TITLE: ***LISTERIA MONOCYTOGENES*** IN FOOD PRODUCTS AND PROCESSING INDUSTRIES

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ABSTRACT:

This work aimed to detect *L. monocytogenes* in animal products and equipment from processing industries using Real-Time PCR and VIDAS LMO 2 techniques. The sampling consisted of 95 dairy products, 74 chicken meat, 31 ice cream, 14 beef cuts, 13 mechanically separated chicken meat, 10 fresh giblets, 09 processed meat products, and 13 equipment from sausage processing plants).The swabs were resuspended in 10mL of UVM broth, incubated at 30°C/24h-26h in a B.O.D. A 1 mL aliquot of the enriched culture was transferred to a 10 mL Fraser broth (FB-Fraser) (Difco™), followed by incubation as described. Then, 0.5mL was transferred to the cap of the VIDAS LMO 2® equipment (bioMérieux, Lyon, France) and the manufacturer's recommendations were followed. For isolation of genomic DNA, the "High Pure PCR Template Preparation Kit" (Roche®, Manheim, Germany) was used, from the sediment of 1.5 mL of culture in centrifuged UVM broth (10,000 rpm/10min), resuspended in 450µL of TE buffer (10mM Tris, 1mM EDTA, pH 8.0) and lysate with lysozyme (10mg/ml) at 37°C/2h in a water bath for cell wall lysis. From this point onwards, the kit manufacturer's recommendations were followed. DNA was eluted in 200µL of elution buffer prewarmed to 70°C and solution stored at -20°C for later use. As a negative control of the PCR reaction, ultrapure water was used to replace the DNA and, as a positive control, the *L. monocytogenes* ATCC® 19114 strain was used. List primers were used. monocy-F-1 (5' CATGGCACCACCAGCATC 3'), List. monocy-R-1 (5' CATCCGCGTGTTTCTTTTC 3') (Genebank Accession Code: FF183456.1) and the List. monocy.-S-1 (5' FAM CGCCTGCAAGTCCTAAGACGC TAMRA 3'), which targets the *L. monocytogenes hly* gene, which encodes the production of listeriolysin O. Primers and probe were synthesized by Life Technologies™ (São Paulo, Brazil). The PCR reaction was performed on the StepOnePlus Real-Time PCR System (Applied Biosystems California, USA), with initial denaturation at 95°C/5 min, 40 heating cycles at 95°C/15sec (denaturation), 54°C/1min (annealing), 60°C/30sec (extension) and final extension at 60°C/30sec. The microorganism was detected in 18/259 (6.95%) samples associating the two methodologies, in 16/259 (6.18%) by Real-Time PCR and in 10/259 (3.86%) by the other method. The presence of the bacteria in cheeses, chicken meats, and sausages indicates that these foods can pose risks to public health and emphasizes the need to implement control measures during processing.

INDEX TERMS: real-time PCR, VIDAS, animal products, public health