International Journal of Food Microbiology*, *350*, 109247. https://doi.org/10.1016/j.ijfoodmicro.2021.109247

Guillet, C., Join-Lambert, O., Le Monnier, A., Leclercq, A., Mechaï, F., Mamzer-Bruneel, M. F., … Lecuit, M. (2010). Human listeriosis caused by *Listeria ivanovii*. *Emerging Infectious Diseases*, *16*(1), 136–138. https://doi.org/10.3201/eid1601.091155

Islam, M. S., Husna, A. A., Islam, M. A., & Khatun, M. M. (2016). Prevalence of *Listeria monocytogenes* in beef, chevon, and chicken in Bangladesh. *American Journal of Food Science and Health*, *2*(4), 39–44.

Jalali, M., & Abedi, D. (2008). Prevalence of *Listeria* species in food products in Isfahan, Iran. *International Journal of Food Microbiology*, *122*(3), 336–340. https://doi.org/10.1016/j.ijfoodmicro.2007.11.082

Jones, M., Arnaud, E., Gouws, P., & Hoffman, L. C. (2019). Effects of the addition of vinegar, weight loss, and packaging method on the

physicochemical properties and microbiological profile of biltong. *Meat Science*, *156*, 214–221. https://doi.org/10.1016/j.meatsci.2019.06.003

Kayode, A. J., Igbinosa, E. O., & Okoh, A. I. (2020). Overview of listeriosis in the southern African hemisphere. *Journal of Food Safety*, *40*(1), e12732. https://doi.org/10.1111/jfs.12732

Kayode, A. J., & Okoh, A. I. (2022). Assessment of the molecular epidemiology and genetic multiplicity of *Listeria monocytogenes* recovered from ready-to-eat foods following the South African listeriosis outbreak. *Scientific Reports*, *12*(1), 20129. https://doi.org/10.1038/s41598-022-20175-x

Khen, B. K., Lynch, O. A., Carroll, J., McDowell, D. A., & Duffy, G. (2015). Occurrence, antibiotic resistance, and molecular characterization of *Listeria monocytogenes* in the beef chain in the Republic of Ireland. *Zoonoses and Public Health*, *62*(1), 11–17. https://doi.org/10.1111/zph.12106

Koopmans, M. M., Brouwer, M. C., Vázquez-Boland, J. A., & van de Beek, D. (2022). Human listeriosis. *Clinical Microbiology Reviews*, *36*(1), e00060–e00019. https://doi.org/10.1128/cmr.00060-19

Latorre, L., Parisi, A., Fraccalvieri, R., Normanno, G., La Porta, M. C., Goffredo, E., … Santagada, G. (2007). Low prevalence of *Listeria monocytogenes* in foods from Italy. *Journal of Food Protection*, *70*(6), 1507–1512. https://doi.org/10.4315/0362-028x-70.6.1507

Ledlod, S., Bunroddith, K., Areekit, S., Santiwatanakul, S., & Chansiri, K. (2020). Development of a duplex lateral flow dipstick test for the detection and differentiation of *Listeria spp*. and *Listeria monocytogenes* in meat products based on loop-mediated isothermal amplification. *Journal of Chromatography B*, *1139*, 121834. https://doi.org/10.1016/j.jchromb.2019.121834

Levine, P., Rose, B., Green, S., Ransom, G., & Hill, W. (2001). Pathogen testing of ready-to-eat meat and poultry products collected at federally inspected establishments in the United States, 1990 to 1999. *Journal of Food Protection*, *64*(4), 1188–1193. https://doi.org/10.4315/0362-028x-64.8.1188

Li, F., Ye, Q., Chen, M., Shang, Y., Zhang, J., Ding, Y., & Wu, Q. (2021). Real-time PCR identification of *Listeria monocytogenes* serotype 4c using primers for novel target genes obtained by comparative genomic analysis. *Lebensmittel-Wissenschaft und-Technologie*, *138*, 110774. https://doi.org/10.1016/j.lwt.2020.110774

Liu, Y., Sun, W., Sun, T., Gorris, L., Wang, X., Liu, B., & Dong, Q. (2020). The prevalence of *Listeria monocytogenes* in meat products in China: A systematic literature review and novel meta-analysis approach. *International Journal of Food Microbiology*, *312*, 108358. https://doi.org/10.1016/j.ijfoodmicro.2019.108358

Lopez-Valladares, G., Danielsson-Tham, M. L., & Tham, W. (2018). Implicated food products for listeriosis and changes in serovars of *Listeria monocytogenes* affecting humans in recent decades. *Foodborne Pathogens and Disease*, *15*(7), 387–397. https://doi.org/10.1089/fpd.2017.2419

Maćkiw, E., Stasiak, M., Kowalska, J., Kucharek, K., Korsak, D., & Postupolski, J. (2020). Occurrence and characteristics of *Listeria monocytogenes* in ready-to-eat meat products in Poland. *Journal of Food Protection*, *83*(6), 1002–1009. https://doi.org/10.4315/JFP-19-525

Mafuna, T., Matle, I., Magwedere, K., Pierneef, R. E., & Reva, O. N. (2021). Whole genome-based characterization of *Listeria monocytogenes* isolates recovered from the food chain in South Africa. *Frontiers in Microbiology*, *12*, 669287. https://doi.org/10.3389/fmicb.2021.669287

Maktabi, S., Pourmehdi, M., Zarei, M., & Fooladgar, A. A. (2016). Detection of antibiotic resistant *Listeria* spp. in beef burgers distributed in Ahvaz City, Iran. *Jundishapur Journal of Health Sciences*, *8*(2). https://doi.org/10.17795/jjhs-34780

Makumbe, H. H., Tabit, F. T., & Dlamini, B. C. (2021). Prevalence, molecular identification, antimicrobial resistance, and disinfectant susceptibility of *Listeria innocua* isolated from ready-to-eat foods sold in Johannesburg, South Africa. *Journal of Food Quality and Hazards Control*, *8*(3), 131–139. https://doi.org/10.18502/jfqhc.8.3.7200

Manqele, A. (2018). Prevalence and characterization of *Salmonella* spp. in slaughter cattle, abattoir environment, and meat sold at retail outlets in Gauteng, South Africa (M.Sc. Thesis). University of Pretoria, South Africa.

Manqele, A., Gcebe, N., Pierneef, R. E., Moerane, R., & Adesiyun, A. A. (2023). Identification of *Listeria* species and multilocus variable-number tandem repeat analysis (MLVA) typing of *Listeria innocua* and *Listeria monocytogenes* isolates from cattle farms and beef and beef-based products from retail outlets in Mpumalanga and north west provinces, South Africa. *Pathogens*, *12*, 147. https://doi.org/10.3390/pathogens12010147

Matle, I., Mbatha, K. R., Lentsoane, O., Magwedere, K., Morey, L., & Madoroba, E. (2019). Occurrence, serotypes, and characteristics of *Listeria monocytogenes* in meat and meat products in South Africa between 2014 and 2016. *Journal of Food Safety*, *39*(4), e12629. https://doi.org/10.1111/jfs.12629

Matsheka, M. I., Mpuchane, S., Gashe, B. A., Allotey, J., Khonga, E. B., Coetzee, S. H., & Murindamombe, G. (2014). Microbial quality assessment and predominant microorganism of biltong produce in butcheries in Gaborone, Botswana. *Food and Nutrition Sciences*, *5*, 1668–1678 http://hdl.handle.net/10311/1280.

Mogomotsi, T. K., & Chinsembu, K. C. (2012). Occurrence of *Listeria monocytogenes* in ready-to-eat foodstuffs in Windhoek, Namibia. *Journal of Research in Microbes*, *1*(2), 65–70.

Morobe, I. C., Obi, C. L., Nyila, M. A., Matsheka, M. I., & Gashe, B. A. (2012). Molecular characterization and serotyping of *Listeria monocytogenes* with a focus on food safety and disease prevention. In *Biochemical Testing* (Vol. 8, pp. 197–216). Rijeka: InTech.

Moura, A., Disson, O., Lavina, M., Thouvenot, P., Huang, L., Leclercq, A., … Lecuit, M. (2019). Atypical hemolytic *Listeria innocua* isolates are virulent, albeit less than *Listeria monocytogenes*. *Infection and Immunity*, *87*(4), e00758–e00718. https://doi.org/10.1128/IAI.00758-18

Nadon, C. A., Woodward, D. L., Young, C., Rodgers, F. G., & Wiedmann, M. (2001). Correlations between molecular subtyping and serotyping of *Listeria monocytogenes*. *Journal of Clinical Microbiology*, *39*(7), 2704–2707. https://doi.org/10.1128/JCM.39.7.2704-2707.2001

Okorie-Kanu, O. J., Anyanwu, M. U., Ezenduka, E. V., Mgbeahuruike, A. C., Okorie-Kanu, C. O., Ugwuijem, E. E., … Vidal, M. (2020). Occurrence and antibiogram of *Listeria* species in raw pork, beef, and chicken meats marketed in Enugu State, Southeast Nigeria. *Veterinary World*, *13*(12), 317–325. https://doi.org/10.14202/vetworld.2020.317-325

Öktem, A. B., Bayram, G., Ceylan, A. E., & Yentür, G. (2006). Prevalence of *Listeria monocytogenes* in some Turkish foodstuffs. *Journal of Food Quality*, *29*(1), 76–86. https://doi.org/10.1111/j.1745-4557.2006.00057.x

Olanya, O. M., Hoshide, A. K., Ijabadeniyi, O. A., Ukuku, D. O., Mukhopadhyay, S., Niemira, B. A., & Ayeni, O. (2019). Cost estimation of listeriosis (*Listeria monocytogenes*) occurrence in South Africa in 2017 and its food safety implications. *Food Control*, *102*, 231–239. https://doi.org/10.1016/j.foodcont.2019.02.007

Onyeka, L. O., Adesiyun, A. A., Keddy, K. H., Madoroba, E., Manqele, A., & Thompson, P. N. (2020). Shiga toxin-producing *Escherichia coli* contamination of raw beef and beef-based ready-to-eat products at retail outlets in Pretoria, South Africa. *Journal of Food Protection*, *83*(3), 476–484. https://doi.org/10.4315/0362-028X.JFP-19-372

Osaili, T. M., Al-Nabulsi, A. A., Shaker, R. R., Jaradat, Z. W., Taha, M., Al-Kherasha, M., … Holley, R. (2014). Prevalence of salmonella serovars, *Listeria monocytogenes*, and *Escherichia coli* O157:H7 in Mediterranean ready-to-eat meat products in Jordan. *Journal of Food Protection*, *77*(1), 106–111. https://doi.org/10.4315/0362-028X.JFP-13-049

Paduro, C., Montero, D. A., Chamorro, N., Carreño, L. J., Vidal, M., & Vidal, R. (2020). Ten years of molecular epidemiology surveillance of

*Listeria monocytogenes* in Chile 2008–2017. *Food Microbiology*, *85*, 103280. https://doi.org/10.1016/j.fm.2019.103280

Perrin, M., Bemer, M., & Delamare, C. (2003). Fatal case of *Listeria innocua* bacteremia. *Journal of Clinical Microbiology*, *41*, 5308–5309. https://doi.org/10.1128/JCM.41.11.5308-5309.2003

Peter, A. (2016). Prevalence and antimicrobial susceptibility of *Listeria monocytogenes* isolated from beef, pork, and chicken sold in Makurdi metropolis. *Microbiology Research Journal International*, *14*(11), 1–7.

Pinto, A. D., Novello, L., Montemurro, F., Bonerba, E., & Tantillo, G. (2010). Occurrence of *Listeria monocytogenes* in ready-to-eat foods from supermarkets in southern Italy. *New Microbiologica*, *33*(3), 249–252.

Pournajaf, A., Rajabnia, R., Sedighi, M., Kassani, A., Moqarabzadeh, V., Lotfollahi, L., & Irajian, G. (2016). Prevalence and virulence determination of *Listeria monocytogenes* strains isolated from clinical and non-clinical samples by multiplex polymerase chain reaction. *Revista da Sociedade Brasileira de Medicina Tropical*, *49*, 624–627. https://doi.org/10.1590/0037-8682-0403-2015

Quereda, J. J., Morón-García, A., Palacios-Gorba, C., Dessaux, C., Portillo, F. G.-d., Pucciarelli, M. G., & Ortega, A. D. (2021). Pathogenicity and virulence of *Listeria monocytogenes*: A trip from environmental to medical microbiology. *Virulence*, *12*(1), 2509–2545. https://doi.org/10.1080/21505594.2021.1975526

Rawool, D. B., Malik, S. V., Shakuntala, I., Sahare, A. M., & Barbuddhe, S. B. (2007). Detection of multiple virulence-associated genes in *Listeria monocytogenes* isolated from bovine mastitis cases. *International Journal of Food Microbiology*, *113*(2), 201–207. https://doi.org/10.1016/j.ijfoodmicro.2006.06.029

Ripolles-Avila, C., Hascoët, A. S., Martínez-Suárez, J. V., Capita, R., & Rodríguez-Jerez, J. J. (2019). Evaluation of the microbiological contamination of food processing environments through implementing surface sensors in an iberian pork processing plant: An approach towards the control of *Listeria monocytogenes*. *Food Control*, *99*, 40–47. https://doi.org/10.1016/j.foodcont.2018.12.013

Ristori, C. A., Rowlands, R. E., Martins, C. G., Barbosa, M. L., Yoshida, J. T., & Franco, B. D. (2014). Prevalence and populations of *Listeria monocytogenes* in meat products retailed in Sao Paulo, Brazil. *Foodborne Pathogens and Disease*, *11*(12), 969–973. https://doi.org/10.1089/fpd.2014.1809

Ryu, J., Park, S. H., Yeom, Y. S., Shrivastav, A., Lee, S. H., Kim, Y. R., & Kim, H. Y. (2013). Simultaneous detection of *Listeria* species isolated from meat processed foods using multiplex PCR. *Food Control*, *32*(2), 659–664. https://doi.org/10.1016/j.foodcont.2013.01.048

Sereno, M. J., Viana, C., Pegoraro, K., da Silva, D., Yamatogi, R. S., Nero, L. A., & Bersot, L. (2019). Distribution, adhesion, virulence, and antibiotic resistance of persistent *Listeria monocytogenes* in a pig slaughterhouse in Brazil. *Food Microbiology*, *84*, 103234. https://doi.org/10.1016/j.fm.2019.05.018

Sergeant, E. S., Love, S. C., & McInnes, A. (1991). Abortions in sheep due to *Listeria ivanovii*. *Australian Veterinary Journal*, *68*(1), 39. https://doi.org/10.1111/j.1751-0813.1991.tb09846.x

Shourav, A. H., Hasan, M., & Ahmed, S. (2020). Antibiotic susceptibility pattern of Listeria spp. isolated from the cattle farm environment in Bangladesh. *Journal of Agriculture and Food Research*, *2*, 100082. https://doi.org/10.1016/j.jafr.2020.100082

Snapir, Y. M., Vaisbein, E., & Nassar, F. (2006). Low virulence but potentially fatal outcome-*Listeria ivanovii*. *European Journal of Internal Medicine*, *17*(4), 286–287. https://doi.org/10.1016/j.ejim.2005.12.006

Soumet, C., Ermel, G., Fach, P., & Colin, P. (1994). Evaluation of different DNA extraction procedures for the detection of *Salmonella* from chicken products by polymerase chain reaction. *Letters in Applied Microbiology*, *19*(5), 294–298. https://doi.org/10.1111/j.1472-765x.1994.tb00458

Strawn, L. K., Fortes, E. D., Bihn, E. A., Nightingale, K. K., Gröhn, Y. T., Worobo, R. W., … Bergholz, P. W. (2013). Landscape and meteorological factors affecting prevalence of three food-borne pathogens in fruit and vegetable farms. *Applied and Environmental Microbiology*, *79*(2), 588–600. https://doi.org/10.1128/AEM.02491-12

Syne, S. M., Ramsubhag, A., & Adesiyun, A. A. (2011). Occurrence and genetic relatedness of *Listeria* spp. in two brands of locally processed ready-to-eat meats in Trinidad. *Epidemiology and Infection*, *139*(5), 718–727. https://doi.org/10.1017/S095026881000172X

Terentjeva, M., Šteingolde, Ž., Meistere, I., Elferts, D., Avsejenko, J., Streikiša, M., … Bērziņš, A. (2021). Prevalence, genetic diversity, and factors associated with distribution of *Listeria monocytogenes* and other *Listeria* spp. in cattle farms in Latvia. *Pathogens*, *10*(7), 851. https://doi.org/10.3390/pathogens10070851

Thomas, J., Govender, N., McCarthy, K. M., Erasmus, L. K., Doyle, T. J., Allam, M., … Blumberg, L. H. (2020). Outbreak of Listeriosis in South Africa associated with processed meat. *The New England Journal of Medicine*, *382*(7), 632–643. https://doi.org/10.1056/NEJMoa1907462

Thrusfield, M. (2007). *Veterinary epidemiology* (3rd ed., pp. 214–265). London: Blackwell Science, A Blackwell Publishing Company.

Todd, E. C. D., & Notermans, S. (2011). Surveillance of listeriosis and its causative pathogen, Listeria monocytogenes. *Food Control*, *22*(9), 1484–1490. https://doi.org/10.1016/j.foodcont.2010.07.021

Van Coillie, E., Werbrouck, H., Heyndrickx, M., Herman, L., & Rijpens, N. (2004). Prevalence and typing of *Listeria monocytogenes* in ready-to-eat food products on the Belgian market. *Journal of Food Protection*, *67*(11), 2480–2487. https://doi.org/10.4315/0362-028x-67.11.2480

Wieczorek, K., Dmowska, K., & Osek, J. (2012). Characterization and antimicrobial resistance of *Listeria monocytogenes* isolated from retail beef meat in Poland. *Foodborne Pathogens and Disease*, *9*(8), 681–685. https://doi.org/10.1089/fpd.2012.1137

Wu, S., Wu, Q., Zhang, J., Chen, M., Yan, Z. A., & Hu, H. (2015). *Listeria monocytogenes* prevalence and characteristics in retail raw foods in China. *PLoS One*, *10*(8), e0136682. https://doi.org/10.1371/journal.pone.0136682

Yehia, H. M., Ibraheim, S. M., & Hassanein, W. A. (2016). Prevalence of *Listeria* species in some foods and their rapid identification. *Tropical Journal of Pharmaceutical Research*, *15*(5), 1047–1052. https://doi.org/10.4314/tjpr.v15i5.21

Zamuz, S., Munekata, P. E. S., Dzuvor, C. K. O., Zhang, W., Sant'ana, A. S., & Lorenzo, J. M. (2021). The role of phenolic compounds against *Listeria monocytogenes* in food. A review. *Trends in Food Science & Technology*, *110*, 385–392. https://doi.org/10.1016/j.tifs.2021.01.068

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Moabelo, K. C., Gcebe, N., Gana, J., Ngoshe, Y. B., & Adesiyun, A. A. (2023). Contamination of beef and beef products by *Listeria* spp. and molecular characterization of *L. monocytogenes* in Mpumalanga, South Africa. *Journal of Food Safety*, *43*(5), e13055. https://doi.org/10.1111/jfs.13055