

# Detecting *Salmonella* Dublin in environmental samples through endpoint multiplex PCR and culture

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## Introduction

*Salmonella* Dublin (*S. Dublin*), a host-adapted serovar, is capable of causing disease in both humans and cattle. Infected animals are prone to becoming long-term carriers, shedding the pathogen, contaminating the environment, and sustaining the disease cycle on the farm. Traditional culture methods are often less sensitive for detecting the pathogen in environmental samples, limiting our ability to understand its prevalence and epidemiology. Therefore, integrating culture-independent techniques is imperative to enhance our capacity to identify affected areas, provide insights into transmission routes, and contribute to mitigating the risk of disease transmission. This study aims to ascertain the prevalence of *S. Dublin* in environmental samples from calf production systems, including calf marketing operations, veal and dairy-beef farms, using multiplex endpoint polymerase chain reaction (PCR) and culture. We hypothesize that PCR will enhance the specific detection of *S. Dublin* in cattle environments.

## Materials and methods

A total of 331 samples from 3 different cattle environments, including one calf marketing operation (n = 125), 19 veal farms (n = 106), and 19 dairy-beef farms (n = 100) located in the mid-west U.S., were collected between May and November 2023. Pre-weaned pens, alleyways, milk mixing rooms and post-wean barns were each sampled once by walking approximately 300 steps while wearing EnviroBootie™ (Hardy Diagnostics). Subsequently, *S. Dublin* was detected using an in-house developed PCR method in addition to traditional culture following the standard procedure of the One Herd Lab.

## Results

*S. Dublin* was identified via PCR in environmental samples from the calf marketing operation (22.4% [28/125]) and in (26% [26/100]) of dairy-beef farms, corresponding to 11 out of the 19 tested, while it was absent in samples from veal farms. The detection of *S. Dublin* through PCR indicates exposure to the pathogen but does not directly confirm animal illness. However, the identification of affected premises can serve as an early warning system, prompting further investigation and implementation of control measures to prevent the potential spread of infection.

In contrast, using traditional culture methods, *S. Dublin* was only recovered from a small number of dairy-beef environmental samples (4.0% [4/100]). Interestingly, one isolate originated from the milk mixing room, pointing out a possible lapse in farm biosecurity measures and raising concerns about potential contamination in feed. The remaining isolates were found in post-weaned barns across 4 different farms, suggesting either a recent introduction or establishment in the environment. Overall, the presence of *S. Dublin* in the farm environment highlights a potential risk of spread and infection for both animals and humans.

## Significance

The use of the in-house PCR method enhanced the detection of *S. Dublin* from environmental samples compared to culture-based methods. PCR detection offers insights into the environmental prevalence of *S. Dublin* and has the potential to enhance our understanding of its transmission dynamics. Subsequent studies will focus on genomic characterization, encompassing sequence typing, analysis of virulence factors, and identification of antibiotic resistance markers in the dairy-beef environmental isolates, as well as investigating their relatedness with clinical strains.

