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Observations on the distribution and control of *Salmonella* in commercial broiler hatcheries in Great Britain

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Abstract

Salmonella can enter hatcheries via contaminated eggs and other breaches of biosecurity. The study examined the prevalence and distribution of *Salmonella* in commercial hatcheries and assessed the effects of providing advice on *Salmonella* control. Intensive swab sampling was performed throughout 23 broiler hatcheries in Great Britain (GB). Swabs were cultured using a modified ISO6579:2017 method. After each visit, tailored advice on biosecurity and cleaning and disinfection procedures was provided to the hatchery managers. Repeat sampling was carried out in 10 of the 23 hatcheries. *Salmonella* prevalence ranged between 0% and 33.5%, with the chick handling areas, hatcher areas, macerator area, tray wash/storage areas, external areas and other waste handling areas being more contaminated than the setter areas. *Salmonella* Senftenberg and *Salmonella* 13,23:i:- were the most commonly isolated serovars. There was a reduction in *Salmonella* prevalence at the second visit in eight out of 10 premises, but prevalence values had increased again in all of the improved hatcheries that were visited a third time. One hatchery harboured a difficult-to-control resident *Salmonella* 13,23:i:- strain and was visited six times; by the final visit, *Salmonella* prevalence was 2.3%, reduced from a high of 23.1%. In conclusion, the study found low-level *Salmonella* contamination in some GB broiler hatcheries, with certain hatcheries being more severely affected. Furthermore, it was shown that *Salmonella* typically is difficult to eradicate from contaminated hatcheries, but substantial reductions in prevalence are possible with improvements to biosecurity, cleaning and disinfection.

KEYWORDS
broiler, eggs, hatchery, poultry, *Salmonella*

Claire E. Oastler and Christopher Nichols should be considered joint first author.

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1 | INTRODUCTION

Salmonella is a food-borne pathogen of global significance. Worldwide, over 90 million enteric infections and 150,000 diarrhoeal deaths have been attributed to it each year (Majowicz et al., 2010), and salmonellosis is the second most commonly reported zoonosis in the European Union (EU) (EFSA & ECDC, 2021). Wildlife is a reservoir for *Salmonella*, and environmental contamination can also arise from the disposal of poultry litter, wash water and abattoir waste (Maurischat et al., 2015; Mughini-Gras et al., 2014). Poultry meat, in particular from the broiler sector, is frequently implicated as a source of *Salmonella* infection in people (Bryan & Doyle, 1995; Hird et al., 1993; Pires et al., 2014).

Previously, *Salmonella enterica* subsp. *enterica* serovar Enteritidis (SE) was a common problem in the broiler breeding and production sectors in Great Britain (GB) (Davies et al., 1997), but more recently the control of SE and another regulated serovar, *S*. Typhimurium (ST), has been very successful. From National Control Programme data, in 2019 the estimated prevalence of regulated serovars in breeding and broiler flocks was well below the EU target of 1% (APHA, 2020). This has been the result of generally good biosecurity combined with widespread use of vaccination against SE and ST in broiler parent flocks (APHA, 2015; Lane et al., 2014; Majowicz et al., 2010; Marier et al., 2014; O'Brien, 2013).

Despite the advent of EU-wide National Control Programmes for *Salmonella*, poultry meat remains the food product in which *Salmonella* is most often detected (EFSA & ECDC, 2021). Sources of *Salmonella* contamination for broiler flocks include hatcheries (Heyndrickx et al., 2002, 2007), breeding farms (Wierup et al., 2017) and feed mills, with some serovars in tracking studies being isolated from both hatcheries and either carcasses or finished meat products (Bailey et al., 2002; Bhatia & McNabb, 1980; Kim et al., 2007; Wales & Davies, 2020). Thus, *Salmonella* contamination in chicken meat intended for human consumption may originate in hatcheries.

Salmonella can enter hatcheries from infected breeding flocks, for instance, via faeces on newly laid eggs (Mine et al., 2003). Such contamination may disseminate widely throughout the hatchery (Crabb et al., 2018; Davies et al., 2001; Wilkins et al., 2002), with some hatcheries showing frequent contamination of equipment and/or residence of the pathogen in areas where cleaning and disinfection is more difficult to implement, such as within ventilation systems (Christensen et al., 1997; Cox et al., 1990; Davies et al., 2001; H. Davies & Breslin, 2004).

Once present within hatcheries, horizontal transmission of *Salmonella* can occur via cross-contamination of eggs or chicks (Cox et al., 2000). Hatcher incubators provide a particularly high-risk environment for this (Cason et al., 1994). Despite the commonly practised disinfection of eggs upon arrival at the hatchery, *Salmonella* can persist in viable eggs if it has already penetrated the shell or is present in pores or defects in the shell (Bailey et al., 1996). During the hatching process, one contaminated egg can spread contamination widely to hatched chicks (Mueller-Doblies et al., 2013), with newly hatched chicks being highly susceptible to colonisation by even low doses of *Salmonella* (Milner & Shaffer, 1952). The proportion of infected chicks leaving the hatchery can reach 9%, whereas only 0.01%–0.05% of eggs entering the hatchery are likely to be *Salmonella*-positive (Bailey et al., 1994; Mueller-Doblies et al., 2013).

Impacts

- *Salmonella* contamination in broiler hatcheries was generally found to occur at low levels, although a small number of hatcheries had more substantial *Salmonella* contamination.
- The most commonly isolated serovars were *Salmonella* Senftenberg and *Salmonella* 13:23:i:-.
- The provision of targeted advice on *Salmonella* control given to hatchery managers was initially successful in reducing the overall prevalence of *Salmonella* at follow-up visits, but this reduction was not sustained.

The tendency for the poultry industry to organise itself into fewer and larger enterprises has increased the risk of dissemination of *Salmonella* contamination once it arises in a hatchery (Davies & Breslin, 2004). Effective interventions at early stages of the hatching process in commercial hatcheries could improve the microbial quality of poultry production and, consequently, the safety of food consumed by the public (Rehkopf et al., 2017). Control options in hatcheries include good biosecurity and rigorous cleaning and disinfection programmes (McMullin, 2009).

The ability of some *Salmonella* strains to pass from breeding flocks to progeny, via temporary contamination or long-term colonisation of hatcheries, is poorly understood. Most relevant studies have focussed on *S*. Gallinarum and SE, with many strains of the latter having a strong propensity for vertical transmission via infection of the forming egg (Liljebjelke et al., 2005). Hatcheries are acknowledged to be an important source of other serovars, but many of the risk pathways involved remain uncertain and this has hampered the implementation of effective intervention measures (Sivaramalingam et al., 2013). The present study aimed to investigate the *Salmonella* status of 23 GB broiler hatcheries and to investigate the changes in prevalence and distribution of *Salmonella* in contaminated hatcheries after advice on its control was provided.

2 | MATERIALS AND METHODS

2.1 | Hatchery selection and sample collection

Twenty-three premises, representing all the commercial-scale broiler chicken (*Gallus gallus)* hatcheries operating in GB at the time of the study, were sampled. Hatcheries were coded H01 to H23. The visits took place between August 2016 and September 2019, with each hatchery being sampled at least once. Ten of these 23 hatcheries were selected for longitudinal sampling to assess the impact

of advice on the control of *Salmonella*, and these hatcheries were visited between a further one to five times, depending on individual circumstances. In total, two visits were made to H03, H04, H09, H10 and H11, three visits were made to H05, H07, H13 and H14, and six visits were made to H01. Thirteen hatcheries were not included in the longitudinal study based on initial *Salmonella* prevalence and historic data indicating there was unlikely to be a significant problem with resident *Salmonella* contamination.

At each visit, intensive sampling was performed, covering the following areas and categories: egg handling areas, egg transfer areas, setter (incubator) areas, hatcher (incubator) areas, chick handling areas, cleaning tools, tray wash/tray store areas, macerator area, service areas, the area outside the hatchery (external areas) and other waste handling areas (areas that did not easily fit into another category). Samples were collected from occupied hatchers and setters during incubation and, where possible, after C&D. The egg handling area is where the eggs are received and stored for short periods of time. Eggs are then automatically turned in incubators for 18 days in the setter incubator area. In multi-stage setters, eggs can be of different ages and from multiple breeding flocks. After 18 days, the eggs are transferred from a setter to a hatcher, where they are incubated for a further three days by which time all fertile eggs will normally have hatched. The chick handling area is where chicks are moved into transport crates and dispatched to their destination farm. In the tray wash/tray store areas, the egg trays, trolleys, chick hatcher baskets and farm chick delivery baskets are washed and stored. Cleaning tools included any piece of equipment used to clean the hatchery such as floor cleaners or brushes. All debris from the hatchery is taken to waste areas and is normally macerated and deposited in an outside skip or tanker prior to dispatch for rendering. Samples collected from the macerator area were categorized separately from other waste handling areas. Outside the hatchery, lorry wash areas and pooled water were targeted for 'external' samples.

Ethical review was not required for the sampling or subsequent procedures. No handling or sampling of animals was performed, and no hatchery procedures for handling fertile eggs or birds were affected by sampling visits.

2.2 | Testing methods

The number of samples collected ranged from 108 to 421 per visit. The number varied according to the size and complexity of the hatchery and accessibility of the areas of interest on the day of the visit. Follow-up visits aimed to replicate the previous sampling where possible. Samples were collected with large (900 cm^2), sterile, moist, hand-held gauze swabs, used to swab 0.5 m^2 of the area of interest and then placed into 225 ml of pre-enrichment culture media (Buffered Peptone Water [BPW]; Merck 10.07228.0500).

Swab samples were tested for the presence of *Salmonella* using a modified version of ISO6579:2017. Briefly, swabs were incubated in BPW at 37 ± 1°C for 16 to 20 hr and then 0.1 ml of the BPW was inoculated onto modified semi-solid Rappaport-Vassiliadis agar (MSRV; Mast DM440D), with the addition of 1 mg/ml of novobiocin (Sigma N1628; Sigma-Aldrich Company Ltd). The MSRV was incubated at 41.5 ± 1°C for 24 ± 3 hr. Spreading growth on MSRV was sub-cultured onto Rambach agar (Merck 1.07500.0002) and incubated at 37 ± 1°C for 24 ± 3 hr. Slide agglutination tests were carried out on suspect colonies to confirm positive results. All *Salmonella*-positive samples were serotyped by the *Salmonella* reference laboratory at the Animal and Plant Health Agency (APHA), Weybridge, according to the White-Kauffmann-Le Minor serotyping scheme (Grimont & Weill, 2007).

2.3 | Provision of advice

After each visit, tailored advice was provided to the hatchery manager in the form of a written report. The advice focussed on biosecurity and hygiene measures as observed during the visit, and how this related to *Salmonella* control. Advice was provided on disinfectant usage, cleaning and disinfection procedures, the hatchery's workflow and waste management procedures. During follow-up visits, any changes or issues with the implementation of advice were noted and described in the next report.

2.4 | Statistical analysis

All statistical analyses were conducted using R x64 3.5.1 (R Core Team, 2018). *Salmonella* prevalence was defined as the number of samples positive for *Salmonella* divided by the total number of samples collected. To analyse differences in *Salmonella* prevalence between visits, generalised linear models were used with a binomial error structure to account for the inherent issues of using proportion data (boundedness, non-constant variance and non-normal errors). The random effect used was hatchery ID. To analyse the effect of sample category (i.e. location of a sample from within the hatchery) on the percentage of *Salmonella*-positive samples, a generalised linear mixed-effects model was used to avoid temporal pseudoreplication, again with a binomial error structure. The random effects used were hatchery ID, nested within visit number.

3 | RESULTS

3.1 | *Salmonella* isolations by the hatchery and serovar

Across the 41 sampling visits to 23 hatcheries, 14 different *Salmonella* serovars were found. The proportion of positive samples at the initial sampling visit for all 23 hatcheries is summarised in Table 1, the proportion of positive samples for the nine hatcheries visited more than once is summarised in Table 2, and the *Salmonella* serovars identified at the *Salmonella*-contaminated hatcheries at all

sampling visits are summarised in Table 3. The prevalence of positive samples ranged from 0% to 33.5% between hatcheries. Across all 23 hatcheries sampled, overall *Salmonella* detection was 8.5% of samples. At least one *Salmonella* serovar was isolated from 18 out of the 23 (78%) hatcheries visited, whilst more than one serovar was recovered from 10 hatcheries (43%). From one hatchery (H10), seven different serovars were isolated over two visits.

The following *Salmonella* serovars were isolated on more than one occasion: *S*. 13,23:i:- (five hatcheries, 14 visits and 493 isolations), *S*. Senftenberg (seven hatcheries, 13 visits and 238 isolations), *S*. Mbandaka (four hatcheries, six visits and 217 isolations), *S*. Montevideo (four hatcheries, five visits and 78 isolations), *S*. Kedougou (three hatcheries, three visits and seven isolations), *S*. Livingstone (two hatcheries, four visits and 46 isolations), and *S*. O-rough:g,s,t:- (two hatcheries, two visits and two isolations). The following serovars (± phage types) were each isolated from a single hatchery only: *S*. Derby, *S*. Idikan, SE PT8, ST DT41, ST DT193, *S*. 6,7:-:- and *S*. 6,7:Z10:-.

3.2 | *Salmonella* isolations by area within hatcheries

Findings are illustrated in Figure 1. After the random effects of individual hatchery and sampling time point were taken into account, there was significant variation in *Salmonella* prevalence relating to the sample origins. Setter areas were used as the reference as the greatest number of samples were collected from this area. Samples taken from the setter areas were significantly more likely ($p < .05$) to be positive for *Salmonella* compared with egg handling areas and egg transfer areas and significantly less likely ($p < .05$) to be positive than many other hatchery locations (chick handling areas, hatcher areas, the macerator area, tray wash/stores areas, external areas and other waste handling areas). No significant difference was identified in *Salmonella* prevalence between the setter areas and the service areas or cleaning tools. Contamination was often found after C&D in those hatcheries with significant contamination problems, particularly within ventilation ducting and fan belt ducting within hatchers. Visits to hatcheries where no *Salmonella* was detected in any area were not included, as these did not add explanatory power to analysis of *Salmonella* distribution within hatcheries.

3.3 | Impact of advice provided to hatcheries

The interval between follow-up visits ranged from two months to two years (average 8.5 months). The length of time between the first and second visits ranged from 2 to 24 months, and between 2 and 11 months for the second and third visits. H01 was visited more often than other hatcheries because of a resident strain of *S*. 13,23:i:- that proved difficult to control. After repeated advice on improvements and changes to cleaning and disinfection protocols, the overall prevalence of *Salmonella* was reduced to 2.3%, after a peak of 23.1% on the second visit.

In the 10 hatcheries that received follow-up sampling visits plus advice on cleaning and disinfection, and considering each hatchery premises separately, there was a significant reduction in *Salmonella* prevalence between the first and second visits (14.4% and 8.7%, respectively; $p < .001$). However, at the third visit a significant increase was seen (17.2%, $p < .001$), compared with both the earlier visits. This pattern is illustrated in Figure 2.

4 | DISCUSSION

The present study describes the *Salmonella* status of 23 broiler hatcheries and the changes in prevalence and distribution of *Salmonella* after the provision of tailored advice. The consolidation of broiler companies has resulted in fewer broiler hatcheries in GB, and the study includes all of the commercial-scale hatcheries owned by integrated companies and independent hatcheries. It is therefore likely to be a representative picture of the overall status of *Salmonella* contamination in broiler hatcheries at the time of the study.

Salmonella was isolated at the first visit from 18 hatcheries (78%). The frequency of contamination and the serovars isolated varied substantially between hatcheries. At the first visits, *Salmonella* was isolated from 8.5% of samples overall. This showed that *Salmonella* can be commonly detected in broiler hatcheries by intensive sampling but at relatively low frequencies. However, some hatcheries had a more severe *Salmonella* problem, as 11 visits to six different hatcheries resulted in 10.8%–33.5% positive samples per visit. Factors that may influence the risk of *Salmonella* contamination include the size (i.e. production volume) of the hatchery (Volkova et al., 2011; Withenshaw et al., 2021), the standard of hatchery hygiene management (EFSA Panel on Biological Hazards et al., 2019), the *Salmonella* status of the supplying breeder flock(s) (Sivaramalingam et al., 2013) and the periodic purchase of imported eggs to satisfy peaks in demand (Racicot et al., 2020).

Although earlier studies into *Salmonella* in GB broiler hatcheries sampled fewer premises, the present data are consistent with these previous reports. A study of two integrated broiler companies found 15.4% of samples positive for *Salmonella* in five visits to two hatcheries (Davies et al., 2001). An investigation into an integrated broiler breeder organisation found an average of 45.6% *Salmonella*-positive samples over two visits to a single hatchery (Davies et al., 1997). The latter study was part of an investigation into persistent SE infections prior to the introduction of vaccination or the National Control Programme, and therefore, the comparatively high *Salmonella* isolation frequency is unsurprising. There has been little research on control of *Salmonella* in GB broiler production for over 15 years, during which time the industry has changed dramatically, including consolidation of smaller companies into larger integrations which utilise fewer but larger hatcheries. However, many longstanding issues associated with *Salmonella* control, such as control of dust and fluff, disinfection of surfaces and equipment and handling of waste remain problematic (Davies & Wray, 1994). The present results indicate that

TABLE 1 Proportion of *Salmonella*-positive samples from surface swabs at 23 hatcheries on first visits

	Positive samples/samples taken											
Hatchery code	Egg areas	Egg transfer areas	Setter areas	Hatcher areas	Chick handling areas	Macerator area	Service areas	Cleaning tools	Trays: wash & store areas	Other waste handling areas	External	Overall %
H01	2/31 (7%)	1/25 (4%)	11/142 (8%)	35/100 (35%)	12/33 (36%)	6/7 (86%)	0/12	3/4 (75%)	2/6 (33%)	2/2 (100%)	-	20.4
H02	0/28	0/13	0/69	0/66	0/76	0/16	0/15	0/8	0/37	0/2	0/12	0.0
H03	0/27	1/34 (3%)	6/105 (6%)	10/85 (12%)	0/46	0/6	0/8	1/6 (17%)	1/32 (3%)	0/5	0/6	5.3
H04	0/30	0/9	0/76	4/34 (12%)	1/20 (5%)	2/2 (100%)	0/4	0/7	1/26 (4%)	-	-	3.8
H05	0/9	-	2/51 (4%)	6/53 (11%)	1/23 (4%)	1/2 (50%)	-	0/7	0/8	0/3	1/3 (33%)	6.9
H06	0/22	0/16	0/76	0/96	0/29	0/6	0/10	0/3	0/26	-	1/2 (50%)	0.3
H07	0/30	0/22	0/59	11/55 (20%)	0/49	0/3	0/7	1/7 (14%)	0/33	0/3	5/14 (36%)	6.0
H08	0/22	0/15	0/43	0/57	0/38	0/3	1/21 (5%)	0/21	0/17	0/6	0/4	0.4
H09	0/17	0/7	0/93	5/86 (6%)	10/18 (56%)	5/5 (100%)	1/8 (13%)	0/3	8/35 (23%)	3/5 (60%)	0/19	10.8
H10	1/23 (5%)	0/10	32/155 (21%)	58/129 (45%)	21/38 (55%)	8/8 (100%)	0/4	5/14 (36%)	5/17 (29%)	5/5 (100%)	-	33.5
H11	1/62 (2%)	1/18 (6%)	37/78 (47%)	18/97 (19%)	2/40 (5.0%)	0/4	0/16	2/10 (20%)	0/25	1/8 (13%)	-	17.3
H12	0/17	0/22	0/108	3/160 (2%)	0/43	0/6	-	0/10	0/24	0/4	0/6	0.8
H13	0/21	0/11	0/64	0/51	26/59 (44%)	6/9 (67%)	0/12	0/11	5/15 (33%)	-	-	14.6
H14	0/15	0/8	1/33 (3%)	0/18	0/14	3/4 (75%)	0/5	0/2	2/4 (50%)	2/4 (50%)	0/1	7.4
H15	0/15	0/13	0/121	13/86 (15%)	3/50 (6%)	0/9	0/21	0/11	0/51	0/3	0/17	4.0
H16	0/34	0/23	0/85	16/100 (16%)	3/54 (6%)	0/13	4/10 (40%)	0/7	2/63 (3%)	0/5	1/4 (25%)	6.5
H17	0/28	0/24	0/57	0/68	0/39	0/8	0/29	0/10	0/68	0/4	0/6	0.0
H18	0/29	0/4	0/86	0/85	0/43	0/6	0/25	0/4	0/71	0/2	0/5	0.0
H19	0/20	1/30 (3%)	16/119 (13%)	6/109 (6%)	0/35	5/11 (46%)	1/14 (7%)	0/3	0/49	0/2	0/3	7.3
H20	0/22	-	0/36	0/35	0/66	0/12	0/30	0/5	0/32	0/14	0/13	0.0
H21	0/31	0/31	0/90	0/159	0/41	0/6	0/6	0/9	0/31	0/11	0/6	0.0
H22	0/33	0/18	0/105	2/73 (3%)	0/43	0/7	0/33	0/10	0/22	0/2	1/3 (33%)	0.9
H23	0/56	0/56	0/50	0/67	0/37	0/12	-	0/9	0/57	0/3	1/11 (9%)	0.3

TABLE 2 Proportion of *Salmonella*-positive samples from surface swabs at all hatcheries visited more than once

Hatchery (H) and visit (V) codes		Positive samples/samples taken											
		Egg areas	Egg transfer areas	Setter areas	Hatcher areas	Chick handling areas	Macerator area	Service areas	Cleaning tools	Trays: wash & store areas	Other waste handling areas	External	Overall %
H01	V1	2/31 (7%)	1/25 (4%)	11/142 (8%)	35/100 (35%)	12/33 (36%)	6/7 (86%)	0/12	3/4 (75%)	2/6 (33%)	2/2 (100%)	-	20.4
	V2	3/31 (10%)	0/25	7/142 (5%)	36/93 (39%)	24/31 (77%)	6/7 (86%)	1/12 (8%)	0/4	3/4 (75%)	1/2 (50%)	-	23.1
	V3	0/26	1/20 (5%)	13/135 (10%)	30/102 (29%)	13/38 (34%)	5/7 (71%)	0/4	0/4	6/38 (16%)	3/4 (75%)	5/12 (42%)	19.5
	V4	0/19	0/19	0/126	3/107 (3%)	2/39 (5%)a	3/7 (43%)	0/2	0/6	14/53 (26%)	7/8 (88%)	6/10 (60%)	8.8
	V5	0/20	0/19	2/125 (2%)	30/99 (30%)	25/42 (60%)	7/7 (100%)	0/2	0/7	9/54 (17%)	2/8 (25%)	3/9 (33%)	19.9
	V6	0/25	0/19	0/119	0/97	0/51	0/5	0/2	0/9	0/43	3/13 (23%)	6/11 (55%)	2.3
H03	V1	0/27	1/34 (3%)	6/105 (6%)	10/85 (12%)	0/46	0/6	0/8	1/6 (17%)	1/32 (3%)	0/5	0/6	5.3
	V2	0/23	0/22	1/107 (0.9%)	2/102 (2%)	1/74 (1%)	0/7	0/4	0/8	0/45	1/10 (10%)	2/14(14%)	1.7
H04	V1	0/30	0/9	0/76	4/34 (12%)	1/20 (5%)	2/2 (100%)	0/4	0/7	1/26 (4%)	-	-	3.8
	V2	0/41	0/13	0/82	7/41 (17%)	1/49 (2%)	0/6	0/3	0/10	3/32 (10%)	0/10	3/16 (19%)	4.6
H05	V1	0/9	-	2/51 (4%)	6/53 (11%)	1/23 (4%)	1/2 (50%)	-	0/7	0/8	0/3	1/3 (33%)	6.9
	V2	0/7	-	0/70	1/106 (0.9%)	0/20	0/21	0/9	0/11	0/22	0/10	-	0.4
	V3	0/6	-	0/79	4/88 (5%)	0/51	5/8 (63%)	0/20	1/4 (25%)	0/20	2/2 (100%)	-	4.3
H07	V1	0/30	0/22	0/59	11/55 (20%)	0/49	0/3	0/7	1/7 (14%)	0/33	0/3	5/14 (36%)	6.0
	V2	0/32	0/21	0/55	4/81 (5%)	0/35	0/3	2/6 (33%)	1/12 (8%)	0/52	-	1/14 (7%)	2.6
	V3	0/32	3/21 (14%)	1/56 (2%)	26/56 (46%)	20/58 (35%)	3/8 (38%)	2/8 (25%)	5/8 (63%)	13/40 (33%)	-	0/10	24.6
H10	V1	1/23 (5%)	0/10	32/155 (21%)	58/129 (45%)	21/38 (55%)	8/8 (100%)	0/4	5/14 (36%)	5/17 (29%)	5/5 (100%)	-	33.5
	V2	0/25	0/12	71/163 (44%)	27/120 (23%)	11/34 (32%)	8/8 (100%)	-	1/11 (10%)	0/21	4/6 (67%)	0/2	30.3
H11	V1	1/62 (2%)	1/18 (6%)	37/78 (47%)	18/97 (19%)	2/40 (5.0%)	0/4	0/16	2/10 (20%)	0/25	1/8 (13%)	-	17.3
	V2	0/68	0/14	3/74 (4%)	2/99 (2%)	0/37	0/5	0/9	0/10	0/30	2/9 (22%)	-	2.0
H13	V1	0/21	0/11	0/64	0/51	26/59 (44%)	6/9 (67%)	0/12	0/11	5/15 (33%)	-	-	14.6
	V2	0/22	0/18	0/63	1/48 (2%)	5/61 (8%)	7/10 (70%)	0/4	1/10 (10%)	3/17 (18%)	-	-	6.7
	V3	0/22	7/18 (39%)	0/63	8/49 (16%)	30/63 (48%)	7/10 (70%)	1/4 (25%)	1/11 (9%)	12/17 (71%)	-	-	25.7
H14	V1	0/15	0/8	1/33 (3%)	0/18	0/14	3/4 (75%)	0/5	0/2	2/4 (50%)	2/4 (50%)	0/1	7.4
	V2	0/15	0/9	0/34	0/22	0/37	0/5	0/6	0/5	0/3	4/4 (100%)	0/2	2.8
	V3	1/15 (7%)	0/4	0/44	0/22	1/26 (4%)	1/5 (20%)	0/6	0/6	2/3 (67%)	2/4 (50%)	0/2	5.1

TABLE 3 Detail of serovars isolated from all 18 *Salmonella*-contaminated hatcheries

Hatchery (H) and number of visits		Serovar(s) isolated* [visit identifier number(s)]										
		Egg areas	Egg transfer areas	Setter areas	Hatcher areas	Chick handling areas	Macerator area	Service areas	Cleaning tools	Trays: wash & store areas	Other waste handling areas	External
H01	6	Ax [1,2]	Ax [1,3]	Ax [1,2,3,5]	Ax [1,2,3,4,5]	Ax [1,2,3,4,5]	Ax [1,2,3,4,5]	Ax [2]	Ax [1]	Ax [1,2,3,4,5]	Ax [1,2,3,4,5,6]	Ax [3,4,5,6]
H03	2		Livstn [1]	Livstn [1,2]	Livstn [1,2]	Livstn [2]			Livstn [1]	Livstn [1]	Livstn [2]	Senf [2] Mvideo [2]
H04	2				Livstn [1,2]	Livstn [1,2]	Livstn [1]			Livstn [1,2]		Livstn [2]
H05	3			SE PT8 [1] Kedou [1]	SE PT8 [3] Derby [1,2,3]	Derby [1]	SE PT8 [1,3]		Derby [3]		SE PT8 [3]	Kedou [1]
H06	1											Kedou
H07	3		Senf [3]	Senf [3]	Senf [1,2,3] Bx [3]	Senf [3]	Senf [3]	Senf [2,3]	Senf [1,2,3]	Senf [3]		Senf [2] Kedou [1] ST DT41 [1]
H08	1							Senf				
H09	1				Senf	Senf	Senf			Senf	Senf	
H10	2	Mvideo [1]		Senf [1,2] Mvideo [1] Mban [1,2] Cx [2] Dx [2]	Senf [1,2] Mvideo [1,2] Mban [1] Bx [2]	Senf [1] Mvideo [1] Mban [1]	Senf [2] Mban [1,2]		Senf [2] Mvideo [1] Mban [1]	Mban [1]	Senf [2] Mban [1,2] Mvideo [2] Ax [2]	
H11	2	Mban [1]	Mban [1]	Mban [1,2]	Senf [1,2]	Senf [1]			Senf [1] Mban [1]		Mban [1,2]	
H12	1				Senf							
H13	3		Ax [3]		Ax [2,3]	Ax [1,2,3] Idikan [3]	Ax [1,2,3]	Ax [3]	Ax [2,3]	Ax [1,2,3]		
H14	3	Ax [3]		Ax [1]		Ax [1]	Ax [1,3]			Ax [1,3]	Ax [1,2,3]	
H15	1				Mvideo	Mvideo						
H16	1				Mvideo	Mvideo		Mvideo		Mvideo		Mvideo
H19	1		Mban	Mban	Senf		Mban	Mban				
H22	1				Mban							ST DT193
H23	1											Ax

**Salmonella* serovars: 'Ax' = 13,23:i:-, 'Bx' = O-rough:g,s,t:-, 'Cx' = 6,7:-:-, 'Dx' = 6,7:Z10:-, 'Kedou' = Kedougou, 'Livstn' = Livingstone, 'Mban' = Mbandaka, 'Mvideo' = Montevideo, 'Senf' = Senftenberg, 'SE PT8' = Enteritidis PT8, 'ST DT41' = Typhimurium DT41, 'ST DT193' = Typhimurium DT193.

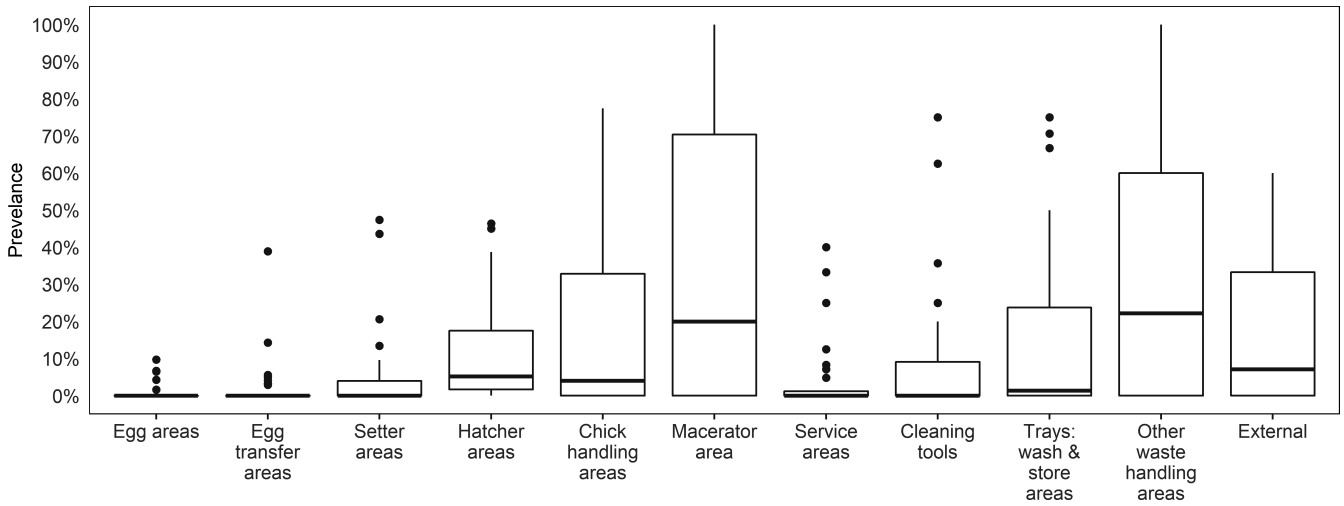

FIGURE 1 *Salmonella* from surface swabs: prevalence values by location, collected from all hatcheries at visits where *Salmonella* was detected from at least one swab. Box shows median and interquartile range (IQR). Whiskers show values up to 1.5 times above IQR. Data beyond the end of the whiskers are outliers and are plotted individually.

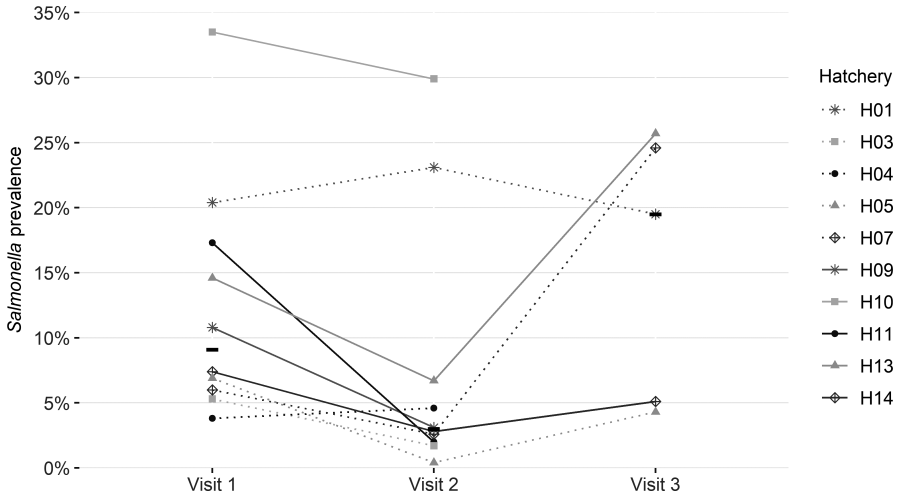

FIGURE 2 Overall *Salmonella* prevalence values from the first, second and third visits (H01, H05, H07, H11 and H13 only) for each hatchery visited more than once. Visits 1 and 2 were to 10 hatcheries and visit 3 was to 5 hatcheries. Compared with initial visits, *Salmonella* prevalence was significantly lower at second visits ($p < .001$), but significantly higher at the third visits ($p < .001$). The median *Salmonella* prevalence percentage across all hatcheries at each visit is represented by the three short black lines

broiler hatcheries still pose a risk for *Salmonella* dissemination in the broiler industry.

Recent studies of broiler hatcheries in other countries echo the *Salmonella* problems identified by the GB data. *Salmonella* was isolated from 4.3% of samples taken in two broiler hatcheries in China (Ren et al., 2016) and 34% of samples from five broiler hatcheries (including two broiler breeder hatcheries) in Korea (Ha et al., 2018). This highlights the variability of *Salmonella* contamination in broiler hatcheries, which was also reflected in our results. By contrast, data from the Dutch monitoring and control program for *Salmonella* showed an overall positive frequency of just 0.3% samples from chicks leaving the hatchery over a 4-year period (Van Der Fels-Klerx et al., 2008). Whilst this was not the intensive sampling of multiple areas employed in our study, the findings do suggest that extending the *Salmonella* Control Programmes to include sensitive hatchery monitoring has the potential to effect microbiological improvements. Each hatchery should have a HACCP-based hygiene management approach that should be validated and monitored by effective sampling and testing for *Salmonella* and indicator organisms. An effective monitoring programme for *Salmonella* would allow managers to be fully aware of the extent of the *Salmonella* contamination issues within the hatchery and can be based on regular testing of hatcher debris and macerated waste.

Salmonella contamination appears also to be present in hatcheries of other poultry types, at intensities similar to those encountered in the present study. From a survey of five GB duck hatcheries, 9.9% of samples overall were positive across 11 visits, with a slightly higher proportion of samples positive (15.1%) at the first visit (Martelli et al., 2016). A comparable situation was found across eight visits to four GB turkey hatcheries, with *Salmonella* being isolated from 5.1% of samples overall (Mueller-Doblies et al., 2013).

There was a trend for contamination to increase in hatcheries along the line of workflow, from egg areas through to setters and hatchers. An increase in *Salmonella*-positive samples from setters onwards was also found by R. H. Davies et al. (1997). This is consistent with a report by Pradhan et al. (2005) in which it was noted that incubated broiler eggs have an increase in bacterial load between incubation and hatching. Furthermore, eggs contaminated by

Salmonella are likely to contaminate other chicks in the same hatcher when they hatch and release fluff (Cason et al., 1994). This contamination can then carry over to chick handling areas, which is consistent with the correlation seen between the intensity of *Salmonella* recovery from hatchers and chick handling areas observed in the current study.

Hatcher chick crates have also been found to be focal points for contamination (Bailey et al., 2001), often due to inadequacies in cleaning and disinfection of equipment and/or in protocols (Corry et al., 2002). This was also reflected in the present data in which tray wash/stores areas were frequently found to be contaminated, posing a risk for persistence and recycling of *Salmonella* during subsequent hatching cycles. Additionally, samples taken in macerator areas, external hatchery areas and other waste handling areas were more likely to be *Salmonella*-positive than setter areas. Waste areas, such as macerators and skips, concentrate *Salmonella* contamination already present in the hatchery. Furthermore, *Salmonella* can persist in outdoor areas which are subject to less frequent cleaning and disinfection than internal areas. *Salmonella* can be newly introduced into the hatchery environment as a result of contaminated eggs, movement of vehicles and equipment between farms, animal by-product plants and hatcheries. Wild birds which may carry *Salmonella* can also contaminate external areas of the premises, including equipment held outside the building, or potentially loft spaces when roof repairs occur (De Lucia et al., 2018). This highlights the need for high biosecurity at broiler hatcheries.

In addition to the frequency of contamination, a further important aspect of *Salmonella* in the hatcheries is the range of serovars present. The current study and results of operator sampling in many of the hatcheries and broiler flocks supplied by them suggest that some serovars (*S*. 13,23,i:-, *S*. Livingstone, *S*. Derby, *S*. Mbandaka) were persisting in the hatchery environment between sampling visits, see Table 3, but this cannot be confirmed without further subtyping. Serovars resident in hatcheries are often also widely distributed throughout broiler flocks (Liebana et al., 2002). The present investigations recovered 15 different *Salmonella* serovars, with *S*. Senftenberg and *S*. 13,23:i:- being the most common. These serovars, in addition to Mbandaka, Kedougou and Montevideo (which were also found) were consistently in the top five serovars found in broiler flocks in GB during the study period, with the exception of Senftenberg in 2018 (APHA, 2019).

Salmonella Senftenberg is commonly found in broiler hatcheries (Grépinet et al., 2012) and was repeatedly isolated from hatcheries in the USA over multiple visits (Bailey et al., 2002). It was the fourth most common serovar isolated from fluff samples in broiler breeder hatcheries in Canada between 1998 and 2008 (Sivaramalingam et al., 2013) and is also common in broiler hatcheries in South Korea (Kim et al., 2007; Shang et al., 2021). It appears that the biofilm-forming capability and desiccation resistance (Pedersen et al., 2008) of some strains may assist *S*. Senftenberg to persist in hatcheries for prolonged periods, and this resident contamination can infect newly hatched chicks (Mueller-Doblies et al., 2013). Such resident *Salmonella* serovars may persist for many years (Christensen et al., 1997; Davies & Wray, 1994; Wilkins et al., 2002) and can be very difficult to eliminate.

Salmonella 13,23:i:-is thought to be a recently emerged monophasic variant of *S*. Idikan, an animal feed-related serovar that can become readily established in feed mills, hatcheries and on broiler farms. This serovar was particularly prevalent in H1 and proved extremely difficult to eradicate. Maertens et al. (2020) found that in laboratory studies the use of sub-inhibitory concentrations of benzalkonium chloride reduced the susceptibility of *Escherichia coli* isolates to the fluoroquinolone antibiotic ciprofloxacin. It is believed that similar mechanisms were occurring in H1, with *S*. 13,23:i:- in this hatchery becoming less susceptible to benzalkonium chloride disinfectants (which were being used widely at the time) and to the quinolone compound nalidixic acid (data not shown). Advice was, therefore, tailored to include a change in disinfectants to more effectively tackle the problem.

Bespoke visit reports were issued to hatcheries, with suggested improvements generally focussing on the choice and concentration of disinfectants and on deep physical cleaning of hard-to-clean areas such as hatcher vents. The extent to which advice was followed was based on conversation with hatchery managers and observations by sampling staff at the follow-up visits. When the advice was followed in full, it was associated with a reduction in hatchery contamination. This pattern was also evident in similar previous studies (Davies & Breslin, 2004; Martelli et al., 2016). However, whilst *Salmonella* contamination was found to be significantly lower on the second sampling visit to hatcheries, this was not found to be sustained when a third visit was carried out.

The transient nature of observed improvements in *Salmonella* contamination was probably a consequence of several factors. For two of the hatcheries visited three times, breeding flocks supplying eggs had recently been identified as being infected with *S*. 13,23:i:-. In another hatchery, the tray washer was undergoing maintenance during the third visit, which could have resulted in a temporary increase in the risk of contamination. It is also possible that in some hatcheries, recommendations were no longer being followed rigorously by the time of the third sampling visit after initial reductions in *Salmonella* prevalence had been achieved, and in some hatcheries the management team had changed. The most common fault was failure to use disinfectants at a concentration that would be effective for *Salmonella*. Increases in hatchery throughput in some cases resulted in reduced cleaning standards and insufficient drying time between washing and disinfection, thereby diluting applied disinfectants. Decisions on disinfection practices were largely driven by time pressure, concerns about cost, corrosion of equipment or Health and Safety concerns. Additionally, the most common fault with tray washers was not operating them at a temperature that avoids the establishment of *Salmonella* because of concerns about energy costs and generation of steam.

The diligence of the hatchery manager was observed to be crucial in terms of maintaining a low prevalence of contamination. Eradication of *Salmonella* was not achieved in any of the investigated hatcheries that had harboured contamination at the initial visit. The

length of time between sampling visits was not identified as a factor affecting biosecurity and increased *Salmonella* prevalence. This suggests that the above factors counteracting enhanced *Salmonella* control were not governed simply by the time elapsed since the control measures were introduced.

Hatcheries that were either new, totally refurbished or were being managed to a very high standard had no *Salmonella* present. One hatchery with old equipment and relatively poor hygiene standards also had no *Salmonella* detected. The reason for this is unknown, but there was no history of infection in breeding flocks in the area supplying the hatchery.

The study highlights the frequency with which commercial broiler hatcheries are contaminated with *Salmonella* and the difficulty of eliminating it. However, with careful and sustained attention to biosecurity and hygiene standards, it can be seen that significant reductions in contamination can be achieved. Specific recommendations for control of *Salmonella* in commercial broiler hatcheries in GB would be to take care when sourcing eggs from outside the company, to apply proven C&D protocols using effective disinfectants at adequate concentration, with regular and thorough decontamination of setters, hatcher areas and chick handling equipment, focussing especially on inaccessible areas such as ducting for ventilation and fan belts. Particular attention should be paid to decontamination of tray wash/stores areas to prevent recontamination of hatchery equipment, and of waste areas (such as egg waste or macerator areas) to prevent accumulation of contamination. Furthermore, as high levels of *Salmonella* contamination were observed in external hatchery areas, biosecurity practices must be in place to more regularly decontaminate external areas and prevent transfer of contamination into the hatchery from these external sources during transport of eggs/chicks and other materials in and out of the hatchery. To ensure that levels of *Salmonella* contamination remain low, training and supervision of biosecurity practices, including enhanced C&D, should be maintained and regularly reinforced by hatchery management.

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CONFLICT OF INTERESTS

No potential conflict of interest was reported by the authors.

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