Environmental monitoring of *Salmonella* Enteritidis on positive layer farms

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Abbreviated title: *Salmonella* Enteritidis on layer farms

Summary

Vaccination of laying hens has proven to reduce the fecal shedding of *Salmonella* Enteritidis (SE) in layers. However, it does not eliminate infection, especially when the environment of the flock is contaminated. The present study describes a longitudinal monitoring of the environmental contamination on two persistently SE contaminated layer farms. At both farms up to 100 places in the three stables, the egg collecting area and the outside environment were sampled on different occasions (during the laying periods and after cleaning and disinfection). Samples were analyzed semi-quantitatively to determine the degree of contamination at the different places. The percentage of contaminated samples at the end of the laying period was quite high for both farms. Cleaning and disinfection following the disposal of the spent hens did not eliminate SE completely, causing the overall percentage of contaminated samples to increase again at the beginning of the laying period of the subsequent flock. Results will be used to take control measures to eliminate the SE contamination.

Keywords: *Salmonella* Enteritidis, environment, layer farms, cleaning and disinfection

Introduction

Since 2004, the total number of human *Salmonella* cases in Belgium is decreasing spectacularly. This decrease is mainly attributed to the reduction of the human SE cases and is most likely caused by the vaccination of laying hens resulting in a reduced fecal shedding of *Salmonella* (Collard et al, 2008). Still, it will be important to focus on the remaining SE positive layer farms to repulse their obstinate contamination.

The objectives of the present study are (i) to determine the prevalence of SE contaminated environmental samples on two farms during the laying period and after cleaning and disinfection (ii) to define by semi-quantitative analysis the degree of SE contamination at the different places.

Materials and Methods

Sample collection

Since July 2008, two persistently SE Belgian layer farms are extensively screened for SE presence. Farm A has three hen houses with cage systems, farm B has also three houses of which two contain cage systems and one contains an aviary. At each sampling occasion (during the laying period and after cleaning and disinfection) about hundred places in the three stables, the egg collecting area and the outside environment were sampled. Surfaces (approx. 0,5 m²) were swabbed using sterile cotton swabs soaked in Buffered Pepton Water (BPW). Samples also consisted of water, feed (25 g), eggs (25 g), feces (25 g), flies, rodent droppings, rodents, dust and air. A volume of 225 ml of BPW was added to each sample. Decimal dilution series were prepared until 10^{-3}, adding consequently 25 ml of each previous dilution to 225 ml BPW.

Isolation and identification of *Salmonella* Enteritidis

Salmonella was isolated according to the ISO 6579-2002 Amd protocol. The serogroup was determined by the Poly A-I - Vi test. A specific PCR targeting the SdfI region was applied to confirm the isolates belonging to the D-serogroup as

SE (Agron et al, 2001; Botteldoorn et al, 2008; Trafny et al, 2006). The Salmonella Enteritidis isolates were phage typed by National Phage Typing Centre of Belgium (Brussels).

Results

In Table 1, the results obtained from July 2008 till May 2009 are summarized per layer farm. The percentage of contaminated samples at the end of the laying period was quite high at both farms, 44% and 22% for farm A and farm B respectively. Cleaning and disinfection following the disposal of the spent hens did not eliminate SE completely; still 5% of the samples on both farms remained positive. At the beginning of the subsequent laying period the percentage of contaminated samples increased to 26% for farm A, followed by a decrease to 3 % in the mid laying period (data farm B are not known yet).

Table 1: Percentage of contaminated samples on both farms (n.k. = not known yet)

Sampling moment	Location	Farm A	Farm B
End laying period	Henhouse 1	35 % (n = 26)	46 % (n = 26)
	Henhouse 2	50 % (n = 22)	7 % (n = 27)
	Henhouse 3	21 % (n = 19)	27 % (n = 26)
	Egg collection area	71 % (n = 7)	20 % (n = 5)
	Others	42 % (n = 12)	8 % (n = 12)
After disinfection	Henhouse 1	9 % (n = 23)	0 % (n = 23)
	Henhouse 2	15 % (n = 20)	5 % (n = 20)
	Henhouse 3	0 % (n = 19)	0 % (n = 20)
	Egg collection area	0 % (n = 10)	/
	Others	0 % (n = 6)	/
Beginning laying period	Henhouse 1	24 % (n = 25)	n.k.
	Henhouse 2	29 % (n = 28)	0 % (n = 22)
	Henhouse 3	0 % (n = 24)	0 % (n = 23)
	Egg collection area	63 % (n = 8)	n.k.
	Others	13 % (n = 8)	n.k.
Mid laying period	Henhouse 1	4 % (n = 25)	n.k.
	Henhouse 2	11 % (n = 26)	n.k.
	Henhouse 3	0 % (n = 24)	n.k.
	Egg collection area	0 % (n = 8)	n.k.
	Others	0 % (n = 11)	n.k.

Some remarkable SE contaminated samples were faeces of a cat and the dog, a fork lift, a brush, crates and clefts in wall and floor. Semi-quantitative analysis has shown that on farm A following places were highly contaminated with SE: faeces of a cat, boots, flies, laying hens faeces, feed and clefts in the wall. On farm B the ceiling, blood lice, the floor, gaps in the floor and the drinking system were highly contaminated. After cleaning and disinfection on farm A, following samples were still SE positive: the feed system, a drain well, clefts in the floor and the floor. On farm B the manure belt was still contaminated.

Phage types isolated on farm A were mainly dominated by PT8, but also PT51, PT7a, PT23, RDNC23, PT28, PT21c and PT13a were found. On farm B especially PT4b was present, but also PT35, PT7 and PT28 occurred.

Discussion

Semi-quantitative analyses of the samples discriminates heavily and less contaminated places or materials. The high prevalence of SE at both farms indicates the need of a regular and sensitive monitoring of persistently SE positive flocks. Phage typing of the SE isolates of both farms demonstrates that different phage types can occur on a layer farm, although there is a main phage type circulating on each farm. Results also demonstrate that the performed disinfection did not lead to a complete elimination of SE, concluding that there is a substantial scope for improving the cleaning and disinfection practices on both farms.

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