TITLE: *Listeria* spp.: FORMATION OF BIOFILM IN PRODUCTS OF ANIMAL ORIGIN

AUTHORS: ANDRADE, J.M.; MELO, N.S.S.; SILVA, M.G.V.; MOURA, F.M.L.; LEAL, C.A.S.; MEDEIROS, E. S.

INSTITUTION: UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO, RECIFE, PE (RUA DOM MANOEL DE MEDEIROS S/N, DEPARTAMENTO DE MEDICINA VETERINÁRIA, CEP 52171-900, RECIFE-PE, BRASIL)

ABSTRACT:

Biofilms are microbial communities that develop in a hydrated extracellular polymer matrix, which adheres to an inert or living surface. The ability to form biofilms is a determining factor for the persistence of *Listeria* spp. in environments for long periods, especially in areas of food handling. A condition that allows the contamination of food products and causes impacts on public health, since the microorganism is the causative agent of listeriosis. A disease that can be transmitted by ingestion of food contaminated by the bacteria. Eight strains of *Listeria monocytogenes* and two *Listeria innocua* strains isolated from samples of animal products belonging to the Laboratory of Inspection of Meat and Milk of the Federal Rural University of Pernambuco were tested. The colonies were inoculated into tubes containing 3mL of Tryptic Soya Broth until turbidity of 0.5 MacFarland and incubated at 37°C for 24 hours. 100 µl of each solution was inoculated, in triplicate, into a 96-well microdilution plate and incubation was carried out at 37°C for 24 hours. The contents of each well were aspirated and washed three times with sterile distilled water. Plate drying occurred at room temperature and adhered cells were stained with 200µl of gentian violet for five minutes. Thereafter, the washing and drying process was again performed as described above. Finally, 200µl of alcohol: acetone was added. The optical density (OD) of the wells was measured by spectrophotometry at 620 nm. Tryptone Broth Soy was used as negative control, a strain of *Escherichia coli* ATCC® DH 5α as positive control. In order to classify the isolates for biofilm production, the mean OD (ODc) of the negative control (ODcn) and the OD of the isolates (OD_{IS}) were measured as negative ($OD_{IS} < OD_{CN}$); weak ($OD_{CN} < OD_{IS} < 2.\ OD_{CN}$); ($2.\ OD_{CN} < OD_{IS} < 4.\ OD_{CN}$); strong ($4.\ OD_{CN} < OD_{IS}$) biofilm builders. All tested strains were able to form biolfilm. Of the colonies of *L. monocytogenes* 87.5% (7/8) were classified as weak and 12.5% (1/8) as moderate. Of the two strains of *L. innocua* analyzed 50% (1/2) were classified as moderate and the same percentage value as weak. The presence of *Listeria* spp. biofilm forming agents from samples of animal products suggests that the microorganism is able to remain in food handling environments for a longer period of time.

Keywords: *Listeria monocytogenes, Listeria innocua,* public health