INVESTIGATION OF THE ORIGIN AND ATTEMPTED CONTROL OF *SALMONELLA* ENTERITIDIS PT6 INFECTION IN TABLE EGG PRODUCTION

Robert H. Davies, Ernesto Leibana and Mark F. Breslin
Veterinary Laboratories Agency-Weybridge, Department of Food and Environmental Safety, New Haw, Addlestone, Surrey KT15 3NB

Introduction

Ovarian or vertical transfer of infection from breeding hens to progeny has been an important aspect of the epidemiology of *Salmonella enterica* serovar Enteritidis (*S.*Enteritidis) infection within the poultry industry. Although both poultry meat production and egg production have been involved in *S.*Enteritidis infection in humans, contaminated eggs are currently thought to be the biggest risk in the UK (ACMSF, 2001). There is little published work relating to the layer breeder sector. This paper describes studies on the distribution and attempted control of *S.*Enteritidis PT6 at various stages in layer breeding and commercial egg production.

Material and Methods

S.Enteritidis PT6 in the hatchery, breeder rearing, parent and commercial rearing farms was identified from notifications to the Zoonoses Order Database. *Salmonella* on the commercial laying farm was identified by follow up but not by routine monitoring. 200-400 samples were taken at each farm. The samples were taken directly into 225 ml of buffered peptone water (BPW) using gauze surgical swabs and consisted of approximately 25 g faecal material or 10 to 15 g dust or other dry environmental samples or surface swabs.

The BPW was returned to the laboratory under ambient conditions on the day of collection and incubated at 37^{o}C for 18 hours. Subsequent culture was in Diasalm (41.5^{o}C, 24/48 h) and Rambach Agar (37^{o}C, 24 h). Isolates were confirmed biochemically and serologically. Genetic typing of *Salmonella* was carried out using a combination of plasmid profile analysis, pulsed field gel electrophoresis and PsH.Sph1 Ribotyping as previously described (Leibana et al, 2001).

Results

The layer breeder hatchery was visited on three occasions after hatching the last eggs from the breeding flock which was infected with *S.*Enteritidis PT6 (PT6) had been terminated. At the first visit significant contamination was found in samples from hatcher incubators (4/96 [7.1%]), chick handling areas (9/58 [15.5%]), tray wash machine and surroundings (6/32 [18.7%]) and waste handling areas (8/9 [88.9%]). This contamination resulted from overdilution of disinfectants by inaccurate metering devices in the power washer and tray wash machine. Once these faults were corrected repeat sampling four weeks later identified only three *Salmonella* isolates from 340 samples and these were in waste disposal areas only. A third visit found only one isolate, in the footwell of a chick delivery vehicle, and this was *S.*Typhimurium DT208, which is normally associated with pigs.

Samples were taken before and after cleaning and disinfection in the infected breeding farm which previously supplied eggs to the hatchery and in two rearing farms. A low level of contamination was found prior to cleaning but after disinfection and fogging with formaldehyde based disinfectants little PT6 was found and there was no reoccurrence of infection in subsequently housed flocks.

PT6 was identified in chicks orginating from the contaminated hatchery in four of six commercial layer rearing houses. After two rounds of fluoroquinolone/competitive exclusion (FQ/CE) treatment no *Salmonella* was found in 60 faecal and environmental samples from each of five of the houses taken when birds were 16 weeks but in the sixth house no *Salmonella* was found in 48 bird level samples but eight of twelve dust samples contained PT6. Sampling carried out after cleaning and disinfection in this house showed significant residual contamination (2/53 floor surfaces, 4/34 ventilation ducts, 3/14 service area floor, 10/40 house surroundings). The house was redisinfected and no *Salmonella* was found in any of the subsequently housed flocks at 16 weeks of age.

Results of sampling four flocks after placement of laying birds from the pullet house where dust was found positive in a large cage laying house are shown in the table. Initially *S.*Enteritidis PT4 predominated in the cage house after further FQ/CE treatment but later in the life of the flock PT6 had increased. Both PTs survived cleaning and disinfection, which was carried out poorly, using a peroxygen disinfectant, and despite vaccination with killed (second flock) and live *S.*Enteritidis vaccines (third and fourth flocks) infection persisted. The administration of competitive exclusion to the third and fourth flocks after placement was also unsuccessful.

Samples taken from the five other houses on site showed similar, but higher, levels of infection with PT6 and similar lack of efficiency of cleaning and disinfection, vaccination and competitive exclusion. During this study all laying houses were sampled several times and *S.*Enteritidis PT6 had spread to these and persisted in each of the houses despite similar interventions.

Molecular fingerprinting of PT6 and other strains associated with the breeding company showed that two genotypes of PT6 were originally present in an independent breeding farm which has been contracted to the breeding company and this was thought to be the original source of the PT6 for the breeding company. One of these genotypes was not found elsewhere in the company, but one type was also found in the hatchery, rearing and breeding sites. Another related genotype of PT6 was also found in the rearing sites, breeding site, hatchery and commercial pullet rearing and cage layer farms. A further type was only present in the rearing site. PT3, 34 and 25 strains from the hatchery were all the same genotype, which was closely related to the PT6s, whereas one PT4 strain found in the hatchery was quite

Contents

distinct. The PT7 strain was closely related to the PT6 found on the rearing site.

Discussion

The work carried out in this study demonstrated the potential for contamination and cross-contamination in hatcheries and also the mistakes which can be made when too much reliance is placed on disinfectant metering devices. Correction of this problem rapidly curtailed the contamination issue but in other hatcheries contamination of incubators has been a much greater problem. This was not the case here because of routine use of formaldehyde evaporation during hatching and the reduced ability of *S.*Enteritidis to permanently colonise equipment, hatcheries and feedmills. Disinfection with formaldehyde based disinfectants was successful on breeding, and breeder rearing farms but peroxygen products were less successful on the pullet rearing farm and, in particular, on the cage farms. This correlates with previous experiences in which peroxygen disinfectants appear to be readily inactivated by residual organic matter. This can be partially overcome if they are used at high concentrations of 2-5% but then they become excessively corrosive for metallic equipment.

The persistence of *S.*Enteritidis in the cage layer houses is consistent with other problem farms operated as multistage sites. In these cases the combination of inadequate cleaning and disinfection, poor control of farm pests and presence of other infected flocks on site facilitates early infection of incoming pullets despite vaccination with killed bacterin or live *S.*Enteritidis or *S.*Gallinarum vaccines. The use of competitive exclusion in this case was not helpful but because the large houses were repopulated in stages administration to some birds was delayed for several days after exposure to infection.

Use of molecular typing for individual phage types of *S.*Enteritidis is difficult as these are highly clonal but the combined approach used in the study provided sufficient discrimination to demonstrate distribution of the organism via the breeding company and also diversification of genotypes, which also occurred with phage types in the hatchery, during the course of the outbreak.

Acknowledgements

This work was funded by Defra. The authors would also like to thank management and staff in the various poultry companies who facilitated this study.

References

ACMSF (2001) Advisory Committee on the Microbiological Safety of Food : Second Report on *Salmonella* in eggs. London : Stationery Office.

Liebana, E., Garcia-Migura, L., Breslin, M.F., Davies, R.H. and Woodward, M.J. (2001) Diversity of strains of *Salmonella enterica* serotype *Enteritidis* from English Poultry Farms assessed by multiple genetic fingerprinting. *Journal of Clinical Microbiology*, **39**, 154-161.

Distribution of Salmonella in cage layer house containing previously infected pullets

	Ends of Egg Belts	Dust	Floor beneath Cages	Bulked faeces/ Droppings belts	Egg Belts	Mouse Droppings	Rat Droppings	Flies
4th week post move*	3/42 (7.1)^{a}	3/28 (10.7)^{a}	1/14 (7.1)^{b}	1/98 (1.0)^{b}	0/14	0/1	0/4	0/1
Last month of lay	3/28 (10.7)^{a1,c2}	7/28 (25.0)^{a3,c2,d2}	8/42 (19.0)^{a4,c3,d1}	4/84 (4.8)^{a1,c3}	-	-	1/1^{c}	1/2 (50.0)^{c}
Post C&D	1/28 (3.6)^{c}	cages 1/56 (1.8)^{c}	12/84 (14.3)^{a2,c10}	17/42 (40.5)^{a1,c16}	0/28	0/5	0/1	0/4
Second Flock:								
Last month of lay	25/28(89.3)^{ace}	48/56(85.7)^{acf}	49/56(87.5)^{acef}	23/28(82.1)^{ace}	4/11(36.4)^{ae}	1/1^{a}	-	-
Post C&D	-	cages 1/33(3.0)^{a}	16/35(45.7)^{ac}	5/12(41.7)^{ac}	1/23(4.3)^{c}	0/1	1/1^{a}	-
Third Flock:								
Last month of lay	11/15 (73.3)^{acg}	10/15 (66.7) ^{acg}	10/10 (100.0) ^{acg}	4/20 (20.0)^{c}	-	-	-	-
Post C&D	-	cages 0/5	2/15 (13.3)^{c}	1/5 (20.0)^{c}	0/5	-	-	-
Fourth Flock:								
8 weeks after placement†	2/9 (22.2)^{ac}	2/10 (20.0)^{c}	0/5	3/15 (20.0)^{ac}	-	-	0/1	-

Key: * After fluoroquinolone/competitive exclusion treatment; † After chlortetracycline treatment; ^{a} *S.* Enteritidis PT4; ^{b} *S.* Typhimurium DT193; ^{c} *S.* Enteritidis PT6; ^{d} *S.* Agona; ^{e} *S.* Indiana; ^{f} *S.* Mbandaka; ^{g} *S.*Enteritidis PT7; - not sampled; number after superscript = no. of isolates of each serovar/phage type

Contents