Acta vet. scand. 2002, *43,* 21-30.

Comparisons of Sampling Procedures and Time of Sampling for the Detection of *Salmonella* in Danish Infected Chicken Flocks Raised in Floor Systems

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Gradel KO, Andersen J, Madsen M: Comparisons of sampling and time of sampling for the detection of *Salmonella* in Danish infected chicken flocks raised in floor systems. Acta vet. scand. 2002, 43, 21-30. – Bacteriological follow-up samples were taken from 41 chicken *(Gallus gallus)* flocks in floor systems, where *Salmonella enterica* (Salmonella) had been detected either directly in bacteriological samples or indirectly by serological samples. Three types of follow-up samples were compared to each other within each flock: 1) 5 pairs of socks, analysed as 5 samples, 2) 2 pairs of socks, analysed as one sample, and 3) 60 faecal samples, analysed as one pooled sample. Agreement between sampling methods was evaluated by the following statistical tests: 'Kappa', 'The adjusted rand', McNemar´s test for marginal symmetry, Proportion of agreement P_0, P_+, P_-, and Odds Ratio. The highest agreement was found between the 2 types of sock sampling, while the lowest agreement was found by comparing 60 faecal samples with 5 pairs of socks. Two pairs of socks analysed as one pool appeared to be just as effective in detecting *S. enterica* as the 60 faecal samples. In broiler flocks, 5 pairs of socks were used both in the routine samples taken at about 3 weeks of age for the establishment of infection of the flock, and as one of the follow-up samples taken shortly before slaughter age, which means that the only notable differences between the 2 sampling rounds were the age of the broilers and of their litter. *S. enterica* was detected more frequently in samples from broilers about 3 weeks old, than in similar samples taken from broilers a few days prior to slaughter at ca. 33-40 days of age.

sampling methods; sampling time.

Abbreviations:
BPW: Buffered peptone water.
ELISA: Enzyme-Linked Immunosorbent Assay.
ISO: International Organization for Standardization.
LPS: Lipopolysaccharide.
LPS-ELISA: Lipopolysaccharide Enzyme-Linked Immunosorbent Assay.
RVS: Rappaport Vassiliadis broth with soy peptone.

Introduction

Routine Salmonella surveillance has been performed in all Danish broiler flocks since 1989 (*Bisgaard* 1992, *Anon.* 1999a), first as a voluntary programme by the Danish Poultry Council and since 1992 as a component of the statutory Ante-mortem inspection programme for broiler flocks. As from the start of the surveillance programmes caecal tonsils from 16 chickens per flock were tested. Assuming a test sensitivity of 1.00, this sampling procedure could with 90% confidence detect salmonella-

positive flocks with a prevalence of 20% (*Skov et al.* 1999). At the end of 1994 a new sampling procedure was implemented, comprising 60 faecal samples per flock analysed as 12 samples with 5 pooled faecal samples each. This sampling procedure improved the sensitivity to a prevalence detection and confidence of 5% and 95%, respectively (*Skov et al.* 1999). In 1997, five pairs of elastic cotton tubes, worn over the boots during a walk in the broiler house ("sock samples") substituted the faecal samples after *Skov et al.* (1999) carried out an investigation indicating that this did not compromise the sensitivity.

In 1994 the EU Zoonoses Directive (Directive 92/117/EEC) was implemented in Denmark in order to carry out surveillance and control measures in parent rearing and breeder chicken flocks. However, in December 1996 further legislation instituting the Danish Salmonella Control Programme was introduced in order to particularly intensify the surveillance of parent breeder flocks by the implementation of a sampling programme consisting of 60 faecal samples collected from each house with parent flocks every 4 weeks (*Feld et al.* 2000). The Danish Salmonella Control Programme thus lays down statutory requirements for routine Salmonella sampling procedures as well as the consequences for the flocks if Salmonella is detected in these routine samples (Anon. 1999a).

Since 1996 the routine sampling programme and the salmonella situation have been frequently scrutinised by all parties involved in the Salmonella Control Programme in order to possibly improve the sensitivity of detecting Salmonella. Some hatcheries have supplemented the statutory faecal samples with voluntary sock samples in the hope of detecting a Salmonella infection at an earlier stage, and in May 2000 sock samples have substituted the 60 faecal samples as sampling material in the Danish Salmonella Control Programme.

The main aim of the study was to compare one pool of 60 faecal samples to one pool consisting of 2 pairs of socks, collected from chicken houses where Salmonella had been detected, and where the animals were housed in floor systems. Five pairs of socks were also included in the study as reference samples.

In broiler houses 5 pairs of socks were used both in routine samples, taken at about 3 weeks of age (*Anon.* 1999a) and used for the primary identification of infected flocks to be included in the study, and in the follow-up samples, typically taken a few days before slaughtering the animals. This provided an opportunity for studying infection dynamics in broiler flocks by comparing the number of Salmonella positive samples between 2 different ages of broilers within the same house and flock.

Materials and methods

Chicken flocks

The Danish Veterinary Institute receives all Salmonella samples submitted under the Danish Salmonella Control Programme for parent and table-egg producing flocks, as well as Antemortem Salmonella samples from broiler flocks prior to slaughter. Thus it is possible centrally to follow the Salmonella infection status of all commercial Danish chicken flocks.

The analytical unit of this study was the Salmonella-infected flock, defined as a group of chickens of the same age, raised in a floor system in the same house during the same period, and infected with *S. enterica* prior to sampling. Houses with broilers, rearing stock on floor, and table egg layers in either free range, deep litter or organic systems fulfilled the criteria for the animals being raised in floor systems. Altogether 41 flocks were included in the study, which covered the period from 16 August 1999 until 5 May 2000. Among the 41 flocks, 29 were broiler flocks, one was a rearing flock while the remaining 11 flocks were table layers

Table 1. Chicken flocks under study as distributed on production category and detected *Salmonella enterica* serovars.

Category (number of flocks)	No. of flocks	Serovar(s) detected at inclusion time	Serovar(s) detected at follow-up (number of flocks)
Broilers (29)	6	4.12:b:-	4.12:b:- (2)
	1	41:z4,z24:-	None
	1	Brevis	None
	1	Derby	None
	1	Indiana Infantis	Typhimurium, DT177[1], Indiana (1)
	4	Infantis	Infantis (3)
	2	Kentucky	None
	1	Senftenberg	None
	1	Typhimurium, DT110	Typhimurium, DT110 (1)
	1	Typhimurium, DT12	None
	1	Typhimurium, DT177, Indiana	Typhimurium, DT177 (1)
	8	Typhimurium, DT41	Typhimurium, DT41 (5)
	1	Typhimurium, DT66	Typhimurium, DT66 (1)
Layer poults (1)	1	Enteritidis, PT8[2]	None
Deep litter egg layers (6)	1	Enteritidis, PT RDNC[3]	Enteritidis, PT21 (1)
	3	Enteritidis, PT6	Enteritidis, PT RDNC, rough (2)
	1	Enteritidis, PT8	None
	1	Serological confirmation only	Enteritidis, PT2 (1)
Organic egg layers (4)	3	Enteritidis, PT6	Enteritidis, PT6 (1)
	1	Enteritidis, PT8	Enteritidis, PT8 (1)
Free range egg layers (1)	1	Serological confirmation only	Enteritidis, PT8 (1)

1) DT = definitive type. 2) PT = phage type. 3) Routine dilution, no conformity.

(6 flocks on deep litter, 4 organic flocks and 1 free range flock). The number of investigated flocks distributed on production category and Salmonella serovar is shown in Table 1.

Sampling procedures

Broiler flocks: As part of the statutory Ante-mortem programme 5 pairs of sock samples are routinely submitted from each broiler flock when the broilers are about 3 weeks old, according to procedures described by *Skov et al.* (1999). Whenever Salmonella was detected in routine sock samples, either the person in charge of the broiler flock or the local District Veterinary Officer was contacted in order to obtain follow-up samples for this study. Typically, the samples were taken a few days prior to slaughtering the broilers.

Layer flocks: During the period of the study all layer flocks were monitored under the Danish Salmonella Control Programme by bacteriological analysis of 60 faecal samples as well as by serological analysis by an indirect lipopolysaccharide (LPS) ELISA including Salmonella 1,4,5,9,12 O-antigens (MIX-ELISA) (*Feld et al.* 2000) of 60 eggs or 60 blood samples for antibodies against Salmonella, at a frequency of 6 times/year. Positive findings in routine samples were con-

firmed once more either by bacteriological analysis (nine flocks) or by positive serological reactions (2 flocks) before inclusion in the study.

Samples investigated: The samples investigated for all the 41 flocks were:

- 5 pairs of socks, analysed as 5 samples ("5-sock-samples").
- 2 pairs of socks, analysed as one sample ("2-sock-samples").
- 60 faecal samples, analysed as one sample. Each faecal sample contained about one gram of fresh faecal material.

However, 2-sock-samples were not received from 2 flocks, and 5-sock-samples were not received from one flock.

Sample collections: Owners of infected flocks, all of whom were familiar with collecting Salmonella samples, were contacted in order to obtain the samples for this study. In order for sampling procedures to reflect ordinary sample collections, these owners received no additional instructions on how to collect the samples. All samples were collected in houses with animals, with the exception of samples from one table egg layer house on deep litter where the animals had just been removed, and from 3 organic layer houses where only few animals remained at the time of sampling.

Laboratory procedures

Bacteriological samples were analysed by a modified ISO 6579 method (*Anon.* 1993). Briefly, the samples were immersed in buffered peptone water (BPW) (Merck 07228) at a weight ratio of 1:10. After incubation at 37 °C for 16-20 h, 100 μl of BPW was transferred to 10 ml of Rappaport Vassiliadis broth with soy peptone (RVS) (OXOID CM 866) and incubated at 42 °C for 18-24 h. 10 μl of RVS was then spread on Rambach agar (Merck 07500) and incubated at 37 °C for 20-24 h. Up to 5 suspected colonies were tested serologically by Kaufmann methods (*Popoff & Le Minor* 1997).

Data analysis and statistics

Data were recorded in a database programme (*Anon.* 1997a). Statistical analysis was performed in an Excell spreadsheet (*Anon.* 1997b), in Splus-2000 (*Anon.* 1999b) and in SAS (*SAS Institute Inc.* 1999).

The observed data were summarised in contingency tables, cross-classified as Salmonella positive/negative. Several methods are available for testing and comparing the agreement of the applied sample types. In the current study the following methods were chosen: Cohen's Kappa (κ) (*Cohen* 1960), The Adjusted Rand *R'* (*Hubert & Arabie* 1985), McNemars test for marginal symmetry (*Fleiss* 1981), Proportion of agreement P_0, P_+,P_-, and Odds Ratio *OR* (*Fleiss* 1981).

Comments on and interpretation of the statistics

Cohen's Kappa (κ) is a popular way of quantifying level of agreement, however, a few comments should be added to this frequently applied statistic. In the calculation of κ, the proportion of chance agreement is calculated and corrected for, this would only be appropriate and relevant under the conditions of statistical independence – which is clearly not the case. *Landis & Koch* (1977) suggest that κ be interpreted as (see the table).

The Rand statistic *R* is an objective criterion for

Kappa Value	Below 0	0-0.2	0.21-0.40	0.41-0.6	0.61-0.8	0.81-1.0
Interpretation	Poor	Slight	Fair	Moderate	Substantial	Almost perfect

evaluation of classification. It measures the proportion of coplaced pairs (*Hubert & Arabie* 1985). The Adjusted Rand statistic *R'* is normalised so that it is zero when classification is selected by chance and 1 when a perfect match is achieved.

McNemar's test statistic is used to test the null hypothesis of marginal symmetry, namely that the probability of an observation being classified into cell [i,j] in the contingency table is the same as the probability of being classified into cell [j,i] (*Fleiss* 1981). The p-value should be interpreted carefully. Its validity depends on the assumption that the cell counts are at least moderately large. Even when cell counts are adequate, the chi-square is only a large-sample approximation to the true distribution of McNemar's statistic under the null hypothesis.

Proportion of overall agreement P_0 and proportion of specific agreement P_+ and P_- are important descriptive statistics. P_0 is the proportion of samples for which the sample types agree (it does not distinguish between positive and negative agreement). P_+ and P_- is the proportion of specific agreement for positive and negative ratings, respectively. They may be interpreted as conditional probabilities – if both are high the agreement is high.

The Odds Ratio can be interpreted as the relative increase in the odds of one sample type making a given classification given that the other sample type made the same classification. Since all statistics have pros and cons we have chosen to list several statistics in order to interpret the results.

Table 2. Comparisons between three sample types for the detection of Salmonella in infected chicken flocks

1a: Faecal samples and 2-sock-samples

Faecal samples[1]	2-sock-samples[2]		Total
	+ Salm	- Salm	
+ Salm[4]	10	3	13
- Salm[5]	6	20	26
Total	16	23	39

1b: Faecal samples and 5-sock-samples

Faecal samples	5-sock-samples[3]		Total
	+ Salm	- Salm	
+ Salm	9	3	12
- Salm	9	19	28
Total	18	22	40

1c: 2-sock-samples and 5-sock-samples

2-sock-samples	5-sock-samples		Total
	+ Salm	- Salm	
+ Salm	13	2	15
- Salm	4	19	23
Total	17	21	38

Numbers in the tables show the number of flocks.

[1)] 60 faecal droppings, analysed as one sample; [2)] 2 pairs of socks, analysed as one sample; [3)] 5 pairs of socks, analysed as 5 samples; [4)] Salmonella detected; [5)] Salmonella not detected.

Results

General

Thirty-nine of the 41 flocks under study were included due to positive results of bacteriological culture for Salmonella in routine surveillance samples. In 37 of the 39 flocks there was no discrepancy between the *S. enterica* serovar found in routine samples and in the Salmonella positive follow-up samples. The remaining 2 flocks were broiler flocks where 2 Salmonella serovars were found in the routine sock samples from each flock. Only one of the detected Salmonella serovars was found in the follow-up samples, cf. Table 1.

Two flocks were included in the study due to serologically positive routine samples for Salmonella. In both of these flocks *S. Enteri-*

Table 3. Statistics used for, and results of comparisons between three sample types for the detection of Salmonella in infected chicken flocks.

Statistics	Faecal samples and 2-sock-samples	Faecal samples and 5-sock-samples	2-sock-samples and 5-sock-samples
κ[1] [95% confidence interval]	0.51 [0.23 ; 0.78]	0.38 [0.10 ; 0.65]	0.68 [0.44 ; 0.94]
R' [2]	0.27	0.14	0.45
Mcnemar (p-value)[3]	0.317	0.083	0.414
OR[4] [95% confidence interval]	11.1 [2.28 ; 54.0]	6.33 [1.37 ; 29.2]	30.9 [4.91 ; 194]
P_o, P_+, P_-[5]	0.77 , 0.68, 0 .82	0.70 , 0.70 , 0.76	0.84, 0.81 , 0,86

[1)] Cohen´s Kappa; [2)] Adjusted Rand R´; [3)] McNemar´s test for marginal symmetry; [4)] Odds Ratio; [5)] Proportion of agreement.

tidis, a serovar sharing O-antigens with the routine LPS-ELISA employed, were found in the follow-up samples.

Comparing different sample types

Table 2 presents comparisons between the 3 sample types in this study. No single sample type was able to detect Salmonella in all the flocks that were found Salmonella positive in the routine samples (faecal samples: 32%, 2-sock-samples: 41% and 5-sock-samples: 45%). Table 3 lists the statistics applied to evaluate the agreement between the sample types. Using the 5-sock-samples as reference (or the "golden standard"), the lowest level of agreement, for all statistics, was found between faecal samples and 5-sock-samples. The 9 flocks where Salmonella was found in the 5-sock-samples, but not in the faecal samples, contributed to this relatively low level of agreement. The highest level of agreement, for all statistics, was found between 2-sock-samples and 5-sock-samples, showing a substantial agreement between these two tests. In 4 flocks Salmonella was found in 5-sock-samples, but not in 2-sock-samples. There was a moderate agreement between faecal samples and 2-sock-samples. In 6 flocks Salmonella was detected in 2-sock-samples, but not in the faecal samples, while the opposite was the case for 3 flocks.

Comparing routine sock samples and 5-sock-samples in broiler flocks

More than one Salmonella serovar was found in

Table 4. Comparison of results of sampling of 26 Salmonella infected broiler flocks at 3 weeks of age, and at slaughter age by 5 pairs of sock samples.

		No. of Salmonella positive samples at slaughter age						
		0	1	2	3	4	5	
No. of Salmonella positive samples at 3 weeks of age	1	10						10
	2	2						3
	3	2						2
	4	2	1			1		4
	5		1			2	4	7
		16	2			3	5	26

Numbers in the table show the number of flocks.

Table 5. Statistics used for, and results of comparisons between sampling of 26 Salmonella infected broiler flocks at 3 weeks of age, and at slaughter age by 5 pairs of sock samples.

Statistics	Routine 5-sock-samples and 5-sock-samples
κ [95% confidence interval]	-0.01 [-0.27 ; 0.24]
R'	0.32
OR [95% confidence interval]	0.89 [0.13 ; 6.16]
P_0	0.11

Legends: See Table 3.

2 of the flocks and this may possibly bias the number of Salmonella positive socks. Moreover, 5-sock-samples were not submitted from one flock. These 3 flocks were consequently excluded from further analyses.

Table 4 compares the numbers of Salmonella positive flocks in routine samples and in 5-sock-samples for the remaining 26 broiler flocks. In 16 flocks, where Salmonella was detected in the routine samples (ranging from 1 to 4 Salmonella positive sock samples), Salmonella was not detected in 5-sock-samples. From 2 flocks, where Salmonella was found in 4 and 5 of the routine sock samples, respectively, Salmonella was only detected in one 5-sock-sample. Among 7 flocks, which were all highly infected in the routine samples, there was a good correlation between routine samples and 5-sock-samples. A lower number of Salmonella positive socks in the routine samples than in the 5-sock-samples was only detected in one flock. The agreement statistics, shown in table 5, indicate a very poor – if any – level of agreement.

For the 26 flocks the time elapse between receiving the routine sock samples and the 5-sock samples was in the range of 10-24 days, with "time elapse peaks" at 14, 15, 16 and 20 days, for 5, 3, 4 and 5 flocks, respectively (data not shown).

Discussion

Several studies have compared the sensitivity and power of sampling methods in chicken houses (*Kingston* 1980, *Higgins et al.* 1982, *Caldwell et al.* 1995, *Byrd et al.* 1997, *Caldwell et al.* 1998, *Skov et al.* 1999). *Skov et al.* (1999) concluded that 5 pairs of socks seemed to be as effective in detecting Salmonella as 12 faecal pools, each pool consisting of 5 faecal samples. The same study also compared one pair of socks to faecal samples and concluded that this sample type was inferior to the 12 × 5 faecal samples for the purpose of detecting Salmonella in broiler flocks. In the period between March 1998 and May 2000 one Danish broiler hatchery submitted voluntary sock samples from all its parent stock houses (1-3 pairs of socks from each house, depending on the number of animals) in addition to the statutory samples under the Danish Salmonella Control Programme. During that period Salmonella was found in sock samples submitted from 3 different parent stock houses, which were situated at 3 different farms while during the same period no Salmonella was found in the corresponding statutory 1 × 60 faecal samples. This difference in Salmonella detection ability between the 2 sample types could be due to the fact that the private sock samples were submitted every week, whereas the obligatory faecal samples were only submitted every 4 weeks, but it could also be due to an improved ability of the sock samples to detect Salmonella as compared to faecal samples. Because Salmonella is rarely detected in samples from parent stock in Denmark, both egg layers, layer poults and broiler chickens raised in floor systems were included in this study in order to provide positive material for the investigation. The results show that 2 pairs of socks, analysed as one pool, do not seem to be inferior in detecting Salmonella when compared to 1 × 60 faecal samples, i.e. they can detect a 5% Salmonella prevalence

with 95% confidence, given that the laboratory sensitivity is 1.00.

Comparison of 5 pairs of socks in broiler flocks between about 3 weeks of age and a few days prior to slaughter clearly indicated that many Salmonella positive flocks will not be detected if the sampling is postponed until a few days prior to slaughter. This may be due to changes in Salmonella excretion by the chickens during the period they are reared in the house, and/or it may be due to adverse factors for Salmonella survival in the litter. Most studies, which describe how different factors may influence the Salmonella excretion rates in chickens, are experimental (*Snoeyenbos et al.* 1978, *Weinack et al.* 1979, *Gustafson & Kobland* 1984, *Desmidt et al.* 1998, *Muir et al.* 1998, *Skov et al.* 2000). The age of the inoculated chickens, using new or used litter, Salmonella inoculation dose, and presence or absence of feed additives are a few examples of factors which may influence the time and amount of Salmonella being excreted by chickens. It is difficult to estimate the importance of these factors in this study because we do know neither the time of infection nor the infective dose, the latter of which is probably lower than in the experimental studies (*Muir et al.* 1998). However, based on Ante-mortem results and hatchery records, 8 of the 26 broiler flocks received *S. Typhimurium* definitive type 41 (ST41) from a Swedish parent flock, 11 flocks were in broiler houses with persistent Salmonella infections, while the remaining 7 flocks had Salmonella types, which occurred sporadically, making it difficult to trace the source of infection (*Kim Gradel,* pers. obs.). There was no difference between reduction rates from routine samples to 5-sock-samples when these 3 groups were compared to each other (data not shown), which can be a genuine tendency, but which may also be due to the low number of flocks within each of the groups. However, the day-old chicks were Salmonella positive in the flocks with ST41, and it is likely that the chicks in the persistently infected houses became Salmonella positive within a few days after their arrival to the houses, because they are more prone to get a Salmonella infection before the intestinal microbial flora is established (*Desmidt et al.* 1998). Several studies have described conditions in the litter which can influence the Salmonella status of broiler flocks (e.g. *Turnbull & Snoeyenbos* 1973, *Weinack et al.* 1979, *Opara et al.* 1992, *Carr et al.* 1995). An increase in ammonia contents and pH in the litter is generally seen during the period when the broilers are raised in the houses, and these increases are detrimental to the survival of Salmonella (*Turnbull & Snoeyenbos* 1973). Other factors such as water activity and moisture content also have a great impact on the survival of Salmonella in litter (*Turnbull & Snoeyenbos* 1973, *Opara et al.* 1992, *Carr et al.* 1995), however, as these factors are not clearly related to the age of the litter it is more difficult to estimate their practical relevance in this study.

In conclusion, this study indicated that sampling faecal material from Salmonella infected chicken flocks by means of 2 pairs of socks worn on the footwear and analysed as one pool, was at least as effective in detecting Salmonella as hand collection of 60 samples of fresh faecal material analysed as one pool. Moreover, it has clearly been shown that, under the present conditions in Danish broiler production, sock samples taken in broiler houses at about 3 weeks of age are more effective in detecting Salmonella than sock samples taken a few days prior to slaughter.

Acknowledgements

The Salmonella laboratory technicians and Jens Christian Jørgensen at the Danish Veterinary Institute in Aarhus, the District Veterinary Officers and the poultry farmers are thanked for excellent collaboration.

References

Anonymous: Microbiology – General guidance on methods for the detection of *Salmonella. ISO 6579: 1993(E),* 1-16. 1993.

Anonymous: Microsoft Access, version 97, 1989-1997. Microsoft Corporation. 1997a.

Anonymous: Microsoft Excel 97 SR-2. 1985-1997. Microsoft Corporation. 1997b.

Anonymous: Annual report on zoonoses in Denmark 1999, 1-28. 1999a.

Anonymous: S-plus 2000 professional Release 2, Mathsoft inc. 1999b.

Bisgaard M: A voluntary *Salmonella* control programme for the broiler industry, implemented by the Danish Poultry Council. Int. J. Food Microb. 1992, *15,* 219-224.

Byrd JA, Corrier DE, DeLoach JR, Nisbet DJ: Comparison of drag-swab environmental protocols for the isolation of *Salmonella* in poultry houses. Avian Dis. 1997, *41,* 709-713.

Caldwell DJ, Hargis BM, Corrier DE, Vidal L, DeLoach JR: Evaluation of persistence and distribution of salmonella serotype isolation from poultry farms using drag-swab sampling. Avian Dis. 1995, *39,* 617-621.

Caldwell DJ, Hargis BM, Corrier DE, DeLoach JR: Frequency of isolation of *Salmonella* from protective foot covers worn in broiler houses as compared to drag-swab sampling. Avian Dis. 1998, *42,* 381-384.

Carr LE, Mallinson ET, Tate CR, Miller RG, Russek-Cohen E, Stewart LE, Opara OO, Joseph SW: Prevalence of Salmonella in broiler flocks: Effect of litter, water activity, house construction and watering devices. Avian Dis. 1995, *39,* 39-44.

Cohen J: A coefficient of agreement for nominal scales. Educ. Psychol. Meas. 1960, *20,* 37-46.

Desmidt M, Ducatelle R, Haesebrouck F: Serological and bacteriological observations on experimental infection with *Salmonella hadar* in chickens. Vet. Microbiol. 1998, *60,* 259-269.

Feld NC, Ekeroth L, Gradel KO, Kabell S, Madsen M: Evaluation of a serological *Salmonella* Mix-ELISA for poultry used in a national surveillance programme. Epidemiol. Infect. 2000, *125,* 263-268.

Fleiss JL: Statistical Methods for Rates and Proportions. 1981, 2. ed. Wiley. New York, 1981.

Gustafson RH, Kobland JD: Factors influencing salmonella shedding in broiler chickens. J. Hyg. Camb. 1984, *92,* 385-394.

Higgins R, Malo R, René Roberge R, Gauthier R: Studies on the dissemination of *Salmonella* in nine broiler-chicken flocks. Avian Dis. 1982, *26,* 26-33.

Hubert LJ, Arabie P: Comparing proportions. J. of Classification 1985, *2,* 193-218.

Kingston DJ: A Comparison of culturing drag swabs and litter for identification of infections with *Salmonella* spp. in commercial chicken flocks. Avian Dis. 1980, *25,* 513-516.

Landis J, Koch GG: The measurement of observer agreement for categorical data. Biometrics 1977, *33,* 159-174.

Muir WI, Bryden WL, Husband AJ: Comparison of *Salmonella typhimurium* challenge models in chickens. Avian Dis. 1998, *42,* 257-264.

Opara OO, Carr LE, Russek-Cohen E, Tate CR, Mallinson ET, Miller RG, Stewart LE, Johnston RW, Joseph SW: Correlation of water activity and other enviromental conditions with repeated detection of salmonella contamination on poultry farms. Avian Dis. 1992, *36,* 664-671.

Popoff Michel Y, Le Minor Léon: Antigenic formulas of the *Salmonella* serovars, 2-151. WHO Collaborating Centre for Reference and Research on *Salmonella.* Paris, 1997.

SAS Institute Inc.: SAS STAT, version 8.00. 1999.

Skov MN, Carstensen B, Tornøe N, Madsen M: Evaluation of sampling methods for the detection of Salmonella in broiler flocks. J. Appl. Microbiol. 1999, *86,* 695-700.

Skov MN, Feld NC, Carstensen B, Madsen M: The serological response to *Salmonella* Enteritidis and S. Typhimurium in experimentally infected chickens, followed with an indirect LPS-ELISA and bacteriological examinations through a one-year period. Avian Dis. 2000 (submitted).

Snoeyenbos GH, Weinack Olga M, Smyser CF: Protecting chicks and poults from salmonellae by oral administration of "normal" gut microflora. Avian Dis. 1978, *22,* 273-286.

Turnbull PCB, Snoeyenbos GH: The roles of ammonia, water activity, and pH in the salmonellacidal effect of long-used poultry litter. Avian Dis. 1973, *17,* 72-86.

Weinack Olga M, Smyser CF, Snoeyenbos GH: Evaluation of several methods of detecting salmonellae in groups of chickens. Avian Dis. 1979, *23,* 179-193.

Sammendrag

Påvisning af Salmonella i danske salmonellainficerede kyllinge- og hønseflokke i gulvsystemer: Sammenligning af prøvemetoder og -tidspunkter.

Opfølgende bakteriologiske prøver blev udtaget fra 41 hønse-/kyllingeflokke *(Gallus gallus)* på dybstrøelse, i hvilke *Salmonella enterica* enten var påvist direkte ved bakteriologiske eller indirekte ved hjælp af serologiske undersøgelser. Følgende 3 typer af opfølgende prøver blev sammenlignet indbyrdes inden for flokkene: 1) 5 par sokker, analyseret som 5 prøver, 2) 2 par sokker, analyseret som én prøve, og 3) 60 gødningsprøver, analyseret som én prøve. Overensstemmelse mellem prøvetagningsprocedurerne blev undersøgt ved hjælp af følgende statistiske tests: 'Kappa', 'The adjusted rand', McNemar´s test for marginal symmetry, Proportion of agreement P_0, P_+, P_- samt Odds Ratio. Den højeste overensstemmelse blev fundet mellem de 2 typer sokkeprøver, mens den laveste overensstemmelse blev fundet, da 60 gødningsprøver blev sammenlignet med 5 par sokker. To par sokker analyseret som én pool fremstod i undersøgelsen som lige så effektive til at detektere *S. enterica* som de 60 poolede gødningsprøver. I slagtekyllingeflokke blev 5 par sokker anvendt både i de rutinemæssige ante mortem undersøgelser ved ca. 3 ugers alderen og som én af de opfølgende prøver, hvor de eneste betydende forskelle mellem prøvetagningsrunderne udgjordes af slagtekyllingernes og strøelsens alder. Inden for den samme flok blev *S. enterica* oftere fundet i 5 par sokker fra slagtekyllinger, som var cirka 3 uger gamle, end i 5 par sokker fra slagtekyllinger, som skulle slagtes inden for få dage.

(Received October 11, 2000; accepted October 8, 2001)

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