

# Identification of routes and mechanisms for distribution and establishment of *Listeria monocytogenes* and *Listeria* spp. in avocado packing environments



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## Summary

This project focuses on the study of the potential for *Listeria* spp. to colonize avocado packing environments at plants that practice dry packing, which consists of completing the packing process without including any avocado wash or wet treatments. At these plants, sanitation procedures involve wet cleaning and sanitizing (**Figure 1**), which may introduce some moisture in the environment, potentially favoring the attachment and likely colonization by *Listeria* spp. The project aims to determine the potential for *Listeria* spp. to establish in the avocado packing environment and the distribution within the avocado packing environment. Results this far show a relatively low prevalence of *Listeria* spp. in the avocado packing plant environment. Subsequent DNA sequencing will allow mapping the potential distribution of *Listeria* spp.

## Objectives

1. Determine harborage sites and spreading mechanisms for *Listeria* spp. and *Listeria monocytogenes* (LM) in the environment inside avocado dry packing facilities.
2. Develop a map of areas of potential *Listeria* spp. and LM cross-contamination to avocado fruits during packing.
3. Evaluate the effect of plant layout, avocado contact surface materials, and environmental factors on attachment and biofilm formation on avocado-contact surfaces during packing.
4. Develop a simulation model to predict the levels of cross-contamination in a fresh avocado processing facility with visualization of the whole processing line

## Methods

Three avocado packing facilities in Mexico were included in the study, conducting sample collection and DNA analysis as follows:

- Sampling of non-food contact surfaces by swabbing with sponges or swabs pre-moistened with D/E broth. Sample testing for *Listeria* spp. by enrichment and screening (3M Molecular detection system), with confirmation of positive samples by cultural methods.
- To develop the *Listeria* distribution map, DNA sequencing will be used for whole genome sequencing (WGS) and multi locus sequence typing (MLST).

## Methods (continued)

In-laboratory studies will include inoculation approach for: determining attachment potential of LM onto avocado contact surfaces, and biofilm formation.

Model development:

- Data collected will be used for developing transfer models using simulation software. *Listeria* prevalence, attachment, biofilm development, temperature/humidity, and transfer will be embedded in a discrete event simulation model.

## Results to Date

A total of 446 environmental samples have been tested for *Listeria* spp. and *Enterobacteriaceae* levels. *Listeria* spp. were isolated from 22 (4.9%) samples. As expected, most of the positive samples were collected from floors, while 2% of the non-floor samples tested positive for *Listeria* spp., including drains, side structures in equipment, mats, and containers (**Figures 2 and 3**). All samples of air and cleaning utensils were negative for *Listeria* spp. DNA from *Listeria* isolates and from enriched samples was extracted, and sequencing is in process. Counts of *Enterobacteriaceae* (EB) were obtained from all samples as an indicator of plant sanitation; results showed relatively low levels of EB, consistent with an adequate level of plant sanitation (**Figure 4**).

## Benefits to the Industry

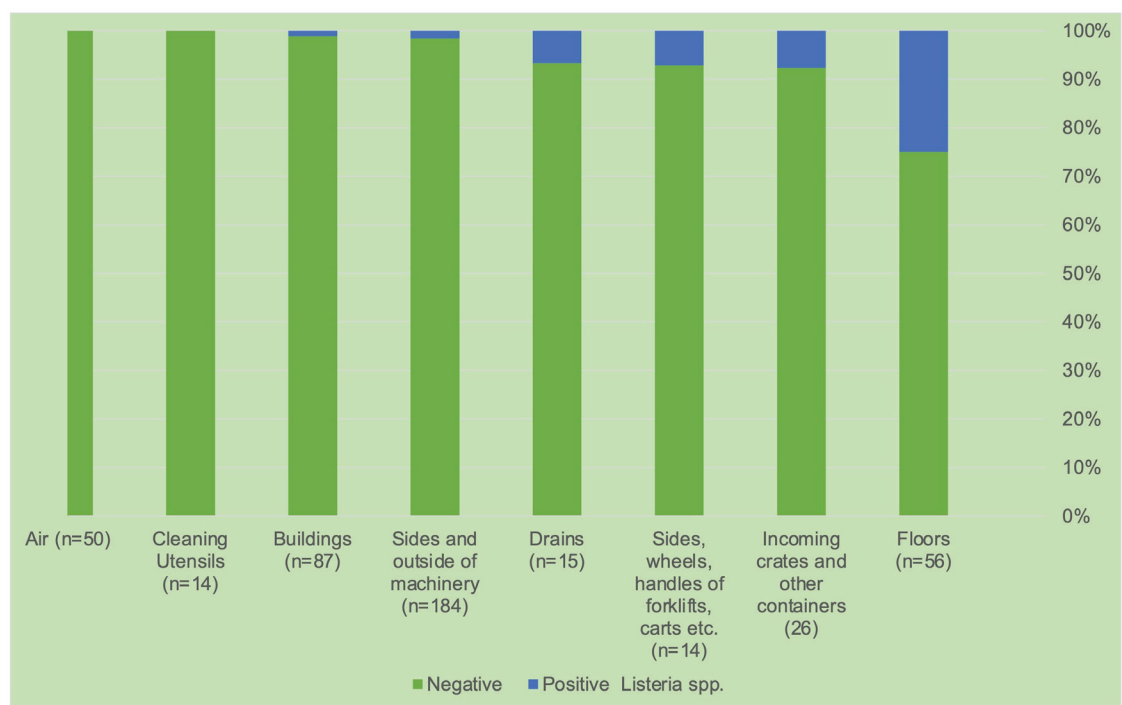
This project aims at tracking *Listeria* spp. in avocado packing plants where fruit washing is eliminated but wet cleaning and sanitation practices are applied. Additional laboratory studies comparing dry cleaning vs. wet cleaning are in process, and the data will permit evaluation of current sanitation practices and possible room for improvement in preventing *Listeria* spp. in the avocado packing environment. After completion of all studies, the potential for *Listeria* to colonize the packing plant environment and the areas of the plant that potentially facilitate harborage at avocado dry packing plants in Mexico will be better understood. Results will help packers to implement practices that improve food safety during avocado dry packing.



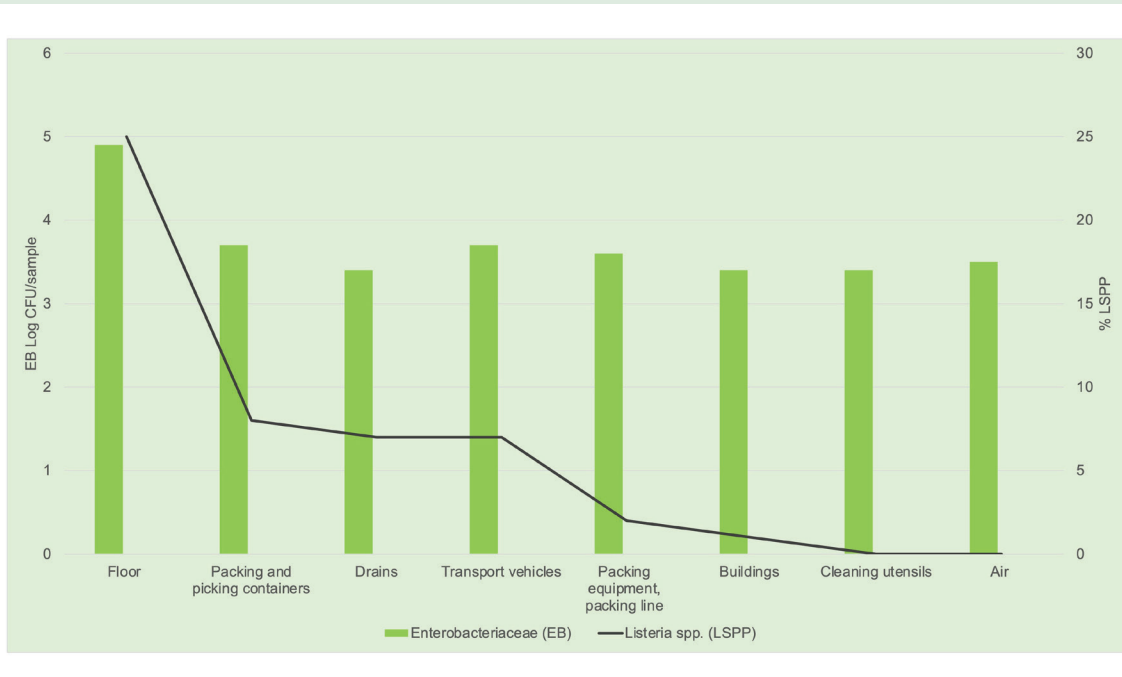
**Figure 1.** Example of wet cleaning in dry avocado packing plants



**Figure 2.** Examples of areas sampled



**Figure 3.** Occurrence of *Listeria* spp. in environmental samples during dry avocado packing



**Figure 4.** Levels of *Enterobacteriaceae* (log CFU/surface) and occurrence of *Listeria* spp. (%) in surface environmental samples from avocado dry packing plants