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Survey of the prevalence of Salmonella species on laying hen farms in Kosovo

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ABSTRACT

A survey on the prevalence of *Salmonella* (*S*.) species was carried out on 39 layer farms in Kosovo between April and September 2012. In total 367 samples, comprising feces, dust, eggs and internal organs from dead birds, were investigated using bacteriological culture methods. Additionally, data on the location of the farm, the total number of birds on the farm, age of birds and laying performance were collected. Salmonella were isolated from 38 samples obtained from 19 (49%) farms. The most common serovar identified was *Salmonella* Enteritidis, found on 18 farms. The most common *S.* Enteritidis phage type was PT29 followed by PT6, PT7, PT21, PT13a, PT8, PT14b and PT4. One *S.* Enteritidis isolate was not typable. Six farms had more than one phage type. Furthermore, serovar *S.* Bovismorbificans was also found in samples from three farms. Flock size or production stage was not associated with the probability of isolating *Salmonella*. The only flock factor found to be significantly associated was percent hen/day production: it was 2.8 times more likely to isolate *Salmonella* from flocks with production above 80% hen/day production compared to flocks producing at a lower level. Analysis of antimicrobial resistance patterns of 30 isolates revealed that all isolates were sensitive to gentamicin, ampicillin, sulphamethoxazole trimethoprim and oxytetracycline, and 29 (97%) were sensitive to ciprofloxacin. All isolates showed intermediate resistance or were resistant to minocycline and cloxacillin. Twenty six isolates (86%) had intermediate resistance to amoxicillin and 27 isolates (90%) were fully resistant to streptomycin. The present survey revealed a high prevalence of *Salmonella* Enteritidis in layer flocks in Kosovo, indicating that table eggs have to be suspected as an important source of human salmonellosis.

Key words: Salmonella, Kosovo, prevalence, survey, layers

INTRODUCTION

In the 1980's, intensive poultry production based on what is now Kosovo territory ran to about ten million broilers per year plus a standing flock of about one million laying hens. Afterwards, political turbulences led to a decline of the poultry sector but since 2000 the poultry industry has recovered, with currently more than half a million lying hens in about 80 flocks supplying 80% of table eggs consumed in Kosovo (the rest being imported). Layer flock sizes range from 2,000 to 80,000 and most layer farms have only one house, although a few larger farms have up to four.

Human salmonellosis is a major public health concern in Europe, mainly caused by the serovar Enteritidis (EFSA, 2006; EFSA and ECDC, 2012). In Kosovo *S.* Enteritidis was isolated from 45% of 247 cases of human gastro-enteritis reported to the Institute of Public Health in Pristina in 2014 (Institute of Public Health, Pristina, 2014). Outbreaks in humans are often related to contaminated poultry meat and eggs (Patrick et al., 2004; Jackson et al., 2013; Middleton et al., 2014). The link between *S.* Enteritidis in humans and the consumption of contaminated poultry products, especially undercooked and raw eggs, has been well documented (Coyle et al., 1988; Hogue et al., 1997; Palmer et al., 2000; De Buck et al. 2004). Commercial layer farms can be a significant reservoir of *Salmonella* infection and pose a threat to humans (Garber et al., 2003; EFSA, 2005; Dewaele et al., 2012). However, a *Salmonella* infection is usually not associated with clinical signs in chickens arguing for specific strategies by the government or industry to protect public health.

Antimicrobial resistance (AMR) is of growing public health concern, especially with the appearance of multi drug resistant microorganisms. Zoonotic bacteria that are resistant to antimicrobials are of special concern since they might compromise effective treatment regimes in humans. It is therefore relevant to assess the nature and extent of AMR in *Salmonella* found in poultry. In 2009, in the European Union, the occurrence of resistance in

Salmonella isolates from salmonellosis cases in humans was high for ampicillin, tetracyclines and moderate for sulphonamides, whereas resistance to the critically important antimicrobials for human medicine, cefotaxime (a third-generation cephalosporin) and ciprofloxacin (a fluoroquinolone) was relatively low (EFSA and ECDC, 2011). In the U.S.A., Han et al. (2013) found 30 out of 54 (56%) *Salmonella* isolates from a variety of human, chicken meat and egg-associated sources were resistant to at least one antimicrobial agent tested.

The survey reported in this paper was carried out to estimate the prevalence of *Salmonella* in egg-laying farms in Kosovo along with the identification of serotypes, phage types and antimicrobial resistance patterns.

MATERIALS AND METHODS

Sampling Plan

The survey was carried out between April and September 2012. The method used was based on the technical specifications document (SANCO/34/2004 Rev3) annexed to Decision 2004/665/EC published by the European Commission concerning the baseline study to estimate the prevalence of *Salmonella* species in flocks of laying hens across the European Union (EC, 2004). On the basis of an expected 50% farm prevalence, to give 95% confidence interval with a precision of ±10%, a sample size of 44 farms out of the total 80 farms in Kosovo would be needed. Due to some practical limitations it was possible to sample 39 farms, selected randomly across 13 municipalities of Kosovo. This resulted in a 95% confidence interval for the prevalence estimate with a precision of ±15%.

Sample Collection

All layer farms in Kosovo at the time of the survey operated caged systems. All except one of the sampled farms had only one house. Therefore only one house was sampled on all farms

except the largest farm that had 80,000 hens in four houses, where two houses were sampled. As required by the technical specification for caged systems, five samples (each about 60g) of naturally mixed feces representative of the whole house were taken from droppings belts, scrapers or deep pits. Two dust samples (each about 25g) were taken, one from the floor and one from the fan housing. All feces and dust samples were collected into separate sterile containers. Thirty eggs were collected from different places around the house. These numbers and types of samples were taken from each of the two sampled houses on the large farm. The intention was also to collect two fresh carcasses from each farm, but in practice only 11 carcasses (up to 24 hours old) of dead chickens were collected, one from each of 11 farms.

Salmonella Culture and Typing Method

Salmonella culture and typing was carried out in the Food and Veterinary Laboratory of the Kosovo Food and Veterinary Agency. The method used for the culture of *Salmonella* was according to ISO 6579:2002 (ISO 2002). From each feces and dust sample, 25g of feces or dust material was mixed in 225ml of buffered peptone water (BPW, CM 059, Oxoid UK). For the egg samples, pools were created using 1ml of yolk from each of 15 eggs to make two 15ml pools per farm. Each 15ml pool of mixed egg yolk was mixed into 135ml of BPW. From carcasses, the liver, spleen and intestines were harvested and 25g of the pooled and macerated material was mixed into 225ml of BPW. Each of these inoculated BPW mixtures was then incubated initially at 37^{o}C for 18-24 hours.

Three separate and equally-spaced drops of the inoculated broth (0.1ml total) were placed on the surface of a modified semi-solid Rapapport Vassiliadis (MSRV) medium with novobiocin (1868-17 Difco) plate. The plates were examined after 24 and 48 hours incubation at 41.5°C for suspect *Salmonella* growth. Suspected colonies were streaked onto Brilliant Green agar (CM 0263, Oxoid UK), Xylose-Lysine-Desoxycholate Agar (XLD CM

0469, Oxoid UK) , Xylose-Lysine-Tergitol 4 (113919 Merck, Germany) and Brilliance™ *Salmonella* agar (CM 1092, Oxoid, UK) and incubated at 37°C for a further 24 hours.

Suspect *Salmonella* colonies were confirmed by serotyping according to the Kauffman-White scheme (Popoff, 2001). Phage typing of *Salmonella* is a useful typing tool for subcategorizing the more common *Salmonella enterica* serovars, i.e. *S.* Enteritidis and *S.* Typhimurium. Isolates of *S.* Enteritidis, were phage-typed according to the World Health Organization collaboration center Colindale schemes (Ward et al., 1987).

Thirty *Salmonella* isolates were tested by disc diffusion for their in vitro sensitivity to eight antimicrobials. The test was performed using the protocol from Bauer et al. (1966). Antimicrobial discs (Oxoid UK) were placed on inoculated Mueller Hinton Agar plates using a disc dispenser. The discs used contained the following antibiotics: streptomycin (S 10mcg); gentamicin (Cn 10mcg); ampicillin (AMP 10mcg); amoxicillin (AML 2mcg); cloxacillin (OB 5mcg); ciprofloxacin (CIP 1mcg); sulphamethoxazole + trimethoprim (SXT 25mcg); oxytetracycline (OT 30mcg); minocycline (MH 30mcg).

Data Collection and Analysis

For the purposes of estimating the population prevalence, the primary sampling unit was the farm. Farms were subsequently designated as positive or negative according to the presence or absence of *Salmonella* in one or more of the samples. At the time of sample collection a brief information sheet was also filled in. This covered the location, total number birds on the farm, production stage of flock in months (time since start of lay), the percent hen.day egg production, appearance of any clinical disease and the number of carcasses found on the day of sampling.

Ninety five percent confidence intervals for percentage estimates were calculated using the Wilson score intervals method, with correction for population size, (Wilson, 1927; Wallis,

2013) as provided in the statistical toolbox at *OpenEpi.com* (Dean et al., 2015). This method provides exact, non-symmetrical confidence intervals that are robust even when sample size is small or the percentages are close to 0% or 100%. To test for differences in percentages between groups the Chi squared test was used as a test for homogeneity among multiple groups. A Fisher or mid-P exact test was used as a test for difference between two groups, which is also summarized using relative risk (RR) with confidence intervals calculated using the Taylor series method (O'Brien et al., 1994) as provided in the statistical toolbox at *OpenEpi.com*. Statements about statistical significance of differences are based on the probability (*p*) value for the test statistic being less than or equal to 0.05 as the arbitrary criterion for significance.

RESULTS

Salmonella Prevalence

From 367 samples tested, *Salmonella* was isolated from 38 samples: 22 isolates from feces, 13 from samples of dust, 2 from eggs and 1 isolate from poultry internal organs (Table 1). With respect to sample type, the highest prevalence of positive samples was for the pooled dust samples. If samples from positive farms are considered only, 34% of the dust pools tested yielded *Salmonella* isolates, compared with 23% of the pooled feces samples, a relative risk of 1.48 (although this tendency was not statistically significant with a mid-p exact p-value of 0.2038). Pooled egg samples had the lowest prevalence of positive samples, with only 5.3% of the pooled samples from positive farms yielding *Salmonella* isolates, a relative risk compared to feces pools of 0.23 (statistically significant, with a mid-p exact p-value: 0.0119).

Of the 39 farms sampled in the survey, 19 tested positive for *Salmonella* in one or more samples (Table 2) giving an estimated farm level prevalence of *Salmonella* in Kosovo layer

159 farms of 48.7% (95% confidence interval: 33.9% to 63.8%) (Table 3). Only two different
160 serovars were identified: *S.* Enteritidis and *S.* Bovismorbificans. *S.* Enteritidis was found on
161 18 of the 19 positive farms, giving an estimated farm level prevalence of *S.* Enteritidis in
162 Kosovo layer farms of 46.2% (95% confidence interval: 31.6% to 61.4%). *S.*
163 Bovismorbificans was found in three of the farms, giving an estimated farm level prevalence
164 of *S.* Bovismorbificans on Kosovo layer farms of 7.7% (95% confidence interval: 2.7% to
165 20.3%). *S.* Bovismorbificans was found in two farms along with *S.* Enteritidis and on one
166 farm as the only serovar.

167 Table 2 provides details of the types of samples from which *Salmonella* was isolated on
168 the survey farms. On 15 of the 19 positive farms *Salmonella* was isolated from one or more
169 of the feces samples. On 10 of these farms, feces samples were the only samples to be
170 positive. *Salmonella* was isolated from dust samples on 8 farms, on five of which feces
171 samples were also positive. *Salmonella* was isolated from eggs on only one farm (where all
172 other samples were negative) and from dead bird organs on only one farm (of 11 farms where
173 carcasses were collected) where feces and dust samples were also positive.

174 The farm level prevalence of *Salmonella* was calculated for farms grouped according to
175 different categories among the variables captured on the questionnaire: location (grouped into
176 five administrative regions), flock size, the production stage and production level (Table 3).
177 The prevalences were calculated regardless of serovar, although *S.* Enteritidis was found on
178 all but one of the positive farms. Layer farms are unevenly geographically distributed, with
179 'concentrations' of poultry farms in the regions of Prizren, in the south, and Peje, in the west.
180 The distribution of number of birds per farm was highly skewed; with most flocks being less
181 than 6,000 birds (minimum 2,400; median 5,200; maximum 80,000 and interquartile range
182 3,600 to 10,000). There was just one farm with 80,000 birds kept as four flocks in four
183 houses. This was the only farm with more than one house. The flocks sampled were between

four and 18 months into production (median 10; interquartile range 8 to 12). Percent hen.day production at the time of sampling varied between 60% and 95% (median 80%; interquartile range 75% to 85%). There was a trend for production to decrease with increasing time into production: 67% of flocks nine months or less into production had over 80% hen.day production, compared with only 24% of those over nine months (mid-p exact p-value: 0.00958).

Table 3 shows that *Salmonella* prevalence was significantly higher among farms in two regions, Gjilan and Peje, compared with the rest (these two regions are geographically at opposite sides of the country, east and west). Flock size or production stage were not associated with different prevalences. The only flock factor found to be significantly associated with different prevalences was percent hen.day production: it was 2.8 times more likely to isolate *Salmonella* from flocks with production above 80% hen.day production compared to flocks producing at a lower level.

Phage Types

All the isolates of *S.* Enteritidis were phage typed. Table 4 shows the phage types of *S.* Enteritidis identified and the proportion of positive farms from which each phage type was isolated. The most common *S.* Enteritidis phage type was PT29, which was isolated from five (28%) of the positive farms. However, PT6, PT7 and PT21 were also found frequently, each being present on four (22%) of the positive farms (Table 4). The other phage types isolated were PT13a (three farms, 17%), PT8, PT14b (each found on two farms, 11%) and PT4, the least common *S.* Enteritidis phage type, found on only one farm. Six farms had combined infections with more than one phage type: types 7 & 21; types 8 & 21; types 7 & 29; types 6 & 13a; types 4 & 6; types 7, 8 & 13a.

Antimicrobial Resistance Patterns

The results of the antimicrobial sensitivity testing of 30 of the *S.* Enteritidis and *S. Bovimorficans* isolates are shown in Table 5. All isolates were sensitive to gentamicin, ampicillin, sulphamethoxazole trimethoprim and oxytetracycline, and 29 (97%) were sensitive to ciprofloxacin. All isolates showed intermediate resistance or were resistant to minocycline and cloxacillin. Twenty six isolates (86%) had intermediate resistance to amoxicillin and 27 isolates (90%) were fully resistant to streptomycin.

DISCUSSION

This survey found *Salmonella* on almost half of the poultry layer farms sampled in Kosovo. *S.* Enteritidis, the serovar most frequently associated with human illness in relation to eggs (EFSA, 2006; EFSA, 2010), was found on 18 of the 19 positive farms. *S.* Bovismorbificans was the only other serovar isolated. Therefore, of the five serovars given top priority by the EU because of their public health significance, *S.* Enteritidis, *S.* Typhimurium, *S.* Virchow, *S.* Infantis and *S.* Hadar, only one was isolated from the farms.

The high flock prevalence of *S.* Enteritidis, is similar to that found in some EU countries by baseline surveys carried out between October 2004 and September 2005 (EFSA, 2007). In those surveys the flock prevalence of *S.* Enteritidis was similarly high or higher in Czech Republic (59.4%), Poland (54.6%), Spain (48.2%), Portugal (47.7%) and Lithuania (44.4%). High flock prevalence of *S.* Enteritidis infection in layer flocks has also been found outside Europe, for example Min Chin Im et al. (2015) found 34 infected out of 67 flocks (51%) tested in a survey in Korea. This demonstrates that Kosovo is not unusual in facing a high flock prevalence of *S.* Enteritidis in its newly developing poultry sector. Nevertheless, across the EU as a whole the baseline surveys found a range of flock prevalence of *S.* Enteritidis from quite low (for example: Austria, 9.5%; UK, 6.2% and the Netherlands, 6.1%), through

231 intermediate levels (for example: Germany, 22.8% and Hungary, 32.2%) to the high
232 prevalences mentioned above.

233 In the baseline surveys carried out in EU, dust samples had a higher likelihood of being
234 positive compared to feces samples (EFSA, 2007). A similar tendency was found in this
235 survey, although, because more feces samples were taken and tested on each farm, more
236 positive feces samples were found overall and it was more common to find a farm positive on
237 the basis of a positive feces sample than a positive dust result. This result suggests that dust
238 sampling could be a more sensitive method of surveillance for *Salmonella* than feces
239 sampling. Isolation of *Salmonella* from dust may be easier than from fresh feces because
240 *Salmonella* is relatively more resistant to desiccation than many competitor organisms (Miura
241 et al., 1964; Davies and Wray, 1996; Davies and Breslin, 2003a). Dust sampling might pick
242 up presence of infection over a longer retrospective period and also infection in the
243 environment (from contaminated feed and from wild birds) while feces samples reflect more
244 closely the current infection status of the birds present at the time of sampling.

245 Only 5.3% of the pooled egg samples tested from the positive layer flocks in the survey
246 yielded *Salmonella*. The EU member state baseline surveys did not routinely include eggs in
247 the survey sample, but in several other studies of naturally *Salmonella* infected laying flocks
248 the proportion of infected eggs was also found to be low (often below 3%) (Humphrey et al.,
249 1991; de Louvois, 1993; Henzler et al., 1994; Kinde et al., 1996; Schlossar et al., 1999;
250 Advisory Committee on the Microbiological Safety of Food, 2001). Arnold et al. (2012)
251 found similarly low percentages of contaminated eggs from infected layer flocks and the rate
252 of contamination was much higher for shells than for contents. Gole et al. (2014)
253 demonstrated an association between indoor environmental contamination by *S.* enterica and
254 contamination of eggs on layer farms in Australia. Arnold et al. (2012) also found the rate of
255 egg shell contamination was higher per infected bird in flocks with high within flock

prevalence of *Salmonella* infection, possibly due to a correlation between high Salmonella prevalence and poor hygiene standards. This means that high prevalence flocks could contribute disproportionately to eggs with contaminated shells. In a survey in Korea, Min Chin Im et al. (2015) found lower rates of *Salmonella* detection inside eggs (5%) and egg shells (17%) relative to detection from environmental dust samples (40%) on layer farms. Sampling on a *Salmonella* infected layer farm in Spain (Garcia et al., 2011) detected *Salmonella* in 92% of feces samples and 34% of samples from eggshells, but no *Salmonella* spp. were detected in the egg contents. Even what may be perceived as a low proportion of egg production contaminated with *Salmonella* may pose a significant risk for human health considering the large number of eggs consumed. It is therefore important to reduce the risk of egg *Salmonella* contamination and the numbers of *Salmonella* bacteria present.

In this survey, flock size was not associated with the risk of *Salmonella*. This differs from the findings of other surveys. For example in a survey by Snow et al. (2007), the highest prevalence of *Salmonella* occurred in the largest farm size category (30,000 birds or more). In the current survey, most flocks contained less than 6,000 birds. Only two farms had 30,000 birds or more, and of these two, the largest was negative for *Salmonella*. Hence, increased risk was not associated with increasing flock size in this survey. This is possibly related to the fact that in Kosovo the larger flocks tend to be managed by owners who have a higher level of training and knowledge. In comparison, the relatively small-scale flocks of up to 6,000 birds are often managed by non-specialized managers with little training. In particular, understanding and application of biosecurity and hygiene measures are poor. In contrast, a survey in Barbados found that the odds of testing positive for Salmonella were 10 times higher in large farms, compared to small farms and the authors related this to the finding that more small farms cleaned and disinfected poultry facilities quarterly or more often than large farms did (Aimey et al., 2013). All the flocks in Kosovo used caged (battery) systems, which

were also found to have higher risk for *Salmonella* in other surveys (Snow et al., 2007). This survey showed a significantly higher probability of isolating *Salmonella* from flocks with higher production levels (greater than 80% hen.day production). This might be explained by increased physiological stress on the birds leading to increased likelihood of shedding *Salmonella*.

Phage typing of *S.* Enteritidis was performed for the first time in Kosovo during this survey. Nine phage types of *S.* Enteritidis were detected. The most common *S.* Enteritidis phage type was PT29. Phage types PT6, PT7 and PT21 were also frequently found in more than 20% of the positive farms. The least common *S.* Enteritidis phage type was PT4 in contrast to other EU countries where PT4 is the most or more common phage type (EFSA, 2007). Improvement of the regular sampling of flocks would be useful in monitoring infection levels. Phage typing of any *Salmonella* isolates could show possible linkages between seemingly sporadic cases which could help in recognizing the spread of infection between flocks.

The antimicrobial sensitivity testing revealed a mixture of sensitivity and resistance of the isolates to different classes of antimicrobial. Most isolates were resistant to the aminoglycoside, streptomycin, but 100% were sensitive to gentamicin. All were resistant to the penicillinase-resistant penicillin, cloxacillin, and most had intermediate resistance to the aminopenicillin, amoxicillin, but 100% were sensitive to ampicillin. Almost two thirds of the isolates were resistant to the tetracycline, minocycline, but 100% were sensitive to oxytetracycline. 100% were also sensitive to sulphamethoxazole and trimethoprim and all but one were sensitive to ciprofloxacin. In contrast to the findings here, a survey of layer flocks in UK, in which 177 *Salmonella* isolates were tested against 16 antimicrobials, 77% were sensitive to all 16, and no more than 15% of isolates were resistant to any single antimicrobial (Snow et al., 2007). In a survey of layer farms in Korea, 93 out of 101 isolates

were fully susceptible to a range of antimicrobials (Min Chin Im et al., 2015). Although based on only a small number of tested isolates, the high level of resistance observed in this survey is cause for concern.

Because Salmonella is an important cause of food borne disease in humans the EU agreed a programme for the reduction of Salmonella of public health significance in farm animals under Regulation EC No 2160/2003. In view of the findings of this survey Kosovo might consider following a similar programme at least with respect to the commercial poultry sector. Good cleaning and disinfection practice has previously been shown to be effective in reducing Salmonella overall (Davies and Breslin 2003b, Garber et al. 2003). Inactivated *Salmonella* Enteritidis vaccines, when used in conjunction with good hygiene and disinfection practices, have also been shown to decrease the presence of *Salmonella* Enteritidis in layer flocks (Oliveiro Caetano de Freitas Neto et al., 2008). In conclusion, the results of this survey show that Salmonella enterica, particularly *S.* Enteritidis, occurs in the commercial large-scale laying hen production in Kosovo, indicating that table eggs could be an important source of human salmonellosis in Kosovo. Kosovo should consider taking steps to address this threat to human health.

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Table 1: Total samples taken and the numbers of positive samples (isolates), by sample type

Type of sample	Total samples from all farms	Number of samples from positive farms	Number of positive samples	% positive (of all samples)	% positive (of samples taken on positive farms only)
Feces (5 x 60g pools per farm)	200	95	22	11.0%	23.2%
Dust swabs (2 x 25g pools per farm)	80	38	13	16.3%	34.2%
Eggs (2 x 15 eggs pooled per farm)	76	38	2	2.6%	5.3%
Internal organs (up to one carcass per farm)	11	7	1	9.1%	14.3%
Total samples – all types (tested pools)	**367**	**178**	**38**	**10.4%**	**21.3%**

Table 2: Types of samples positive for Salmonella on the survey farms

Types of samples positive for *Salmonella*	Number of farms
All samples negative	**20**
Positive samples	**19**
Egg only	*1*
Dust swab only	*3*
feces only	*10*
feces and dust swab	*4*
feces, dust swab and internal organs	*1*
Total	**39**

Table 3: Farm level prevalence of Salmonella among layer farms in the survey

	number of farms sampled	positive farms: number (%)		(95% c.i.)[1]
Overall	39	19	(48.7%)	(33.9% to 63.8%)
by region				
Ferizaj (south/east)	4	1	(25.0%)	(4.6% to 70.0%)
Gjilan (east)	6	4	(66.7%)	(30.0% to 90.3%)
Peje (west)	13	9	(69.2%)	(42.4% to 87.3%)
Pristina (centre/east)	4	0	(0.0%)	(0.0% to 49.0%)
Prizren (south)	12	5	(41.7%)	(19.3% to 68.1%)
Overall Chi-Square: 7.903	*p-value: 0.995*			
by two groups of regions				
Gjilan + Peje	19	13	(68.4%)	(46.0% to 84.6%)
The rest	20	6	(30.0%)	(14.6% to 51.9%)
		Relative risk: 2.28		*(1.09 to 4.76)*
Fisher exact (2-tail) p-value: 0.03633		*Mid-P exact (2-tail) p-value: 0.02107*		
by flock size category				
<5,000	18	9	(50.0%)	(29.0% to 71.0%)
5,000 < 10,000	10	5	(50.0%)	(23.7% to 76.3%)
10,000 <20,000	7	3	(42.9%)	(15.8% to 75.0%)
>=20,000	4	2	(50.0%)	(15.0% to 85.0%)
Overall Chi-Square: 0.1173	*p-value: 0.990*			
by two flock size groups				
<5,000	18	9	(50.0%)	(29.0% to 71.0%)
>=5,000	21	10	(48.0%)	(28.3% to 67.6%)
		Relative risk: 1.05		*(0.55 to 2.00)*
Fisher exact (2-tail) p-value: >0.9999		*Mid-P exact (2-tail) p-value: 0.888*		
by production stage				
<=9m	18	10	(56%)	(33.7% to 75.4%)
>9m	21	9	(43%)	(24.5% to 63.5%)
		Relative risk: 1.30		*(0.68 to 2.47)*
Fisher exact (2-tail) p-value: >0.6392		*Mid-P exact (2-tail) p-value: 0.4526*		
by hen.day production				
<=80%	22	6	(27%)	(13.2% to 48.2%)
>80%	17	13	(76%)	(52.7% to 90.4%)
		Relative risk: 2.80		*(1.35 to 5.83)*
Fisher exact (2-tail) p-value: >0.005702		*Mid-P exact (2-tail) p-value: 0.003126*		

[1] *c.i.: confidence interval. For proportion/percentage these are Wilson score intervals; for relative risk these are Taylor series.*

Table 4: Phage types of S. Enteritidis identified on 18 Salmonella positive farms

Phage type	number of farms[1]	percentage of the 18 positive farms
nPT29	5	27.8%
nPT6	4	22.2%
nPT7	4	22.2%
nPT21	4	22.2%
nPT13a	3	16.7%
nPT8	2	11.1%
nPT14b	2	11.1%
nPT4	1	5.6%
untypeable	1	5.6%

[1] *six farms had more than one phage type (details in text)*

474 **Table 5: Antimicrobials included in AMR testing of the Salmonella isolates, and the**
475 **resulting sensitivity**

Antimicrobial class and sub-classes	**Active ingredient in the disc**	**sensitivity / resistance**
Aminoglycocide	streptomycin (S 10mcg)	3/30 sensitive 27/30 resistant
Aminoglycocide – *2 deoxystreptamine*	gentamicin (Cn 10mcg)	30/30 sensitive
Penicillin – *aminopenicillin*	ampicillin (AMP 10mcg)	30/30 sensitive
	amoxicillin (AML 2mcg)	4/30 sensitive 26/30 intermediate
Penicillin – *penicillinase-resistant*	cloxacillin (OB 5mcg)	0/30 sensitive 30/30 resistant
2nd generation quinolone (fluoroquinolone)	ciprofloxacin (CIP 1mcg)	29/30 sensitive 1/30 intermediate
Sulphonamide + diaminopyrimidine	Sulphamethoxazole + trimethoprim (SXT 25mcg)	30/30 sensitive
Tetracyclines	oxytetracycline (OT 30mcg)	30/30 sensitive
	minocycline (MH 30mcg)	11/30 intermediate 19/30 resistant

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