

# DuPont Qualicon RiboPrinter® System

## APPLICATION PROFILE

## Investigating Sources of Fatal Disease in Farm Animals with Genetic Fingerprints by the RiboPrinter® Microbial Characterization System

Epidemic outbreaks among farm animals — epizootics — frequently reach far beyond their source. Within only a few days, a single *Listeria monocytogenes* strain can infect an entire herd or flock with rapidly progressing and potentially fatal listerial encephalitis. Determining the pathogen's route of contamination demands accurate and in-depth information as fast as possible.

### Overview

An important pathogen of both animals and humans, *L. monocytogenes* is especially troublesome in farm settings where the bacterium can lead to potentially fatal meningitis.

In animals, the source of illness is most often associated with the consumption of poor quality, incompletely-fermented silage. Properly fermented silage has a low pH, caused by lactic acid fermentation of the *Lactobacillus* spp. present in the silage vegetation or storage container. This low pH, typically less than 5.5, inhibits growth of *L. monocytogenes*. Improper management of the ensilage process or its storage can lead to contamination by the pathogen.

Identifying the source of *L. monocytogenes* contamination in cases of animal listeriosis is often complex because of the bacterium's nature. *L. monocytogenes* studies have established that the organism has five distinct genomic groups. This often results in confused identifications when feed is examined because both pathogenic and non-pathogenic strains — indistinguishable to many conventional methods of analysis — can be present.

Using discriminatory strain typing by DNA-based methods as part of an epizootiologic investigation improves the chances of discovering the contamination source. However, most genetics-based typing methods are far too laborious, unreproducible and time consuming to be of much help.

### The investigation

Researchers at Cornell University used a typing tool that offers accurate, fast data — the RiboPrinter® microbial characterization system — when their colleges of Agriculture and Life Sciences and Veterinary Medicine were contacted about a possible outbreak of



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listerial encephalitis in a flock of 655 sheep. The RiboPrinter® system is capable of characterizing 32 bacterial isolates a day instead of the week or more needed to type isolates by other methods. For the Cornell investigation, this meant that genetics-based, definitive information would be available fast enough to help stop the spread of the disease.

Microbiologists and veterinary technicians began the investigation by evaluating the sick animals and acquiring clinical, feed and environmental samples. The *L. monocytogenes* recovered from the brain specimens of seven stricken sheep with listeriosis and the strains found in feed, silage, equipment and the barns were then characterized using the automated RiboPrinter® system. At first, the evidence was perplexing: Fingerprints — RiboPrint® patterns — of strains from the brain sample isolates could not be matched to any *Listeria* found in the sheep's food. However, a matching strain found was found in feed for the farm's cows. How did this strain get into the sheep's feed?

To answer the question, investigators looked for anything that the two feeds might have in common.

### Solving the mystery

The investigation was resolved when the RiboPrinter® system typed isolates from the front end loader used to transport both the cow's corn silage and the sheep's haylage. The RiboPrint® patterns from the loader's bucket, which was not washed or otherwise decontaminated between tasks, turned out to be the culprit of cross contaminating the feed. By pinpointing the path of contamination, the mystery was solved. The farmer could target decontamination, avoiding costly and unnecessary disposal of feed that was not to blame for the outbreak. Most importantly, future contamination by this route and the subsequent death of valuable livestock, could be prevented.

The RiboPrinter® system's ability to discriminate below the species level returned 50 distinct patterns from the 1,346 isolates characterized. This accuracy was a critical tool for determining the strain responsible for the epizootic. Without DNA typing at this sub-species level, the source of the pathogen could not have been found. The Cornell researchers, with their colleagues from the Northeast Dairy Foods Research Center, concluded that the RiboPrinter® system offers "substantial advantages" over other typing methods.

These same researchers also learned that the pattern database established during their investigation could serve as the basis for an electronic library of human and animal outbreak strains. Because the RiboPrinter® is standardized, patterns could be shared with other laboratories. This capability allows for global epidemiological assessment — and perhaps control — of *L. monocytogenes* and the illnesses it causes.

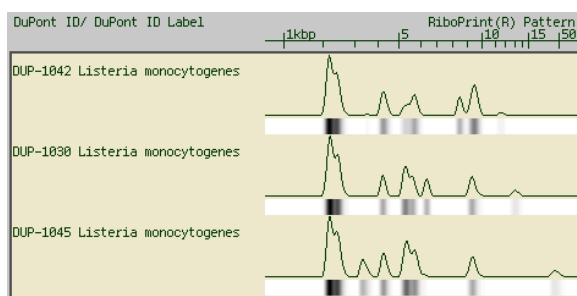


Figure 1. Clinical isolates of *Listeria monocytogenes* from all sheep affected with listerial meningoencephalitis as well as samples from a corn dump and the bucket of a front end loader belonged to the RiboGroup 1042 (top). Patterns from sampling of haylage fed to the sheep (middle, 1030) and from leachate from a corn dump (bottom, 1045) ruled out both as sources of the illness.

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