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Assessment of cleaning and disinfection in *Salmonella*-contaminated poultry layer houses using qualitative and semi-quantitative culture techniques

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Abstract

Salmonella infection of laying flocks in the UK is predominantly a problem of the persistent contamination of layer houses and associated wildlife vectors by *Salmonella* Enteritidis. Methods for its control and elimination include effective cleaning and disinfection of layer houses between flocks, and it is important to be able to measure the success of such decontamination. A method for the environmental detection and semi-quantitative enumeration of salmonellae was used and compared with a standard qualitative method, in 12 *Salmonella*-contaminated caged layer houses before and after cleaning and disinfection. The quantitative technique proved to have comparable sensitivity to the standard method, and additionally provided insights into the numerical *Salmonella* challenge that replacement flocks would encounter. Elimination of *S.* Enteritidis was not achieved in any of the premises examined although substantial reductions in the prevalence and numbers of salmonellae were demonstrated, whilst in others an increase in contamination was observed after cleaning and disinfection. Particular problems with feeders and wildlife vectors were highlighted. The use of a quantitative method assisted the identification of problem areas, such as those with a high initial bacterial load or those experiencing only a modest reduction in bacterial count following decontamination.

Keywords: *Salmonella*; Poultry; Layers; Eggs; Chickens; Environment; Cleaning and disinfection

1. Introduction

Salmonella enterica serovar Enteritidis came to prominence as a major food-borne pathogen in Europe and America during the 1980s (Hogue et al., 1997; Baumler et al., 2000). It is currently the serovar most commonly isolated from gastrointestinal *Salmonella* infections in the UK (Anon., 2005) and is amongst the most significant serovars in public health elsewhere, including North America (CDC, 2004). Poultry products, especially undercooked and raw eggs, have been a major risk factor for human infection with *S.*

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Enteritidis (Coyle et al., 1988; St Louis et al., 1988; Hogue et al., 1997; Palmer et al., 2000; CDC, 2004; De Buck et al., 2004).

Improved biosecurity and hygiene in the UK poultry industry and vaccination of the majority of commercial laying birds and broiler breeders, introduced in the mid to late 1990s, has been followed by a large reduction in reported incidents of *S.* Enteritidis in poultry and in humans (Anon., 2000). Breeder and multiplier flocks in the UK are generally free of *Salmonella* (Anon., 2004), as biosecurity and monitoring resources at this level in the production chain are considerable. However, the situation is different in production flocks and persistence of contamination on commercial laying farms is currently considered to be the predominant problem (van de Giessen et al., 1994; Davies and Breslin, 2003b). Cleaning and disinfection (C&D) following depopulation of broiler and layer houses has been shown previously to have limited effectiveness in many cases (Davies and Wray, 1995, 1996; Davies et al., 1998; Davies and Breslin, 2003b) with technical issues, the choice of disinfectants and the influence of wildlife vectors being identified as significant factors.

Recently, a semi-quantitative most-probable-number technique has been evaluated for use in the monitoring of *Salmonella* in the poultry house environment (Wales et al., in press). The present report compares this method with an established qualitative method in the assessment of C&D in caged layer houses, and provides a comparison of the relative efficacies of differing C&D regimes.

2. Materials and methods

2.1. Sample collection

The owners of caged layer flocks that had previously been identified through the Zoonoses Order Database, by personal contact, or through previous sampling, as having *S.* Enteritidis were approached. When permission for intensive sampling had been obtained, the flocks were visited and environmental samples were taken. At least two visits were made: once in late lay prior to depopulation and once following depopulation, cleaning and disinfection. One farm was also visited after cleaning but before disinfection. For standard qualitative culture (SS), samples were taken directly into 225 ml of Buffered Peptone Water (BPW: Merck) using gauze surgical swabs (Kleenex Readiwipes: Robinson Healthcare). Samples consisted of approximately 25 g faecal material, floor spillage from under cages, dust from within and around cages (10–15 g), surface swabs, rodent faeces (1–10 g) and flies from adhesive paper or contact insecticidal traps (1–2 g). Sterile swabs soaked in BPW were used to sample the surfaces (0.5 m^2) of egg belts, spiral auger, chain- or hopper-fed feeder troughs, cleaned droppings boards and floors beneath cages, and to swab the interiors of empty cages and spillage cups or troughs beneath nipple lines, where composite samples were obtained from eight cages per swab. Similar sites were sampled for semi-quantitative culture (QS) on the same occasions. For this method, bulked faeces (approximately 30 g) and dry environmental samples were collected into dry pots, and surface swabs from 0.1 m^2 surface area of equipment were deposited in 90 ml of chilled BPW.

All solid samples were returned to the laboratory under ambient conditions on the day of collection and processed immediately. Swab samples taken into BPW were kept in a cold box at below 10 °C and also processed on return to the laboratory. Mouse and rat carcasses were collected as available on four occasions and transported to the laboratory where the whole of the liver, spleen and intestines was removed aseptically and chopped with scissors for culture.

2.2. Standard culture technique

Samples in BPW were pre-enriched at 37 °C for 18 h and then cultured on selective and isolation media as for the QS technique. Representative *Salmonella* isolates were confirmed by complete serotyping at the *Salmonella* reference laboratory at VLA, Weybridge according to the Kaufmann-White Scheme (Popoff, 2001).

2.3. Semi-quantitative culture technique

Faeces (10 g) were weighed and mixed with 90 ml BPW. A 10 ml aliquot of this primary preparation was dispensed into a universal container to serve as the first in a series of 10-fold dilutions. The series was continued by taking 1 ml from the primary preparation and mixing with 9 ml BPW and a decimal dilution series was completed by successively repeating this step five