

# Evaluation of bacterial indicators for monitoring the *Salmonella* Enteritidis status of layer farms

Dewaele I.<sup>1</sup> , Ducatelle R.<sup>2</sup>, Herman L.<sup>1</sup>, Heyndrickx M.<sup>1</sup>, De Reu K.<sup>1\*</sup>

<sup>1</sup> Institute for Agricultural and Fisheries Research, Technology and Food Science Unit, Brusselsesteenweg 370, 9090 Melle, Belgium

<sup>2</sup> Ghent University, Faculty of Veterinary Medicine, Department of Bacteriology, Pathology and Poultry Diseases, Salisburylaan 133, 9820 Merelbeke, Belgium

\* Corresponding author: koen.dereu@ilvo.vlaanderen.be

Abbreviated title: Indicator of *Salmonella* Enteritidis on layer farms

## Summary

Cleaning and disinfection of layer farms is important to minimize the possible *Salmonella* Enteritidis (SE) infection pressure. An indicator organism can be useful to verify the efficacy of cleaning and disinfection and maybe to estimate the *Salmonella* status of a layer farm. The present study aims to evaluate *Escherichia coli*, *Enterococcus faecalis* and *Enterococcus hirae* as potential indicator organisms by a quantitative suspension disinfection test. The test was performed according to the EN1656 standard using following commonly used disinfection products in Belgium: Cid 20, D 50 and Virocid. Based on the results, *E.coli* will be chosen as indicator to test in practice.

Keywords: *Salmonella* Enteritidis, disinfection, EN1656, *E. coli*, *Enterococcus*

## Introduction

Cleaning and disinfection of poultry houses between subsequent production rounds is important in the elimination of possible *Salmonella* contamination. Verification of good cleaning and disinfection is performed by taking Aerobic Plate Counts (APC) with Rodac contact plates in the hen house, giving an indication of the remaining aerobic microorganisms. Still, field research has revealed that good hygienic scores (APC) do not imply successful elimination of *Salmonella*. Therefore, a microbiological indicator organism can be an additional tool to check the effectiveness of cleaning and disinfection.

A suitable indicator has to meet several criteria. First, it must be shed in a similar way as SE, that is by faecal excretion. More specifically, if the faecal indicator is isolated, we can conclude that faecal contamination has occurred or is still present, and that it is reasonable to assume that SE could be present. Second, the indicator organism should be more prevalent on layer farms than SE. Third, the indicator should be easily and quickly detected and enumerated (Ghafir et al, 2008). Finally, it is preferred that the indicator is equally or less susceptible for disinfection than SE. This means that if the indicator is not detected after disinfection, there is a high probability that SE is eliminated. Possible indicator organisms can be *E.coli* and *Enterococcus* spp.. The aim of this study is to compare SE with *E.coli* and *Enterococcus* spp. in their susceptibility to disinfection to choose a suitable indicator organism.

## Materials and Methods

### Strains

The following bacteria were used in the test: *Salmonella* Enteritidis (a field strain), *E.coli*, *En. faecalis* and *En. hirae*.

### Disinfection products

The following disinfection products were tested: Cid 20, D 50 and Virocid (Cidlines, Belgium).

### Disinfection test

The disinfection tests were performed according to the EN1656 standard (Anon., 2000) under simulated low soiling conditions and with a contact time of 30 min at 10 °C.

## Results

First, the different concentrations of the disinfection products at which SE is 1/ totally, 2/ partly and 3/ not at all eliminated were determined. Second, the elimination of SE was compared with the elimination of *E.coli*, *En. hirae* and *En. faecalis* using the predetermined concentrations. Each disinfection test was repeated three times (Table 1- 3).

Table 1: Disinfection test Cid 20

Concentration of product	0.50%	0.35 – 0.40%	0.20%
<i>Salmonella</i> Enteritidis	0 CFU/ml (*)	$1.0 \times 10^4$ CFU/ml	$1.3 \times 10^8$ CFU/ml
	0 CFU/ml	$1.8 \times 10^4$ CFU/ml	$6.9 \times 10^7$ CFU/ml
	0 CFU/ml	$8.5 \times 10^2$ CFU/ml	$3.3 \times 10^7$ CFU/ml
<i>Escherichia coli</i>	0 CFU/ml	0 CFU/ml	$1.4 \times 10^7$ CFU/ml
	0 CFU/ml	0 CFU/ml	$3.6 \times 10^6$ CFU/ml
	0 CFU/ml	0 CFU/ml	$1.4 \times 10^7$ CFU/ml
<i>Enterococcus faecalis</i>	0 CFU/ml	0 CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	0 CFU/ml
<i>Enterococcus hirae</i>	0 CFU/ml	0 CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	0 CFU/ml

(\*) Colony counts of target organism present in suspension after applied treatment

Table 2: Disinfection test with D 50

Concentration of product	0.08%	0.03 – 0.04%	0.01%
<i>Salmonella</i> Enteritidis	0 CFU/ml (*)	$3.5 \times 10^3$ CFU/ml	$2.3 \times 10^8$ CFU/ml
	0 CFU/ml	$5.5 \times 10^3$ CFU/ml	$3.3 \times 10^8$ CFU/ml
	0 CFU/ml	$3.5 \times 10^2$ CFU/ml	$3.1 \times 10^8$ CFU/ml
<i>Escherichia coli</i>	$2.9 \times 10^3$ CFU/ml	0 CFU/ml	$2.2 \times 10^8$ CFU/ml
	0 CFU/ml	0 CFU/ml	$6.7 \times 10^7$ CFU/ml
	0 CFU/ml	0 CFU/ml	$2.4 \times 10^8$ CFU/ml
<i>Enterococcus faecalis</i>	0 CFU/ml	$3.9 \times 10^3$ CFU/ml	$4.8 \times 10^8$ CFU/ml
	0 CFU/ml	0 CFU/ml	$8.9 \times 10^6$ CFU/ml
	0 CFU/ml	0 CFU/ml	$3.15 \times 10^8$ CFU/ml
<i>Enterococcus hirae</i>	0 CFU/ml	$1.7 \times 10^5$ CFU/ml	$3.7 \times 10^7$ CFU/ml
	0 CFU/ml	$5.3 \times 10^3$ CFU/ml	$8.6 \times 10^7$ CFU/ml
	0 CFU/ml	$3.3 \times 10^5$ CFU/ml	$4 \times 10^8$ CFU/ml

(\*) Colony counts of target organism present in suspension after applied treatment

Table 3: Disinfection test with Virocid

Concentration of product	0.20%	0.12 – 0.13%	0.06%
<i>Salmonella</i> Enteritidis	0 CFU/ml (*)	$2.8 \times 10^3$ CFU/ml	$3.4 \times 10^7$ CFU/ml
	0 CFU/ml	$5.7 \times 10^3$ CFU /ml	$1.1 \times 10^8$ CFU/ml
	0 CFU/ml	$3.5 \times 10^2$ CFU /ml	$2.3 \times 10^7$ CFU/ml
<i>Escherichia coli</i>	0 CFU/ml	$1 \times 10^3$ CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	$1.6 \times 10^3$ CFU/ml
	0 CFU/ml	0 CFU/ml	$2.8 \times 10^5$ CFU/ml
<i>Enterococcus faecalis</i>	0 CFU/ml	0 CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	$4 \times 10^2$ CFU/ml
<i>Enterococcus hirae</i>	0 CFU/ml	0 CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	0 CFU/ml
	0 CFU/ml	0 CFU/ml	0 CFU/ml

(\*) Colony counts of target organism present in suspension after applied treatment

## Discussion

The SE field strain was more resistant to Cid 20, D 50 and Virocid compared to *E. coli* and *Enterococcus* spp. In general, *E. coli* was more resistant than *Enterococcus* spp.. These findings can be explained by the fact that Gram-negative bacteria are often less sensitive to biocides than Gram-positive bacteria because their outer surface layers consist essentially of LPS and protein-lined diffusion pores. This layer provides a barrier to the penetration of many types of anti-bacterial agents (Fraise et al, 2004).

It is possible that the used SE field strain has acquired resistance to disinfection. In this respect, it would also be interesting to compare the sensitivity of the SE field strain with an ATCC SE strain towards disinfection products.

The disinfection tests have show that none of the studied indicators was equally or less susceptible for the products than SE, although we believe that *E.coli* is still a possible candidate as indicator for the following reasons. First, *E.coli* was more resistant than *Enterococcus* spp. Second, as *E.coli* was more sensitive to disinfection than SE, we can conclude that when *E.coli* is found after cleaning and disinfection, there is a major possibility that SE is also not completely eliminated and might still be present. However, when *E.coli* is not found after cleaning and disinfection, there is still a small chance that SE is present. This is a drawback for *E.coli* as indicator. For this reason further research is necessary. It is important to know if field strains of SE behave in a similar way as ATCC strains and if this is also the case for *E.coli* strains. Meanwhile, also the practical use of *E.coli* as indicator organism will be studied.

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