



HAL
open science

Investigation of the concurrent colonization with and in poultry flocks and assessment of the sampling site for status determination at slaughter

G. Rasschaert, K. Houf, J. van Hende, L. de Zutter

► To cite this version:

G. Rasschaert, K. Houf, J. van Hende, L. de Zutter. Investigation of the concurrent colonization with and in poultry flocks and assessment of the sampling site for status determination at slaughter. *Veterinary Microbiology*, 2007, 123 (1-3), pp.104. 10.1016/j.vetmic.2007.03.011 . hal-00532223

HAL Id: hal-00532223

<https://hal.science/hal-00532223>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Investigation of the concurrent colonization with *Campylobacter* and *Salmonella* in poultry flocks and assessment of the sampling site for status determination at slaughter

Authors: G. Rasschaert, K. Houf, J. Van Hende, L. De Zutter

PII: S0378-1135(07)00148-4
DOI: doi:10.1016/j.vetmic.2007.03.011
Reference: VETMIC 3625

To appear in: *VETMIC*

Received date: 15-11-2006
Revised date: 15-3-2007
Accepted date: 22-3-2007

Please cite this article as: Rasschaert, G., Houf, K., Van Hende, J., De Zutter, L., Investigation of the concurrent colonization with *Campylobacter* and *Salmonella* in poultry flocks and assessment of the sampling site for status determination at slaughter, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.03.011

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



1 **Investigation of the concurrent colonization with *Campylobacter* and *Salmonella* in**
2 **poultry flocks and assessment of the sampling site for status determination at slaughter**

3

4 G. Rasschaert, K. Houf, J. Van Hende, L. De Zutter*

5

6 Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine,

7 Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

8

* Corresponding author. Tel.: +32 2647455; fax: +32 2647491
E-mail address: lieven.dezutter@ugent.be

1 **Abstract**

2 Fifty six broiler flocks and 20 laying hen and breeder flocks were sampled in six
3 slaughterhouses for the presence of *Campylobacter* and *Salmonella*. Samples were taken from
4 three different sites of the gastrointestinal tract, namely from the crop, the duodenum and the
5 ceca. The prevalence of flocks colonized with *Campylobacter* and *Salmonella* was determined
6 and an association between the concurrent colonization with these two pathogens was
7 investigated. Furthermore, the best sampling site for status determination at the
8 slaughterhouse level was evaluated.

9 Of the broiler flocks, 73% were colonized with *Campylobacter*, whereas 13% were
10 *Salmonella* positive at slaughter. Concerning the laying hen and breeder flocks, all flocks
11 were colonized with *Campylobacter* and 65% of the flocks were *Salmonella* positive. No
12 association was found between *Campylobacter* and *Salmonella* occurrence in broiler flocks.
13 Since all laying hen and breeder flocks were colonized with *Campylobacter*, no association
14 between the concurrent colonization with the two pathogens could be determined. At the
15 slaughterhouse level, sampling only the duodena was sufficient to determine the
16 *Campylobacter* status of poultry flocks, whereas the three sampling sites had to be analyzed
17 to detect all flocks colonized with *Salmonella*.

18

19

20 Key words: *Campylobacter*; *Salmonella*; broiler flocks; laying hen and breeder flocks;

21 concurrent colonization

1 **1. Introduction**

2

3 *Campylobacter* and *Salmonella* are two major causes of human bacterial gastroenteritis in the
4 industrialized world. Contaminated poultry meat is an important source of infection for both
5 zoonoses. Many studies have determined the prevalence of broiler flocks colonized with
6 *Campylobacter*, *Salmonella*, or both, at slaughter age. The reported prevalence ranges from
7 3% in Finland to more than 90% in the UK for *Campylobacter*, and from 4% in Canada to
8 67% in Japan for *Salmonella* (Chambers et al., 1998; Limawongpranee et al., 1998; Evans and
9 Sayers, 2000; Perko-Mäkelä et al., 2002). Only few studies have investigated the possible
10 association between the occurrence of *Campylobacter* and *Salmonella* in poultry flocks.
11 Though no association was found in a Danish study (Wedderkopp et al., 2001), a positive
12 correlation was reported in Dutch poultry flocks (Jacobs-Reitsma et al., 1994; Jacobs-
13 Reitsma, 1995).

14 The intestines, especially the ceca are the primary sites of colonization for both
15 *Campylobacter* and *Salmonella* (Fanelli et al., 1970; Beery et al., 1988; Achen et al., 1998).
16 Birds are subjected to feed withdrawal during transport to the slaughterhouse, resting time,
17 and sometimes even a few hours before transport. Some studies have demonstrated that
18 following feed withdrawal more crops than ceca were contaminated with *Campylobacter* and
19 *Salmonella* (Hargis et al., 1995; Byrd et al., 1998). Therefore, it is possible that the ceca are
20 not the best sampling site for determining the *Campylobacter* and *Salmonella* prevalence in
21 flocks at the slaughterhouse level. However, the studies above are performed on individual
22 birds and it is not clear if the results reported can be extrapolated to the flock level.

23 The study aimed to determine the prevalence of *Campylobacter* and *Salmonella* colonized
24 broiler flocks and laying hen and breeder flocks at slaughter age; to investigate an association
25 between colonization with *Campylobacter* and *Salmonella* in poultry flocks; and to evaluate

1 the best sampling site for determining the *Campylobacter* and *Salmonella* prevalence in flocks
2 at the slaughterhouse level.

3

4 **2. Materials and methods**

5

6 *2.1. Sampling*

7 During the period from January 2002 to March 2004, 76 flocks were examined for the
8 presence of *Campylobacter* and *Salmonella*. A flock was defined as all birds reared in the
9 same poultry house on a farm. The sampled flocks consisted of 56 broiler flocks and 20 spent
10 laying hen and breeder flocks. Flock size ranged from 1,100 to 18,000 chickens. Broiler
11 flocks were between 35 and 42 days old when slaughtered, whereas the laying hen and
12 breeder flocks were between 10 and 26 months old. The broiler flocks were slaughtered in
13 four Belgian slaughterhouses, and the laying hen and breeder flocks were slaughtered in three
14 other Belgian slaughterhouses. The flocks were sampled just before slaughter and during
15 processing. From each flock, 30 live birds were randomly chosen from 15 crates from
16 different transport containers. Of each bird, a swab sample of the crop was taken. In addition,
17 30 gastrointestinal tracts per flock (95% CI to detect a prevalence of 10% for more than 1,000
18 birds) were randomly collected from the slaughter line just after mechanical evisceration. All
19 samples were packed in sterile plastic bags, transported to the laboratory under cooled
20 conditions, and processed the same day.

21

22 *2.2. Bacterial culture for Salmonella*

23 From each of the 30 gastrointestinal tracts, 1 g of duodenal content and 1 g of one cecum
24 were aseptically collected. These samples were pooled resulting in three subsamples of 10 g
25 ceca content and three subsamples of 10 g duodenal content. The pooled samples were

1 homogenized for 1 min. with 90 ml of buffered peptone water (BPW, Oxoid, Basingstoke,
2 UK) in a stomacher blender at normal speed. The 30 crop swabs were pooled to three
3 subsamples and homogenized with 25 ml of BPW each. The homogenates were incubated at
4 37°C for 16 to 20 h. One portion of 100 µl of each of the homogenates was plated in the
5 middle of Diagnostic Semi-Solid *Salmonella* Agar plates (Diassalm, LabM 537, Lancashire,
6 UK). Furthermore, 100 µl of each homogenate was added to 10 ml Rappaport-Vassiliadis
7 broth (RV, Oxoid CM669, Basingstoke, UK). After incubation for 24 h at 42°C, a loopful of
8 the discolored Diassalm plates and 10 µl of all RV tubes were plated onto Xylose Lysine
9 Deoxycholate plates (XLD, Oxoid CM469, Basingstoke, UK) and incubated at 37°C for 24h.
10 Presumptive *Salmonella* colonies were confirmed at genus level by PCR using the primers
11 described by Aabo et al. (1993). The reaction mixture and amplification protocol were as
12 described by Botteldoorn et al. (2003). Per flock, one *Salmonella* isolate per pooled sample
13 was further characterized, which resulted in a maximum of nine isolates per flock if all plates
14 showed *Salmonella* growth. The *Salmonella* isolates were serotyped by the Belgian reference
15 laboratory for *Salmonella* according to the Kauffman-White scheme (Popoff and Le Minor,
16 1997).

17

18 2.2. Bacterial culture for *Campylobacter*

19 Of each of the BPW homogenates, 1 ml was added to 9 ml selective Preston broth (Nutrient
20 Broth n°2 CM 67, Oxoid, Basingstoke UK, enriched with 5% (v/v) lysed defibrinated horse
21 blood and 1% Preston Supplement (5000 IU polymixin B, 0.010 g rifampicin, 0.0076 g
22 trimethoprim and 0.010 g amphotericin dissolved in 10 ml ethanol)). The enrichment broths
23 were incubated for 24 to 48 h at 42°C under microaerobic conditions (6% CO₂, 6% H₂, 4% O₂
24 and 84% N₂). After 24 h, 10 µl of each enrichment broth was plated onto modified
25 Cefoperazone Charcoal Deoxycholate Agar (mCCDA; CM 739 plus SR155, Oxoid,

1 Basingstoke, UK) and incubated at 42°C under microaerobic conditions. If there was no
2 *Campylobacter* growth on mCCDA after 24 h of incubation, 10 µl of the 48-h incubated
3 enrichment broth was plated on a new mCCDA plate. Morphologically typical colonies were
4 picked, examined by Gram staining, subcultured on a blood agar plate (CM 965 and L13,
5 Oxoid, Basingstoke, UK and 5% (v/v) defibrinated horse blood) and stored in whole horse
6 blood at -80°C for further examination. Per flock, one *Campylobacter* isolate per pooled
7 sample was stored, which resulted in a maximum of nine isolates per flock if all plates
8 showed *Campylobacter* growth. Identification on species level was performed according to
9 the PCR assay of Vandamme et al. (1997) for *C. jejuni* and *C. coli*, and according to the PCR
10 assay of Linton et al. (1996) for *C. lari*.

11 A flock was considered *Salmonella* or *Campylobacter* colonized, when *Salmonella* or
12 *Campylobacter*, respectively, was detected in at least one of the pooled samples of the crop,
13 the duodenum or the ceca.

14

15 2.3. Statistical analysis

16 The chi square (χ^2) test was used to investigate an association between *Campylobacter* and
17 *Salmonella* occurrence in poultry flocks, and to determine the differences in *Salmonella* and
18 *Campylobacter* colonization of the crops, the duodena and the ceca. Differences were
19 considered to be nonsignificant at $p \geq 0.05$. If the data contained frequencies lower than 5, the
20 Cramers' V test was used. The outcome of Cramers' V ranges from -1 (negative association)
21 to 1 (positive association), with 0 indicating no association.

22

23 3. Results

24

25 3.1. Broiler flocks

1 Forty one (73%) broiler flocks were colonized with *Campylobacter*, whereas *Salmonella* was
2 isolated from seven flocks (13%). Three flocks were positive for both *Campylobacter* and
3 *Salmonella*, and 11 flocks were negative for the two species (Table 1). There was little to no
4 association between *Campylobacter* and *Salmonella* occurrence in broiler flocks
5 (Cramers'V=0.26). Of the 41 *Campylobacter* positive flocks, 32 flocks were colonized with
6 only one *Campylobacter* species: 29 flocks with *C. jejuni*, two flocks with *C. coli* and one
7 flock with *C. lari*. Six flocks carried two species in the gastrointestinal tract: four flocks
8 harboured *C. jejuni* and *C. coli* in the intestines, whereas the other two were colonized with *C.*
9 *jejuni* and *C. lari*. The isolates of the three remaining flocks were not identified on species
10 level. The seven *Salmonella* positive flocks were each colonized with only one *Salmonella*
11 serotype: two flocks were colonized with serotype Typhimurium O5+, two flocks with
12 serotype Infantis, whereas the other three flocks were colonized with serotype London,
13 serotype Hadar and an isolate which was not typeable by serotyping, respectively.

14 The site of colonization for the *Campylobacter* and *Salmonella* positive flocks is shown in
15 Table 2. Concerning the *Campylobacter* colonized broiler flocks, there was a significant
16 difference between the presence of *Campylobacter* in the three sampling sites (χ^2 test,
17 $p=0.038$). More flocks were colonized in the duodena and ceca than in the crops. On the other
18 hand, there was no association between the prevalence of *Salmonella* in the three sampling
19 sites (Cramers'V=0.040). Sampling only the duodena was sufficient to detect all
20 *Campylobacter* positive flocks, whereas the three sampling sites had to be analyzed to
21 determine the *Salmonella* status of flocks at the slaughterhouse level (Table 2).

22

23 3.2. Laying hens and breeder flocks

24 *Campylobacter* was isolated from each of the 20 sampled flocks (100%), whereas *Salmonella*
25 was isolated from 13 (65%) flocks (Table 1). Since all flocks were colonized with

1 *Campylobacter*, it was not possible to determine an association between *Campylobacter* and
2 *Salmonella* occurrence in these flocks. Four flocks were colonized with only one
3 *Campylobacter* species (*C. jejuni*), 11 flocks with two species (*C. jejuni* and *C. coli*), and five
4 flocks with three species (*C. jejuni*, *C. coli* and *C. lari*). From 11 of the 13 *Salmonella*
5 positive flocks, serotype Enteritidis was isolated. The two remaining flocks were colonized
6 with serotype Braenderup and a strain which was not typeable by serotyping, respectively.
7 There was a weak association between the presence of *Campylobacter* in the three sampling
8 sites (Cramers'V=0.51). As for the broiler flocks, the duodena and the ceca were more often
9 colonized than the crops. Sampling the duodena or the ceca was sufficient to determine the
10 *Campylobacter* status. Concerning *Salmonella*, there was no significant difference between
11 the prevalence in the three sampling sites (χ^2 test, $p=0.333$). As for the broiler flocks,
12 sampling the three sampling sites was necessary to detect all flocks colonized with
13 *Salmonella*.

14

15

16 **4. Discussion**

17

18 In the present study, 73% of the broiler flocks and 100% of the laying hen and breeder flocks
19 were colonized with *Campylobacter*. This prevalence of *Campylobacter* colonized broiler
20 flocks is in agreement with another Belgian study (Herman et al., 2003), in which 67% of the
21 broiler flocks were colonized with *Campylobacter* at slaughter. In contrast to broiler flocks,
22 only limited data is available about the *Campylobacter* prevalence in laying hen and breeder
23 flocks. Moreover, the duration of colonization in older birds is not completely clear. It is
24 generally accepted that for broiler flocks, the colonization persists at least until the slaughter
25 age of 6 weeks (Newell and Fearnley, 2003). As the birds get older, the number of infected

1 birds and the level of campylobacters recoverable may gradually reduce (Newell and
2 Wagenaar, 2000). Self-limitation of colonization and the presence of antibodies against *C.*
3 *jejuni* without colonization have also been reported (Newell and Fearnley, 2003). However,
4 instead of reduced colonization, the present study reveals that colonization in laying hen and
5 breeder flocks is higher than in broiler flocks in terms of the number of flocks colonized and
6 the number of species isolated from the gastrointestinal tract.

7 In the present study, 13% of the broiler flocks were colonized with *Salmonella* at slaughter.
8 This prevalence is comparable to the results of the study of van de Giessen et al. (2006), in
9 which approximately 12% of the broiler flocks at slaughter age were colonized with
10 *Salmonella* (van de Giessen et al., 2006). This is a decline compared to a previous Belgian
11 study of Heyndrickx et al. (2002) and an earlier Dutch study of Jacobs-Reitsma et al. (1994)
12 in which 33% and 27% of the examined broiler flocks at slaughter age were colonized with
13 *Salmonella*, respectively. In the present study, 65% of the laying hen and breeder flocks were
14 colonized with *Salmonella*. In execution of regulation EC/2160/2003 on the control of
15 *Salmonella* and other specified foodborne zoonotic agents, the E.U. have published a report
16 about the *Salmonella* prevalence in laying hen flocks during the last nine weeks of their
17 production period in 2004-2005. The prevalence ranged from 0% in Luxembourg and Sweden
18 to 80% in Portugal (EFSA, 2006). The prevalence in Belgium (38%) was almost half of the
19 reported prevalence in the present study. In the EFSA study, the prevalence was determined at
20 the farm by collecting fecal and environmental samples during the last nine weeks of their
21 production period, whereas in the present study three different sites of the gastrointestinal
22 tract of the birds were sampled at slaughter. That way, only flocks shedding *Salmonella* are
23 detected in the EFSA study, whereas flocks carrying *Salmonella* without shedding the
24 bacteria are detected by our sampling method. This may be an explanation for the higher
25 prevalence found in the present study. Another possibility is that flocks, *Salmonella*-free nine

1 weeks before slaughter, acquire an infection during the last weeks of their production period
2 or even during transport as demonstrated by Rigby and Pettit (1980). A last explanation is that
3 in the time span between the present study and the EFSA study, the *Salmonella* prevalence is
4 reduced due to the increased vaccination of laying hen flocks.

5 In the present study, no positive or negative association was found between the presence of
6 *Campylobacter* and *Salmonella* in broiler flocks. This in agreement with the results of
7 Wedderkopp et al. (2001) but in contrast with the outcome of the study of Jacobs-Reitsma et
8 al. (1994). They reported a positive correlation between the colonization with these two
9 organisms in broiler flocks. *Campylobacter* free flocks were more often also *Salmonella* free,
10 and *Campylobacter* positive flocks were more often also positive for *Salmonella*. The lack of
11 an association in the present study may indicate that the transmission routes for flock
12 colonization with *Campylobacter* and *Salmonella* are independent in Belgium. Jacobs-
13 Reitsma (1995) also reported a positive correlation between *Campylobacter* and *Salmonella*
14 occurrence in breeder flocks. However, since all laying hen and breeder flocks were colonized
15 with *Campylobacter* in the present study, no correlation could be demonstrated.

16 In the present study, the duodenum was most often found positive for *Campylobacter* or
17 *Salmonella*, followed by the ceca and finally the crop. This in contrast with the studies of a
18 research group (Hagris et al., 1995; Ramirez et al., 1997; Corrier et al., 1999) which has
19 demonstrated that feed withdrawal in market-age broilers resulted in an increased incidence of
20 *Salmonella*-positive crops and less pronounced in *Salmonella*-positive ceca. The number of
21 *Salmonella*-positive crops may even exceed the number of positive ceca. Byrd et al. (1998)
22 observed the same for *Campylobacter* colonized flocks. However, these studies were
23 performed on individual birds, whereas the present study is performed on flock level, which is
24 a possible explanation for the different outcome. It is remarkable in the present study that for
25 the broiler flocks as well as the laying hen and breeder flocks, sampling only the duodenum

1 was sufficient to detect all flocks colonized with *Campylobacter*, whereas the three sampling
2 sites had to be analyzed to determine the *Salmonella* status of flocks.

3 In conclusion, the present study has shown that there was no association between the
4 concurrent colonization with *Campylobacter* and *Salmonella* in broiler flocks. Sampling only
5 the duodenum has been shown to be sufficient to determine the *Campylobacter* status in
6 poultry flocks at the slaughterhouse level. However, it is still necessary in epidemiological
7 studies to sample the three sites, as the genotypes of the strains isolated from crop, duodenum
8 and ceca may differ (Rasschaert et al., 2006). For determining the *Salmonella* prevalence in
9 poultry flocks at the slaughterhouse level, a combination of the three sampling sites is
10 necessary.

11

12 **Acknowledgements**

13 Special thanks to S. Vangeenberghe for excellent technical assistance. We also want to thank
14 the staff of the seven slaughterhouses to their kind cooperation during sampling.

1 **References**

- 2 Aabo, S., Rasmussen, O.F., Rossen, L., Sørensen, P.D., Olsen, J.E., 1993. *Salmonella*
3 identification by the polymerase chain reaction. *Mol. Cell. Probes* 7, 171-178.
- 4 Achen, M., Morishita, T.Y., Ley, E.C., 1998. Shedding and colonization of *Campylobacter*
5 *jejuni* in broilers from day-of-hatch to slaughter age. *Avian Dis.* 42, 732-7.
- 6 Beery, J.T., Hugdahl, M.B., Doyle M.P., 1988. Colonization of gastrointestinal tracts of
7 chicks by *Campylobacter jejuni*. *Appl. Environ. Microbiol.* 54, 2365-2370.
- 8 Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K., Herman, L., 2003. *Salmonella*
9 on pig carcasses: positive pigs and cross contamination in the slaughterhouse. *J. Appl.*
10 *Microbiol.* 95, 891–903.
- 11 Byrd, J.A., Corrier, D.E., Hume, M.E., Bailey, R.H., Stanker, L.H., Hargis, B.M., 1998.
12 Incidence of *Campylobacter* in crops of preharvest market-age broiler chickens. *Poultry*
13 *Sci.* 77, 1303-1305.
- 14 Chambers, J.R., Bisailon, J-R., Labbé, Y., Poppe, C., Langford, C.F., 1998. *Salmonella*
15 prevalence in crops of Ontario and Quebec broiler chickens at slaughter. *Poultry Sci.* 77,
16 1497-1501.
- 17 Corrier, D.E., Byrd, J.A., Hargis, B.M., Hume, M.E., Bailey, R.H., Stanker, L.H., 1999.
18 Presence of *Salmonella* in the crop and ceca of broiler chickens before and after
19 preslaughter feed withdrawal. *Poultry Sci.* 78, 45-49.
- 20 EFSA, European Food Safety Authority (2006). Report of the task force on zoonoses data
21 collection on the analysis of the baseline study on the prevalence of *Salmonella* in
22 holdings of laying hen flocks of *Gallus gallus*. [Online] [http://www.efsa.europa.eu/en/
23 science/monitoring_zoonoses/reports/report_finlayinghens.html](http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports/report_finlayinghens.html)
- 24 Evans, S.J., Sayers, A.R., 2000. A longitudinal study of *Campylobacter* infection of broiler
25 flocks in Great Britain. *Prev. Vet. Med.* 46, 209-223.

- 1 Fanelli, M.J., Sadler, W.W., Franti, C.E., Brownell, J.R., 1970. Localization of Salmonellae
2 within the intestinal tract of chickens. *Avian Dis.* 15, 366-395.
- 3 Hargis, B.M., Caldwell, D.J., Brewer, R.L., Corrier, D.E., Deloach, J.R., 1995. Evaluation of
4 the chicken crop as a source of *Salmonella* contamination for broiler carcasses. *Poultry*
5 *Sci.* 74, 1548-1552.
- 6 Herman, L., Heyndrickx, M., Grijspeerdt, K., Vandekerchove, D., Rollier, I., De Zutter, L.,
7 (2003). Routes for *Campylobacter* contamination of poultry meat: epidemiological study
8 from hatchery to slaughterhouse. *Epidemiol. Infect.* 131, 1169-1180.
- 9 Jacobs-Reitsma, W.F., Bolder, N.M., Mulder, R.W.A.W., 1994. Cecal carriage of
10 *Campylobacter* and *Salmonella* in Dutch broiler flocks at slaughter: a one-year study.
11 *Poultry Sci.* 73, 1260-1266.
- 12 Jacobs-Reitsma, W.F., 1995. *Campylobacter* bacteria in breeder flocks. *Avian Dis.* 39: 355-
13 359.
- 14 Limawongpranee, S., Hayashidani, H., Okatani, A.T., Ono, K., Hirota, C., Kaneko, K.,
15 Ogawa M., 1998. Prevalence and persistence of *Salmonella* in broiler chicken flocks. *J.*
16 *Vet. Med. Sci.* 61, 255-259.
- 17 Linton, D., Owen, R.J., Stanley, J., 1996. Rapid identification of the genus *Campylobacter*
18 and five *Campylobacter* species enteropathogenic for man and animals. *Res. Microbiol.*
19 147, 707-718.
- 20 Newell, D.G., Fearnley, C., 2003. Sources of *Campylobacter* colonization in broiler chickens.
21 *Appl. Environ. Microbiol.* 69, 4343-4351.
- 22 Newell, D.G., Wagenaar, J.A., 2000. Poultry infections and their control at the farm level, p:
23 497-509. In I. Nachamkin and M.J. Blaser (eds.), *Campylobacter* 2nd edition, American
24 Society for Microbiology, Washington D.C., US.

- 1 Perko-Mäkelä, P., Hakkinen, M., Honkanen-Buzalski, T., Hänninen, M.-L., 2002) Prevalence
2 of campylobacters in chicken flocks during the summer of 1999 in Finland. *Epidemiol.*
3 *Infect.* 129, 187-192.
- 4 Popoff, M.Y., Le Minor, L., 1997. Antigenic formulas of the *Salmonella* serovars, 7th
5 Revision. Report of the WHO Collaborating Centre for Reference and Research on
6 *Salmonella*. Institute Pasteur, Paris, France.
- 7 Ramirez, G.A., Sarlin, L.L., Caldwell, D.J., Yezak, C.R., Mume, M.E., Corrier, D.E.,
8 Deloach, J.R., Hargis, B.M., 1997. Effect of feed withdrawal on the incidence of
9 *Salmonella* in the crops and ceca of market age broiler chickens. *Poultry Sci.* 76, 654-656.
- 10 Rasschaert, G., Houf, K., Van Hende, J., De Zutter, L., 2006. *Campylobacter* contamination
11 during poultry slaughter in Belgium. *J. Food Prot.* 69, 27-33.
- 12 Rigby, C.E., Pettit, J.R., 1980. Changes in the *Salmonella* status of broiler chickens subjected
13 to simulated shipping conditions. *Can. J. comp. Med.* 44, 374-381.
- 14 Vandamme, P., Van Doorn, L.-J, Al Rashid, S.T., Quint, W.G.V., Van Der Plas, J., Chan
15 V.L., On, S.L.W., 1997. *Campylobacter hyoilei* Alterton et al. 1995 and *Campylobacter*
16 *coli* Véron and Chatelain 1973 are subjective synonyms. *Int. J. Syst. Bacteriol.* 47, 1055-
17 1060.
- 18 van de Giessen, A.W., Bouwknegt, M., Dam-Deisz, W.D.C., van Pelt, W., Wannet, W.J.B.,
19 Visser, G. (2006). Surveillance of *Salmonella* spp. and *Campylobacter* spp. in poultry
20 production flocks in The Netherlands. *Epidemiol. Infect.* 134, 1266-1275.
- 21 Wedderkopp, A., Gradel, K. O., Jørgensen, J. C., Madsen, M., 2001. Pre-harvest surveillance
22 of *Campylobacter* and *Salmonella* in Danish broiler flocks: a 2-year study. *Int. J. Food*
23 *Microbiol.* 68, 53-59.

1 **Table 1.** The distribution in the prevalence of *Campylobacter* and *Salmonella* colonized
 2 flocks.

Broiler flocks			
	<i>Campylobacter</i> +	<i>Campylobacter</i> -	total
<i>Salmonella</i> +	3	4	7
<i>Salmonella</i> -	38	11	49
total	41	15	56
Laying hen and breeder flocks			
	<i>Campylobacter</i> +	<i>Campylobacter</i> -	total
<i>Salmonella</i> +	13	0	13
<i>Salmonella</i> -	7	0	7
total	20	0	20

3

1 **Table 2.** The distribution of *Campylobacter* and *Salmonella* colonized flocks for individual
 2 and combined sampling sites.

	crop	duodenum	ceca	crop +duodenum	duodenum +ceca	crop +ceca	total
Broiler flocks							
<i>Campylobacter</i> +	28 (68%)	41 (100%)	36 (88%)	41 (100%)	41 (100%)	39 (95%)	41
<i>Salmonella</i> +	3 (43%)	3 (43%)	2 (29%)	6 (86%)	4 (57%)	5 (71%)	7
Laying hen and breeder flocks							
<i>Campylobacter</i> +	13 (65%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20 (100%)	20
<i>Salmonella</i> +	6 (46%)	10 (77%)	7 (54%)	12 (92%)	12 (92%)	10 (77%)	13

3

4