DETECTION OF PATHOGENIC AND VIRULENT SEROGROUPS OF *LISTERIA MONOCYTOGENES* IN BEEF PRODUCTS POSES A HEALTH CONCERN TO CONSUMERS IN SOUTH AFRICA

James Gana[*,+], Habiba L. Mohammed[µ], Ruth J. Ndagimba[¥], Zakari Mamman[+], Yusuf Garba[£], Musa I. Bunu[√] and Abiodun A. Adesiyun*

[*]Department of Production Animal Studies, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, Pretoria 0110, South Africa

+Department of Agricultural Education, School of Vocational Education, Federal College of Education, P.M.B.39, Kontagora, Niger State, Nigeria

µDepartment of Agricultural Technology, Federal Polytechnic Bida, Niger State, Nigeria

¥Department of Animal Health and Production Technology, Niger State College of Agriculture, Mokwa, Nigeria

£Department of Biology, Federal College of Education, Kontagora, Niger State, Nigeria

√Department of Chemistry, Federal College of Education, Kontagora, Niger State, Nigeria

***Corresponding Author:** jamesgana38@gmail.com & gana.james@fcekg.edu.ng*

ABSTRACT

In 2017-18 South experienced the largest outbreak of human listeriosis in the world caused by L. monocytogenes following the consumption of 'polony', a ready-to-eat meat product. A majority (59%) of the cases originated from Gauteng province, South Africa. As a follow-up study to the outbreak, a study was carried out to determined the prevalence of pathogenic and virulence serogroups of L. monocytogenes in various beef and beef products retailed in Gauteng province, South Africa using standard bacteriological and molecular methods. The overall prevalence of Listeria spp. was 28.0% (112/400), comprising Listeria monocytogenes (9.3%), Listeria innocua (16.3%), and Listeria welshimeri (2.5%) (p<0.001). It is crucial to have detected that the region (p=0.036), type of product (p=0.032), and temperature at storage (p=0.011) significantly affected the occurrence of L. monocytogenes in beef products. The pathogenic serogroups 4b-4d-4e (51.4%), and 1/2a-3a (43.2%), were detected among the isolates of L. monocytogenes, and importantly, they were all carriers of seven virulence-associated genes (hlyA, inlB, plcA, iap, inlA, inlC, and inlJ). Our study also demonstrated that 16.7% of 'polony' samples investigated were contaminated by L. monocytogenes. Considering that pathogenic and virulent L. monocytogenes contaminated beef and beef products retailed in South Africa, the food safety risk posed to consumers still exists and cannot be ignored. Therefore, it is imperative to reduce the contamination of these products with L. monocytogenes during beef production, processing, and retailing to avoid future outbreaks of human listeriosis in the country.

Keywords: *Listeria,* Serogroups, Virulence genes, Beef products, South Africa.

INTRODUCTION

Listeriosis is a well-known food-borne infection that mainly affects humans, particularly immunocompromised, pregnant, young, and old individuals (Buchanan *et al.*, 2017). *Listeria monocytogenes* is the leading cause of human and animal listeriosis, posing the highest risk to food safety and public health and responsible for various clinical manifestations (Schlech, 2019). *L. monocytogenes* has been isolated from several types of foods that serve as vehicles for the transmission of the pathogen to humans and caused listeriosis globally (Lopez-Valladare *et al.*, 2018). The types of food implicated include milk and milk products (Molla *et al.*, 2004), vegetables (Zoellner *et al.*, 2019), and meat and meat products (Matle *et al.*, 2020).

Between 2017 and 2018, South Africa experienced the world's largest outbreak of human listeriosis (Olanya *et al*.,. 2019). Whole genome sequencing (WGS) identified *L. monocytogenes* sequence type 6, which originated from 'polony,' an RTE beef product, as the outbreak's origin (Allam, et al. 2018). Matle *et al.* (2019) reported the prevalence of *L. monocytogenes* to be 10.1%, 13.5%, and 19.5%) for raw intact meat, RTE meat products, and processed meats, respectively, in South Africa. There is however a dearth of current information on epidemiological data on the samples assessed for

contamination by *L. monocytogenes*, the risk posed by RTE foods, and the species of *Listeria* other than *L. monocytogenes* in beef and beef products.

The current study, therefore, detected the effect of human listeriosis posed by the consumption of beef and beef products in Gauteng province, South Africa, by determining the. the prevalence of pathogenic and virulent serogroups of *L. monocytogenes* in RTE foods and the species of *Listeria* other than *L. monocytogenes* in beef and beef products and using polymerase chain reaction (PCR) assays to characterize the isolates of *L. monocytogenes* concerning their serogroups and carriage of virulence genes.

MATERIALS AND METHODS

Study design

The cross-sectional study was conducted at 48 retail outlets in Gauteng province, South Africa. These were selected based on the information obtained from the Consumer Goods Council of South Africa (CGCSA) (https://www.cgcsa.co.za/). The number of samples collected from each type of outlet was proportional to its size and availability during sampling visits using a convenience sampling approach. A sample size of 400 was determined using the formula by Thrusfield (2007). The samples were collected between October 2019 and April 2021 during a single visit to each outlet, including raw, chilled, and frozen beef and dried beef-based RTE products**.**

Sample collection, isolation, and identification of *Listeria* spp.

For the 48 outlets from where samples were collected, for 32 (66.7%) outlets, 8 samples were collected per outlet, while for 16 (33.7%), 9 samples were obtained per isolate. The types of samples collected per outlet were based on the beef and beef products available during the visit.

The procedure described by Matle *et al*. (2019) was used to isolate and confirm suspect *Listeria* spp. using phenotypic assays. For the study, the validated *Listeria* Precis method, according to a protocol by Thermofisher Scientific and reported by Matle *et al*. (2019) was used with some modifications. with minor modifications. Ten grams of each sample was aseptically transferred into a stomacher bag containing 225 mL of ONE broth-*Listeria* (Oxoid, Basingstoke, UK) for enrichment. The samples were homogenized in a Stomacher (Stomacher Lab Blender 400, Seward Ltd., West Sussex, UK) for 5 min at 15,493 x g speed, followed by aerobic incubation at 35^0C for 24 h for enrichment. Thereafter, for isolation of *Listeria*, 10 µL of enriched broth sample was inoculated onto Brilliance-*Listeria* agar (BLA) plates (Oxoid, Basingstoke, UK) and incubated at 35^0C for 24 h. Based on the phenotypic appearance of the colonies on BLA, which presumptively classified green-blue colonies without a halo as *Listeria* spp. while blue colonies with white/cream halo) were tentatively identified phenotypically as *L. monocytogenes*. Single colonies of suspected *Listeria* spp. (colonies that appeared blue without a halo) and *L. monocytogenes* (blue colonies with a white/cream halo) were sub-cultured on BLA for further purification (Jamali *et al.*,. 2013).

Molecular confirmation of *Listeria*

Extraction of DNA from enriched broth cultures and colonies

DNA was extracted by heating method, and the crude extract was used in subsequent PCR protocols (Soumet *et al.*, 1994). Briefly, 2 mL aliquots of samples in enrichment broth were spun at 15,493 x g for 5 minutes in a microcentrifuge (Eppendorf, South Africa). The pellets were suspended in 200 µL of sterile distilled water, heated to 95°C in a dry block for 10 minutes, cooled at room temperature for 5 minutes, and centrifuged at 15,493 x g for 5 minutes. The supernatant was pipetted into sterile tubes, and the pellet was discarded. The DNA in the supernatant was used as template DNA using in PCR assays.

Determination of the species of *Listeria* using mPCR

The multiplex PCR was used to determine the five species of the *Listeria* isolates (*L. grayi, L, innocua, L. ivanovii, L. monocytogenes, L. seeligeri* and *L. welshimeri*) as described by Ryu et al. (2013). The multiplex PCR mix was prepared as follows: 12.5 µL of 2x red Taq master mix 5 µL (Lasec, SA, Pty, Cape Town, South Africa) nuclease-free water, 5 µL DNA template, and 4 µL of 20uM primer mix for PCR assay (Doumith et al. 2004). Multiplex PCR was performed with an initial denaturation at 94 ^{0}C for 5 min, 35 cycles of denaturation at 94^0 C for 30 s, annealing at 60^o C for 30 s, and extension at 72 ^{0}C for 30 s, and final extension at 72 ^{0}C for 5 min. PCR amplicons were electrophoresed on a 3.0% agarose gel using 1×Tris-acetate-EDTA (TAE) buffer and stained with ethidium bromide (Ryu *et al*., 2013). *L. monocytogenes* ATCC 19111, *Listeria innocua* ATCC 33090,

L. w*elshimeri* ATCC 35897, *L. grayi* ATCC 25401, *L. ivanovii* ATCC 19119, and *L. seeligeri* ATCC 35967 were used as positive controls, *Campylobacter fetus* ATCC 27373 as a negative control, and water as a blank

Determination of the serogroups of *L. monocytogenes* isolate

Multiplex PCR was used to classify *L. monocytogenes* strains into serogroups that target genes (*ORF2110, ORF2819, Imo1118, Imo0737, and prs)* as described by Doumith *et al.* (2004). The *prs* gene was used as a target marker for the *Listeria* species. PCR mix was prepared as follows, 12.5 µL of 2x of red Taq master mix (Lasec, South Africa), 5 µL nuclease-free water, 5 µL DNA template, and 4 µL of 20 uM primer mix (containing all oligonucleotides listed in Table 1) for PCR assay. The PCR cycling conditions were as follows: initial denaturation step at 94°C for 3 min; 35 cycles of 94°C for 0.40 min. at 53°C for 1.15 min. and 72°C for 1.15 min., and one final cycle at 72°C for 7 min. and amplification was done in a Thermocycler. The PCR products were subjected to electrophoresis on 3% ethidium bromide-stained agarose gel for 3 h at 100 v. *L. monocytogenes* ATCC 19111 was used as a positive control, *Campylobacter fetus* ATCC 27373 as a negative control, and water as a blank.

Detection of virulence genes in *L. monocytogenes* isolates

Multiplex PCR as described by Rawool et al. (2007) and Xu *et al.* (2009) was used to identify the hlyA, inlB, plcA, actA, and iap virulence genes which are specific to *L. monocytogenes*. The second multiplex PCR was used to identify the extra virulence genes inlA, inlC, and inlJ
(Liu *et al*., 2007). The PCR products were electrophoresed on a 3% agarose gel and results were captured using a gel documentation system (Vacutec, SA)

Data analysis

Laboratory data generated for the prevalence of the six species of *Listeria,* serogroups, and virulence genes were analyzed using Stata software (StataCorp LLC, College Station, Texas, USA), and the association of variables with the detection of *Listeria* or selected characteristics were determined using Fisher's Exact and Chi-square test. The significant difference was evaluated using ($P< 0.05$), and percentages were calculated at a 95% confidence interval. To evaluate the prevalence of the six species by the geographical location of the outlets, type of retail outlets, type of beef and beef products, storage temperature, and status of the product, a descriptive analysis was also performed using StataCorp.

Results

Overall, of the 400 samples of beef and beef products , the prevalence of the *Listeria* genus was 28.0% (112/400). The prevalence of the six species of *Listeria* investigated was 16.3, 9.3, 2.5, 0.0%, 0.0 and 0.0% for *L. innocua*, *L. monocytogenes*, *L. welshimeri, L. grayi, L. ivanovii,* and *L. seeligeri,* respectively ($P<0.05$).

From the 48 outlets, regardless of the type of beef and beef products tested, 26 (54.1%) yielded *L. monocytogenes*, with the positivity rate ranging from 11.1% (2/9) to 33.3% (3/0). Samples from 36 (75%) of 48 outlets were positive for *L. innocua*, with the range of frequency of detection in positive outlets being from 11.1% (2/9) to 44.4% (4/9). Only 8 (16.7%) of the outlets yielded *L. welshimeri* ranging from 11.1% (2/9) to 25% (2/8) in positive outlets.

The prevalence of *L. monocytogenes, L. innocua,* and *L. welshimeri* in beef and beef products and the univariate analyses of associated factors

The prevalence of *L. monocytogenes* was 9.3% (37/400), and statistically significant differences were detected for three variables, namely, the retail outlets' regional locations ($P=0.036$), the type of product ($P=0.032$), and the temperature of storage ($P=0.011$).

The prevalence of *L. innocua* was 16.3% (65/400). The temperatures at which the beef and beef products were kept pre-sale had a statistically significant ($P<0.001$) effect on the prevalence of *L. innocua*.

L. welshineri contaminated 2.5% (10/400) of the samples. None of the variables had a statistically significant ($P>0.05$) effect on the prevalence of *L. welshimeri.*

Overall, the frequency of distribution of the serogroups among 37 isolates of *L. monocytogenes* was 43.2% (16/37), 5.4% (2/37), and 51.4% (19/37) for serogroup 1/2a-3a, 1/2c-3c, and 4b-4d-4e, respectively ($P<0.001$). The distribution of the serogroups by region, type of retail outlets, kind of beef and beef products, storage temperature, and status of product is shown in Table 5. Statistically

significant differences were detected only in the frequency of serogroups by region for 4b-4d-4e (P=0.023) and for the type of outlet for 1/2a-3a (P=0.003).
The detection frequency of the eight virulence genes was 100.0% (37/37) each for seven virulence genes (*hlyA, inlB, plcA, iap, inlA, inlC,* and *inlJ*) but 97.3% for virulence gene *actA*. The frequency of detecting *actA* across the three variables was from 90.0% (regions) to 93.8% (type of product).
Seven virulence genes were detected in the three serogroups (1/2a-3a, 1/2c-3c, and 4b-4d-4e), but the *actA* gene was detected in 93.8% (15/16) of the isolates in serogroup 1/2a-3a.

Discussion
Our findings that 9.3% of the beef and beef products tested were contaminated by *L. monocytogenes* has food safety implications for consumers because *L. monocytogenes*, the most important species of *Listeria,* is frequently associated with cases and outbreaks of human listeriosis (Cartwright *et al.,* 2013). A similar prevalence of 8.3% for *L. monocytogenes* was reported for beef and beef products (raw beef, RTE, milled beef, offal, and organs) sampled at retail outlets in Mpumalanga province, South Africa (Moabelo *et al*., 2023). However, Matle *et al.* (2019) reported a higher prevalence of the pathogen in meat and meat products in the country. The differences in the findings between both studies that used the same detection methods may be due to several factors. These factors include the fact that the current study was conducted on beef and beef products sampled from retail outlets in Gauteng province. In contrast, the study reported by Matle *et al*. (2019) was done on meat and meat products (poultry, cattle, sheep, pork, and game; meat samples collected from the three major ports of the country and abattoirs, meat processing plants, butcheries, and retail outlets. This is because the sources of the meat products have the potential to influence the prevalence of *L. monocytogenes***.** It has been documented that all these variables potentially affect the prevalence of *L. monocytogenes* in meat (Liu *et al*.,. 2020).
The study also documented, for the first time in Gauteng province, South Africa, the prevalence of three species of *Listeria* (*L. monocytogenes*, *L. innocua,* and *L. welshimeri)* in beef and beef products according to retail outlet location, types of outlets, and beef products, and the virulence/pathogenic characteristics of the *L. monocytogenes* isolates.
The prevalence of *L. innocua* (16.3%) in the present study is lower than the 21.3% in RTE food samples in Johannesburg, South Africa (Makumbe *et al*., 2021). The organism has been recovered at varying frequencies in meat products elsewhere, such as in Spain, 13.9% (Gómez *et al*., 2014), and China, 28.9% (Chen *et al*., 2009). It is well established that, unlike *L. monocytogenes*, the most important *Listeria* species implicated in human listeriosis, *L. innocua* is considered non-pathogenic (Guillet, et al. 2010; Koopmans, et al. 2023).
Regarding the variables for the detection of *L. monocytogenes* in the current study, the prevalence of *L. monocytogenes* varied significantly across the district locations of the retail outlets, a finding in agreement with published studies in South Africa (Matle *et al*., 2019) and Bangladesh (Islam *et al*., 2016).
It was no surprise that *L. monocytogenes* was detected at the highest frequency (13.2%) in chilled beef and beef products compared with those kept at room temperature and frozen temperatures. This is because the pathogen can survive and multiply at chilling or refrigeration temperatures, which occur at the retail level (Okada *et al*., 2013) and during transport.
In our study, *L. monocytogenes* contaminated 14.5% of minced beef samples**,** which is higher than the prevalence of 1.0% reported in minced meat in Switzerland (Fantelli and Stephan 2001) and 12.2% in Japan (Inoue *et al*., 2000). However, a considerably higher frequency of contamination of minced meat and products have been documented in Ireland, 29.0% (Khen, et al. 2015), Belgium, 42.1% (Van Coillie *et al.*, 2004), and Brazil, 59.4% (Ristori *et al*., 2014). Minced beef and beef products are known to be contaminated by pathogens, primarily due to the preparation methods (Ristori *et al.*, 2014). Furthermore, minced meat-borne listeriosis outbreaks have been documented (Öktem *et al.*, 2006).
The detection of *L. monocytogenes* in 6.9% of the RTE products is also a food safety concern since RTE beef products have been associated with human listeriosis globally (Lopez-Valladares *et al.*, 2018). . These findings suggest that there is a need to standardize the production of 'biltong' in South Africa to confirm the potential for dry 'biltong' to support the contamination and subsequent multiplication of *L. monocytogenes* since critical control points, specifically, during curing (types and

concentration of agents) and the temperature and length of drying the product, which have the potential to influence the contamination, survival, and multiplication of *L. monocytogenes* in 'biltong' With the detection of *L. monocytogenes* in 9.3% of 'biltong' samples tested positive for L. monocytogenes in the present study, other studies have reported a prevalence 7% and 5% of S*almonella* spp. (Manqele, 2018) and Shiga toxin-producing *Escherichia coli* (Onyeka *et al*., 2020), respectively, in 'biltong' sampled from retail outlets in Gauteng province, again indicating the potential health concern posed to consumers of contaminated products.

It is of epidemiological and clinical relevance that of the four distinct serogroups of *L. monocytogenes* reported by Doumith *et al*. (2004), three (4b-4d-4e, 1/2a-3a, and 1/2c-3c) were detected at significantly different frequencies among the *L. monocytogenes* in our study. The relevance is primarily because these serogroups contain seven serotypes (1/2a, 1/2c, 3a, 3c, 4b, 4d, and 4e), of which serotypes 4b, 1/2a, and 1/2c are most frequently reported in animals and humans (Bergholz *et al.*, 2018).

Of potential virulence and pathogenicity importance is the detection that all (100.0%) of the isolates of *L. monocytogenes* assessed in the current study were positive for seven of the virulence-associated genes, which included the genes encoding specific virulence factors, specifically, internalins (*inlA, inlB, inlC, inlJ*), hemolysin (*hlyA)*, phospholipase (*plcA*), and actin polymerization (*actA*) in 97.3% of the isolates. Matle *et al.* (2019) reported similar findings in a study conducted on meat and meat products across the country.

CONCLUSIONs

In conclusion, the detection of pathogenic serogroups of *L. monocytogenes* in 9.3% of beef and beef products, particularly in RTEs (6.9%) should serve as a concern for consumers of these products. This is important because the country recently experienced a large outbreak of human listeriosis with 59% of the cases who were linked to the consumption of RTE beef products. The fact that *L. monocytogenes* were detected in some of these RTEs in the provinces is indicative that food safety concerns on exposure to listeriosis still exist.

REFERENCES

Allam, M., Tau, N., Smouse, S. L., Mtshali, P. S., Mnyameni, F., Khumalo, Z. T., & Smith, A. M. (2018). Whole-genome sequences of *Listeria monocytogenes* sequence type 6 isolates associated with a large foodborne outbreak in South Africa, 2017 to 2018. *Genome announcements*, *6*(25), 10-1128.

Arya, R., Antonisamy, B., & Kumar, S. (2012). Sample size estimation in prevalence studies. *The Indian Journal of Pediatrics*, *79*, 1482-1488.

Bergholz, T. M., Shah, M. K., Burall, L. S., Rakic-Martinez, M., & Datta, A. R. (2018). Genomic and phenotypic diversity of *Listeria monocytogenes* clonal complexes associated with human listeriosis. *Applied Microbiology and Biotechnology*, *102*, 3475-3485.

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.

Cartwright, E. J., Jackson, K. A., Johnson, S. D., Graves, L. M., Silk, B. J., & Mahon, B. E. (2013). Listeriosis outbreaks and associated food vehicles, United States, 1998–2008. *Emerging infectious diseases*, *19*(1), 1.

Chen, J., Zhang, X., Mei, L., Jiang, L., & Fang, W. (2009). Prevalence of Listeria in Chinese food products from 13 provinces between 2000 and 2007 and virulence characterization of *Listeria monocytogenes* isolates. *Foodborne Pathogens and Disease*, *6*(1), 7-14

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.

Fantelli, K., & Stephan, R. (2001). Prevalence and characteristics of shigatoxin-producing *Escherichia coli* and *Listeria monocytogenes* strains isolated from minced meat in Switzerland. *International Journal of Food Microbiology*, *70*(1-2), 63-69.

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.

Inoue, S., Nakama, A., Arai, Y., Kokubo, Y., Maruyama, T., Saito, A., & Kumagai, S. (2000). Prevalence and contamination levels of *Listeria monocytogenes* in retail foods in Japan. *International Journal of Food Microbiology*, *59*(1-2), 73-77.

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.

Jamali, H., Paydar, M., Ismail, S., Looi, C. Y., Wong, W. F., Radmehr, B., & Abedini, A. (2015). Prevalence, antimicrobial susceptibility and virulotyping of *Listeria* species and *Listeria monocytogenes* isolated from open-air fish markets. *BMC microbiology*, *15*, 1-7.

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.

Koopmans, M. M., Brouwer, M. C., Vázquez-Boland, J. A., & van de Beek, D. (2023). Human listeriosis. *Clinical Microbiology Reviews*, *36*(1), e00060-19.

Lakicevic, B., & Nastasijevic, I. (2017). Listeria monocytogenes in retail establishments: Contamination routes and control strategies. *Food Reviews International*, *33*(3), 247-269.

Liu, D., Lawrence, M. L., Austin, F. W., & Ainsworth, A. J. (2007). A multiplex PCR for species- and virulence-specific determination of Listeria monocytogenes. *Journal of Microbiological Methods*, *71*(2), 133-140.

Liu, Y., Sun, W., Sun, T., Gorris, L. G., 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.

Loo, K. Y., Letchumanan, V., Dhanoa, A., Law, J. W. F., Pusparajah, P., Goh, B. H., & Chan, K. G. (2020). Exploring the pathogenesis, clinical characteristics and therapeutic regimens of *Listeria monocytogenes*. *Microbiology*, *3*, 1-13.

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.

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*.

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.

Matle, I., Mbatha, K. R., & Madoroba, E. (2020). A review of Listeria monocytogenes from meat and meat products: Epidemiology, virulence factors, antimicrobial resistance and diagnosis. *Onderstepoort Journal of Veterinary Research*, *87*(1), 1-20.

Moabelo, K. C., Gcebe, N., Gana, J., Ngoshe, Y. B., & Adesiyun, A. A. (2023). Contamination of beef and beef products by *Listeria* species. and molecular characterization of L. monocytogenes in Mpumalanga, South Africa. *Journal of Food Safety*, e13055.

Molla, B., Yilma, R., & Alemayehu, D. (2004). *Listeria monocytogenes* and other *Listeria* species in retail meat and milk products in Addis Ababa, Ethiopia. *Ethiopian Journal of Health Development*, *18*(3), 208-212.

Montero, D., Bodero, M., Riveros, G., Lapierre, L., Gaggero, A., Vidal, R. M., & Vidal, M. (2015). Molecular epidemiology and genetic diversity of *Listeria monocytogenes* isolates from a wide variety of ready-to-eat foods and their relationship to clinical strains from listeriosis outbreaks in Chile. *Frontiers in microbiology*, *6*, 384.

Okada, Y., Ohnuki, I., Suzuki, H., & Igimi, S. (2013). Growth of *Listeria monocytogenes* in refrigerated ready-to-eat foods in Japan. *Food Additives & Contaminants: Part A*, *30*(8), 1446-1449.

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.

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.

Rabinovich, L., Sigal, N., Borovok, I., Nir-Paz, R., & Herskovits, A. A. (2012). Prophage excision activates Listeria competence genes that promote phagosomal escape and virulence. *Cell*, *150*(4), 792-802.

Rawool, D. B., Malik, S. V. S., 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.

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.

Smith, A. M., Tau, N. P., Smouse, S. L., Allam, M., Ismail, A., Ramalwa, N. R., & Thomas, J. (2019). Outbreak of *Listeria monocytogenes* in South Africa, 2017–2018: Laboratory activities and experiences associated with whole-genome sequencing analysis of isolates. *Foodborne pathogens and disease*, *16*(7), 524-530.

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.

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. *New England Journal of Medicine*, *382*(7), 632-643.

Thrusfield, M. (2007). Sample size determination. *Veterinary epidemiology*, *3*, 185-189

Xu, X. K., Wu, Q. P., Zhang, J. M., Deng, M. Q., & Zhou, Y. H. (2009). Studies on specific detection of *Listeria monocytogenes* in foods by duplex PCR. *Chinese Journal of Health Laboratory Technology*, *19*(6), 1199-1201.

Zhang, Y., Yeh, E., Hall, G., Cripe, J., Bhagwat, A. A., & Meng, J. (2007). Characterization of *Listeria monocytogenes* isolated from retail foods. *International Journal of Food Microbiology*, *113*(1), 47-53.

Zoellner, C., Wiedmann, M., & Ivanek, R. (2019). An assessment of listeriosis risk associated with a contaminated production lot of frozen vegetables consumed under alternative consumer handling scenarios. *Journal of food protection*, *82*(12), 2174-2193.