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Enumeration of *Salmonella enterica* in naturally contaminated animal feed samples by flotation prior to real-time quantitative PCR

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Aim

To extract and quantify *Salmonella* cells directly from feed samples by a combination of flotation and quantitative real-time PCR (qPCR).

Introduction

Animal feed might serve as a reservoir of *Salmonella* contributing to the spread into the food chain. Levels of *Salmonella* in feed samples are low, bacteria are unevenly distributed and stressed and could therefore be hard to recover using standard culture based methods. Due to this, there is a need for accurate, rapid and user-friendly sample preparation methods prior to molecular analyses. Moreover, to facilitate quantitative risk assessment in the feed production chain, there is a need to enumerate *Salmonella* in naturally contaminated feed.

Conclusion

- Quantification of *Salmonella* directly in artificially contaminated feed samples using flotation in combination with real-time PCR was possible with a linear range of 10^2 -10^6 CFU/g feed.
- *Salmonella* was detected in 7/30 naturally contaminated subsamples (levels up to 7.8×10^3 CFU/g feed). This is higher than previously reported, indicating the presence of "hot-spots" and/or non-culturable *Salmonella* cells.

Results

A method to quantify *Salmonella* in feed samples was evaluated. This method consists of flotation[1] in combination with quantification using a real-time PCR assay[2, 3].

Samples withdrawn from the upper interphase after flotation were analysed using growth on selective agar plates (XLD) to check viability and recovery. Samples were also analysed using qPCR (Figures 1 & 2).

Using artificially contaminated samples, the qPCR method estimated that more cells were recovered in the samples compared to selective plating (Figure 1). This effect is most likely due to that damaged cells were unable to grow under the applied selective pressure.

To validate the flotation protocol, *Salmonella* was extracted and quantified from naturally contaminated soya with qPCR (Figure 2). Levels were higher than previously reported, indicating that the flotation method allows for isolation of cells not possible to detect using standard culture based methods and/or the presence of "hot spots" with locally higher levels.

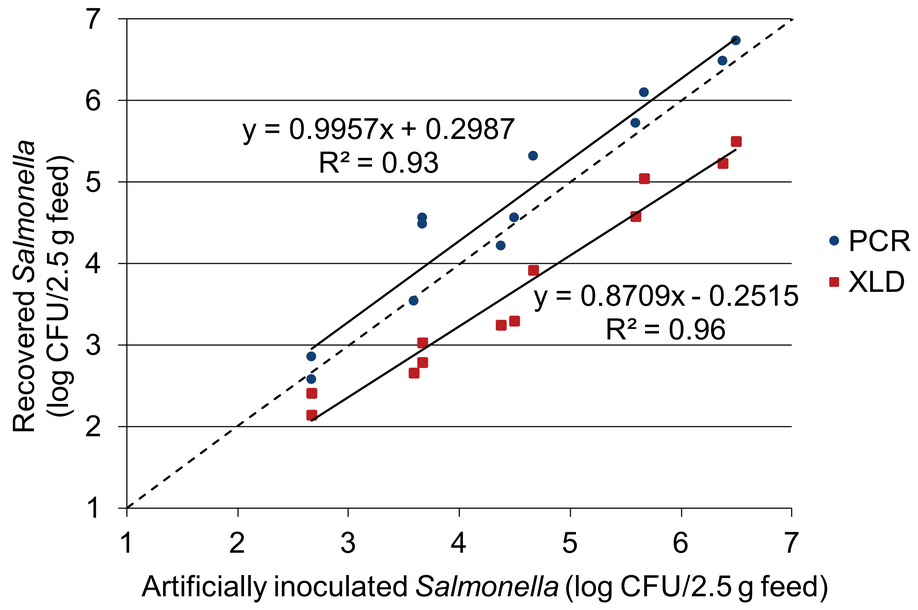


Figure 1. Quantification of cells in samples withdrawn from the upper interphase after flotation using qPCR and plating on selective plates.

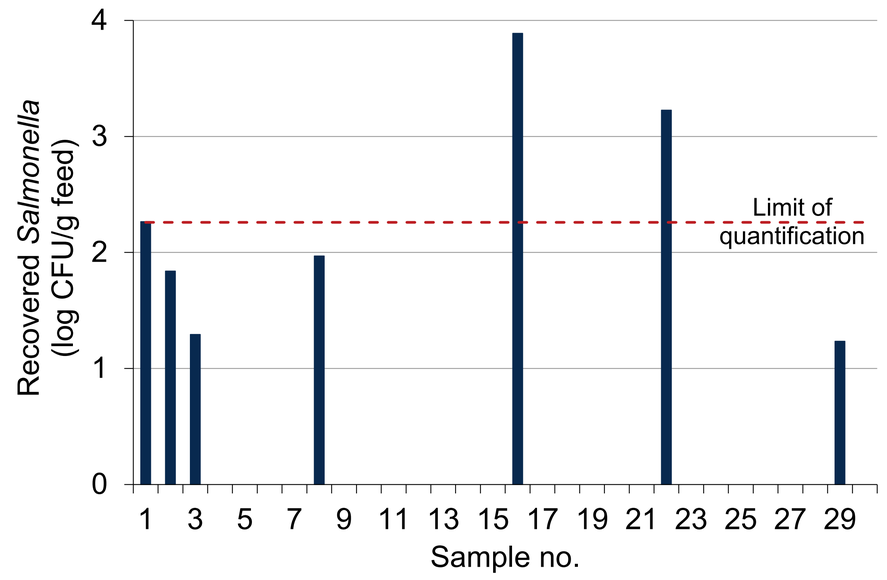


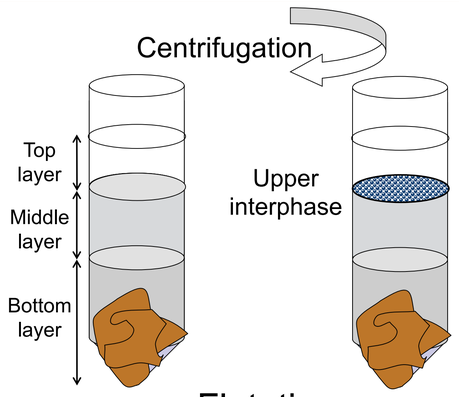
Figure 2. Quantification of cells in naturally contaminated samples using flotation followed by qPCR.

Method

Feed sample

2.5 g feed sample is placed in a tube

- Addition of bottom layer density media.
- Centrifugation.
- Addition of two layers of density media.



Flotation protocol

A discontinuous density gradient separation technique based on the buoyant density of bacteria[1].

1. The sample is loaded at the bottom of the tube.

2.The density media (colloidal solutions of non-toxic silanised silica particles) of different densities are applied in layers according to the designed flotation set-up[1].

3. During centrifugation (~3000 x g), due to the buoyant density of the target bacterium, it floats from the high density bottom layer through the middle layer to be accumulated at the upper interphase.

4. The target bacterium recovered from the upper interphase, separated from inhibitors and matrix particles, can now be used for further analyses.

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