

Surveillance of *Salmonella* spp. and *Campylobacter* spp. in poultry production flocks in The Netherlands

A. W. VAN DE GIESSEN^{1*}, M. BOUWKNEGT¹, W. D. C. DAM-DEISZ¹,
W. VAN PELT², W. J. B. WANNET³ AND G. VISSER⁴

¹ Microbiological Laboratory for Health Protection, National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands

² Centre for Infectious Diseases Epidemiology, RIVM, Bilthoven, The Netherlands

³ Laboratory for Infectious Diseases Diagnostics and Screening, RIVM, Bilthoven, The Netherlands

⁴ Inspectorate for Health Protection and Veterinary Public Health, Zutphen, The Netherlands

(Accepted 23 November 2005, first published online 2 May 2006)

SUMMARY

In The Netherlands, a national programme for the surveillance of zoonotic bacteria in farm animals has been operative since 1997. We describe the results of the surveillance of *Salmonella* spp. in flocks of laying hens and broilers and of *Campylobacter* spp. in broiler flocks in the period 1999–2002. The prevalence of *Salmonella* spp. in laying-hen flocks has significantly decreased from 21·1% in 1999 to 13·4% in 2002. This decreasing trend might indicate that the control measures taken by the poultry industry were effective. *S. Enteritidis* was the predominant serovar in laying hens accounting for one third of the positive flocks. Although prevalence estimates for *Salmonella* spp. in broiler flocks did not yield a significant decreasing trend in 1999–2002, a decrease in *Salmonella* prevalence to 11% was measured in 2002. During the study period, *S. Paratyphi B* var. Java emerged in broilers to become the predominant serovar in 2002 accounting for one third of the positive flocks. The prevalence of *Campylobacter* spp. in broiler flocks did not increase nor decrease continuously between 1999 and 2002, which roughly corresponds with the monitoring results from the poultry industry. In this period, the estimated flock prevalence roughly averaged around 20%, with *C. jejuni* being the predominant species. The approach of monitoring presented in this paper can serve as a blueprint for monitoring schemes in farm animal populations to be developed in the context of the EC Zoonoses Directive.

INTRODUCTION

Salmonella and *Campylobacter* spp. remain the most common bacterial causes of human diarrhoeal illness in many countries [1]. In The Netherlands, in 1996–1999, a sentinel study on gastroenteritis in general practices was conducted in which *Salmonella*

and *Campylobacter* spp. were associated with 4 and 10% of the cases respectively [2]. In addition, in 1999–2000, a study was conducted in the Dutch general population indicating the total number of cases of salmonellosis and campylobacteriosis to be approximately 50 000 and 100 000 respectively [3]. Whereas the number of laboratory-confirmed cases of campylobacteriosis in The Netherlands remained at a fairly constant level, the number of confirmed human *Salmonella* infections has declined consistently during the past decade [4].

* Author for correspondence: Dr A. W. van de Giessen, National Institute of Public Health and the Environment, Department MGB (63), PO Box 1, 3720 BA Bilthoven, The Netherlands.
(Email: arjen.van.de.giessen@rivm.nl)

For many decades, *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S. Typhimurium*) was the predominant *Salmonella* serotype in humans in The Netherlands. However, from 1988, *Salmonella enterica* serovar Enteritidis (*S. Enteritidis*) has emerged as a major serotype in man, with PT4 being the most prevalent phage type. In 1995, *S. Enteritidis* PT4 accounted for approximately 50% of *Salmonella* infections in humans [5], after which the contribution of this phage type has been more than halved to be replaced by other *S. Enteritidis* phage types as in many other European Union countries [6, 7]. *S. Enteritidis* infections have predominantly been associated with the consumption of raw eggs and egg-containing foods [8, 9]. In The Netherlands, the contribution of eggs to human cases of salmonellosis was estimated at approximately 35% between 1999 and 2002 [6], while more than 80% of the *S. Enteritidis* infections were associated with eggs (W. van Pelt, unpublished observations). Further, infections caused by other *Salmonella* serotypes have mainly been associated with consumption of (undercooked) foods of animal origin, and food animals, especially poultry, pigs and cattle, are considered major sources of infection [6].

The main *Campylobacter* species causing disease in humans are *C. jejuni* (accounting for the vast majority of the cases) and *C. coli* [10, 11]. In The Netherlands, *C. jejuni* and *C. coli* accounted for 89% and 8% respectively, of all laboratory-confirmed cases in 2002 (Y. Doorduyn, unpublished observations). In developed countries worldwide, *Campylobacter* infections in humans have mainly been associated with the consumption of undercooked poultry meat, handling of raw poultry, consumption of raw milk or untreated drinking water, direct contact with pets, foreign travel and, to a lesser extent, with consumption of meat other than poultry [12, 13]. In The Netherlands, 30.5, 32.5 and 31.3% of raw chicken products, including whole carcasses and parts of legs and breasts, sampled at retail in 2000, 2001 and 2002 respectively, were contaminated with campylobacters [14], whereas samples of raw pork and beef were rarely found to be contaminated [15].

In 1997, the Dutch Product Boards for Livestock, Meat and Eggs implemented monitoring and control programmes in the poultry meat and egg production chains to reduce *Salmonella* and *Campylobacter* contamination of poultry meat, and *S. Enteritidis* and *S. Typhimurium* contamination of laying hens [16, 17]. These programmes include amongst others microbiological examination of flocks at each stage of the production chain, application of strict hygiene

measures throughout the production chain and a logistic slaughtering procedure for broiler flocks.

The EC Zoonoses Directive [18] obliges all Member States to report on the occurrence of zoonoses and zoonotic agents annually. From this perspective, in 1997, the Dutch Food and Consumer Product Safety Authority/Inspectorate for Health Protection and Veterinary Public Health (VWA/KvW) commissioned the National Institute for Public Health and the Environment (RIVM) to implement a surveillance programme in farm animals in The Netherlands with the main objective to monitor trends in the occurrence of zoonotic bacteria. In this report, the results of the surveillance of *Salmonella* spp. in flocks of laying hens and broilers and of *Campylobacter* spp. in broiler flocks in the period 1999–2002 are described. These results were also used to measure the effects of the control programmes in the poultry industry.

MATERIALS AND METHODS

Programme design

A two-stage sampling scheme was used to accurately estimate the annual prevalences of *Salmonella* and *Campylobacter* spp. at flock level. Each year, the primary sample sizes (number of laying-hen and broiler flocks to be sampled) were calculated for estimation of the predicted prevalences with an accuracy of 5% at a 90% confidence level. This confidence level was chosen to limit the number of sampling units from a logistic point of view. The calculations were made by using the epidemiological computer program WinEpiscope [19]. Appropriate numbers of laying-hen and broiler farms were randomly selected by the Animal Health Service (GD) from a national database stratified according to geographical region. The selected farmers were requested to participate in the voluntary programme. Sampling was conducted by employees of the VWA/KvW according to a sampling protocol. On each farm, one flock (i.e. all birds of similar age housed within one building) was randomly selected for sampling. From the selected flock, 60 samples were taken enabling detection of at least 5% shedding animals at a 95% confidence level. Fresh faecal samples were randomly collected from the floor or – in case of laying hens – the manure conveyer and, subsequently, the samples were aggregated into five pooled samples. The samples were transported to the RIVM in cooled transport boxes by a professional delivery service. Microbiological examination started within 48 h. Approximately 100–200 flocks of laying hens and broilers were sampled

annually. However, due to an epidemic of foot-and-mouth disease (FMD), no samples were obtained from March 2001 until July 2001.

Detection of *Salmonella* spp.

For the detection of *Salmonella* spp. in faecal samples a modification of ISO 6579 [20] was applied, using a combination of Rappaport–Vassiliadis (RV) broth and modified semi-solid Rappaport–Vassiliadis (MSRV) agar for selective enrichment. In summary, from each pooled sample, 25 g was added to 225 ml of buffered peptone water [Nederlands Vaccin Instituut (NVI), Bilthoven, The Netherlands] and incubated for 18 ± 2 h at 37 ± 1 °C. Of the pre-enrichment culture, 0.1 ml was inoculated in 10 ml RV broth (Oxoid, Haarlem, The Netherlands), which was incubated for 24 ± 2 h at 42 ± 1 °C. The culture obtained in RV broth was plated on Brilliant Green Agar (BGA; Oxoid), followed by incubation for 24 ± 2 h at 37 ± 1 °C. If no suspect colonies were obtained on BGA, the RV culture was plated out on BGA again after a second incubation period of 24 ± 2 h at 42 ± 1 °C. Preparation of MSRV (Difco; Becton Dickinson, Alphen a/d Rijn, The Netherlands), containing 0.01 g l^{-1} novobiocine (Sigma-Aldrich Chemie, Wyndreut, The Netherlands), was performed according to the manufacturer's instructions. Three drops of the pre-enrichment culture were inoculated on this medium, followed by incubation for $2 \times 24 \pm 2$ h at 42 ± 1 °C. Suspect white culture from the border of the growth zone was transferred onto BGA and handled as described for the RV procedure. Suspect colonies on BGA were biochemically confirmed using ureum agar with triple sugar iron agar (NVI) and lysine-decarboxylase broth (NVI). Serotyping of *Salmonella* isolates and phage-typing of *S. Enteritidis* and *S. Typhimurium* isolates were performed according to the standard operating procedures of the Dutch National Reference Laboratory for *Salmonella* (RIVM, Bilthoven). Quantitative antimicrobial resistance testing of *Salmonella* isolates was performed at the Dutch National Reference Laboratory for antimicrobial resistance testing (Central Institute for Animal Disease Control, Lelystad).

Detection of *Campylobacter* spp.

For the detection of thermophilic *Campylobacter* spp., each pooled faecal sample was directly plated on *Campylobacter* blood-free selective agar (CCDA; Oxoid) using sterile swabs, followed by incubation for

48 ± 4 h at 42 ± 1 °C using the GENbox Microaer system (bioMérieux, Marcy l'Etoile, France). Subsequently, plates were examined for the presence of suspect colonies and if present, one colony per plate was transferred to CCDA, inoculating colonies from positive pooled samples from the same flock on the same plate. The microaerobic incubation procedure was repeated and characteristic *Campylobacter* colonies were examined by microscope for typical spiral-shaped cells and rapid motility. If the microscopic results were ambiguous, the Indx Campy agglutination test (Bipharma, Weesp, The Netherlands) was performed additionally, as prescribed by the manufacturer. Up to 2001, one *Campylobacter* isolate per positive pooled sample was stored in 2 ml peptone glycerol. At a later stage, these isolates were subjected to the mixed polymerase chain reaction (PCR) method described by Van de Giessen *et al.* [21] to discriminate *C. coli* from *C. jejuni*. In 2002, the species of the *Campylobacter* isolates were not determined.

Data analyses

Annual prevalence estimates, standard errors and confidence limits were calculated by using the methods provided by Thrusfield [22]. Trend analyses were performed using multivariable logistic regression (MLR), by analysing a continuous 'trend-variable' that represented subsequent time periods of 3 months. Applying MLR enabled adjustments for other factors, such as differences in age or flock size distribution between years and the non-sampling period in 2001, thereby excluding possible biases in the prevalence estimates. A trend was assumed to be present if the likelihood ratio test for the trend-variable yielded a significant *P* value at the 95% confidence level. The qualitative geographical analyses were performed by using the SAS/GIS procedure in the statistical software package SAS [23]. Statistics Netherlands [24] provided the geographical spread of farms in The Netherlands that were used for comparison with the geographical spread of sampled farms. The statistical tests for assessing differences between the geographical distribution of farms in the database and the actual distribution of farms in The Netherlands were based on χ^2 distribution.

RESULTS

For both laying hens and broilers, the geographical distribution of the sampled flocks corresponded well

Table 1. Crude annual prevalence estimates (*P*) for *Salmonella* and *Campylobacter* spp. in Dutch poultry production flocks

	1999			2000			2001			2002		
	<i>n</i> *	<i>P</i>	90% CI	<i>n</i>	<i>P</i>	90% CI	<i>n</i>	<i>P</i>	90% CI	<i>n</i>	<i>P</i>	90% CI
Laying hens												
<i>Salmonella</i> spp.	166	21.1	16.0–26.2	166	19.3	14.4–24.2	84	14.3	8.1–20.5	134	13.4	8.7–18.1
<i>S. Enteritidis</i>	166	9.6	7.3–11.9	166	9.0	6.8–11.2	84	4.8	2.5–7.1	134	6.0	4.0–8.0
Broilers												
<i>Salmonella</i> spp.	151	19.9	14.6–25.2	128	16.4	11.0–21.8	123	22.8	16.6–29.0	161	11.2	7.1–15.3
<i>Campylobacter</i> spp.	154	17.5	12.5–22.5	127	23.6	17.4–29.8	123	16.3	10.9–21.8	166	27.1	21.5–32.8

CI, Confidence interval.

* *n* gives the number of flocks examined that year.

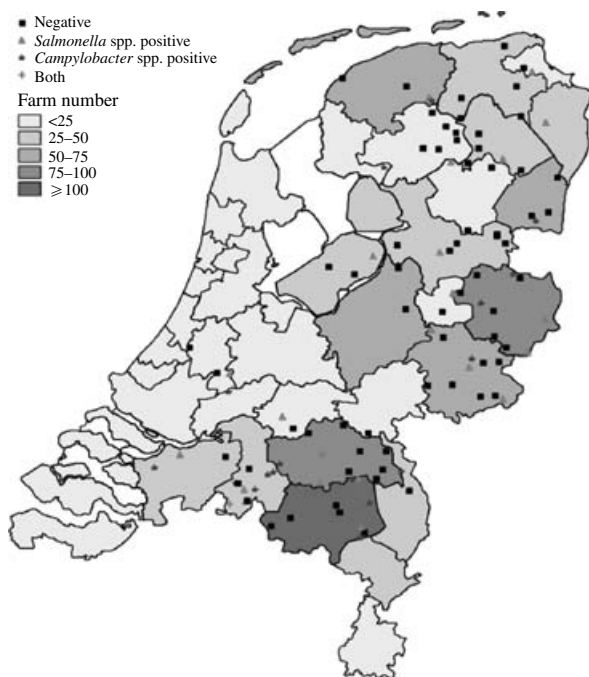


Fig. 1. Geographical distribution of the sampled broiler flocks compared to the distribution of broiler farms in The Netherlands in 2001.

with the actual distribution of farms in The Netherlands. No spatial clustering of positive flocks was observed. *Salmonella*- and *Campylobacter*-positive flocks were observed throughout the country. For illustration, the geographical distribution of the broiler flocks sampled in 2001 is illustrated in Figure 1.

In Table 1, the crude annual prevalence estimates for *Salmonella* and *Campylobacter* spp. as well as their 90% confidence intervals are presented numerically. A graphical representation of the crude annual

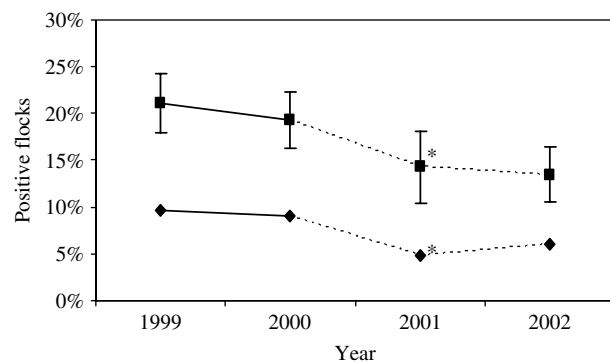


Fig. 2. Crude annual prevalence estimates (with standard errors) for *Salmonella* spp. (—■—) and *S. Enteritidis* (---◆---) in flocks of laying hens. * Inaccurate estimate caused by a non-sampling period from March to July 2001 due to an epidemic of foot-and-mouth disease.

prevalence estimates for *Salmonella* spp. in laying hens is given in Figure 2. A decrease was observed from 21% of positive flocks in 1999 to 13% in 2002. Trend analysis on the data adjusted for age, flock size and the non-sampling period due to the FMD outbreak in 2001, showed a significant decreasing trend ($P=0.02$) within this 4-year period. The prevalence of *S. Enteritidis* was a fairly constant fraction of approximately 45% of the *Salmonella*-positive flocks in all years, with the exception of 2001 (33%). Trend analysis on the prevalence estimates for *S. Enteritidis* yielded a *P* value of 0.08 for a decreasing trend, thus suggesting significance at the selected confidence level. Figure 3 shows the percentage of *Salmonella*- and *S. Enteritidis*-positive flocks according to time present at the farm indicating an increase of *Salmonella*-positive flocks in the last part of the production period. The most frequently isolated *Salmonella* serotypes are shown in Table 2. In laying hens, *S. Enteritidis* was

Table 2. Most frequently isolated *Salmonella* serotypes in Dutch poultry production flocks

	1999		2000		2001		2002					
	Serotype	<i>n</i> *	%†	Serotype	<i>n</i>	%	Serotype	<i>n</i>	%			
Laying hens	Enteritidis	16	46	Enteritidis	15	47	Enteritidis	4	33	Enteritidis	8	44
	Braenderup	5	14	Infantis	6	19	Braenderup	2	17	Infantis	6	33
	Infantis	5	14	Braenderup	4	13	Infantis	2	17	Typhimurium	2	11
	Livingstone	5	14	Mbandaka	2	6	Others	4	33	Others	4	22
	Others	13	37	Livingstone	2	6						
			Others	8	25							
Broilers	Enteritidis	8	27	Java	8	38	Mbandaka	10	36	Java	6	33
	Infantis	5	17	Virchow	4	19	Infantis	6	21	Infantis	3	17
	Java	4	13	Mbandaka	3	14	Java	5	18	Livingstone	2	11
	Mbandaka	3	10	Enteritidis	2	10	Typhimurium	3	11	Mbandaka	2	11
	Uganda	3	10	Manhattan	2	10	Thompson	2	7	Virchow	2	11
	Others	13	43	Others	5	24	Others	4	14	Others	4	22

* *n* gives the number of flocks positive for this serotype.
 † Percentage of the total number of *Salmonella*-positive flocks.

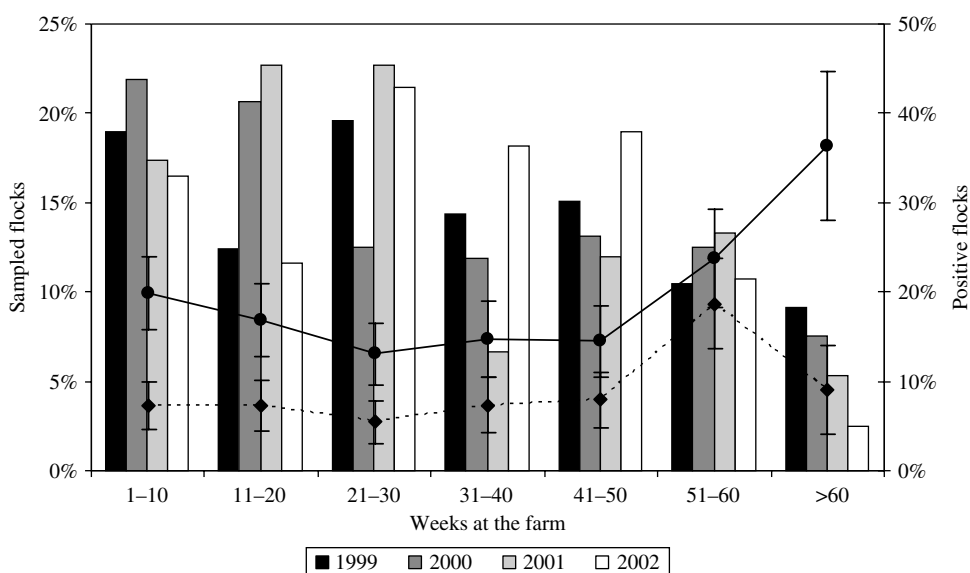


Fig. 3. Percentage of sampled laying-hen flocks and percentage (with standard errors) of *Salmonella*-positive (—●—) and *S. Enteritidis*-positive (—◆—) flocks according to time present at the farm.

the predominantly isolated serotype, PT4 being the predominant *S. Enteritidis* phage type.

The crude annual prevalence estimates for *Salmonella* spp. in broiler flocks are illustrated in Figure 4. The estimates showed comparable values between 1999 and 2001, followed by a decrease in 2002. Trend analysis on data from 1999 until 2002, adjusted for age, flock size and the non-sampling period in 2001, did not show a statistically significant decreasing trend for *Salmonella* spp. ($P=0.20$). In 2001–2, the predominant *Salmonella* serotypes in

broiler flocks were *S. Mbandaka*, *S. Infantis* and *S. Paratyphi B* var. Java (Table 2). The latter serotype increased from 13% of the *Salmonella*-positive flocks in 1999 to one third of the positive flocks in 2002. Multi-resistant *S. Typhimurium* DT104 (corresponding to the Dutch phage types 406 and 501) was isolated from two broiler flocks in 2001 and from one in 2002. Figure 5 shows the percentage of *Salmonella*- and *Campylobacter*-positive flocks according to age. Whereas the percentage of *Salmonella*-positive flocks declined after 3 weeks of age, the percentage of

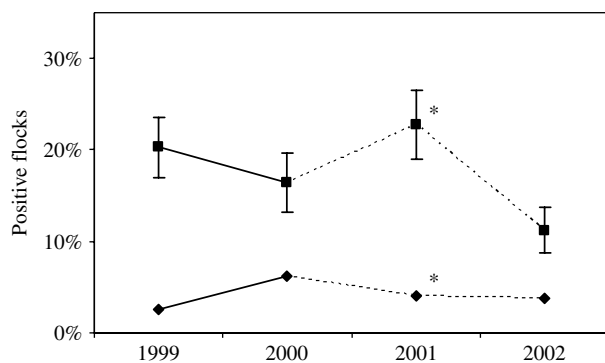


Fig. 4. Crude annual prevalence estimates (with standard errors) for *Salmonella* spp. (—■—) and *S. Paratyphi* B var. Java (---◆---) in broiler flocks. * Inaccurate estimate caused by a non-sampling period from March to July 2001 due to an epidemic of foot-and-mouth disease.

Campylobacter-positive flocks steadily increased towards the end of the broiler period.

The crude annual prevalence estimates for *Campylobacter* spp. in broiler flocks are illustrated in Figure 6. In 2000 and 2002, an increase in the prevalence estimate was observed compared to 1999 and 2001 respectively. Trend analysis of the prevalence estimates, adjusted for age, flock size, season and the non-sampling period due to FMD, gave no indication for a significant trend during the study period ($P=0.18$). A seasonal variation in *Campylobacter* occurrence in broiler flocks was observed with a peak in the summer months (Fig. 6). Molecular analysis of the *Campylobacter* isolates revealed that *C. jejuni* was isolated from 85 to 100% of the positive flocks in different years, whereas *C. coli* was found to be present in 13–20% of the positive flocks.

DISCUSSION

In this surveillance study, basic statistical principles were applied for prevalence estimation of zoonotic bacteria in poultry production flocks. However, several factors may have affected the prevalence estimates. First, the willingness of farmers to participate in this voluntary surveillance programme might have been related to the farm or flock status and thus may have interfered with the randomization of the sample. Furthermore, positive flocks with a low percentage of shedding animals may have falsely tested negative due to the limited number of samples taken per flock. Also, the crude prevalence estimates have not been adjusted for misclassification of flocks due to imperfect diagnostic testing. Since the isolates were

confirmed and subjected to serotyping or species differentiation, false-positive test results are unlikely. However, false-negative testing may have occurred, especially in case of low numbers of bacteria present in the pooled samples. Therefore, the presented crude prevalences are likely to underestimate the true prevalence. Nevertheless, by applying the same sampling scheme and the same sampling and testing methods in consecutive years and by adjusting the data for those factors that may cause biases in the estimates, trends in the prevalence of the target bacteria in poultry production flocks could be analysed.

From 1999 onwards, a significantly decreasing trend in the prevalence of *Salmonella* spp. in flocks of laying hens was observed. As regards *S. Enteritidis*, the limited number of positive flocks might have caused the absence of statistical significance. The estimated *Salmonella* prevalence in 2002 was 13%, with *S. Enteritidis* accounting for one third of the positive flocks. The decreasing trend for *Salmonella* spp. (and a similar tendency for *S. Enteritidis*) in laying-hen flocks might indicate that the control measures taken by the poultry industry are effective. However, the initial aim of the control programme to reduce *Salmonella* contamination in laying hens to <5% of *S. Enteritidis*- or *S. Typhimurium*-positive flocks was not achieved until now. Furthermore, serological examination of laying-hen flocks at the end of production by the Animal Health Service (GD) indicated a decrease in *S. Enteritidis*-positive flocks from 14% in 1997 to 9% in 2002 [6]. The results of this serological monitoring roughly correspond with the results of our surveillance programme, indicating a decrease of *S. Enteritidis* infections in flocks of laying hens. Moreover, the increase in the percentage of *Salmonella*-positive flocks observed in the last part of the production period may well be due to the ageing of the hens, which thereby become more susceptible to infection.

In 1999, an extensive study on the prevalence of *Salmonella* spp. in table eggs in The Netherlands was conducted, yielding an estimate of at least 0.03% and at most 0.3% (14 pools of 10 eggs each out of 4620 pools were found positive). Eleven (77%) of the isolates were *S. Enteritidis* [25]. Since 2002, according to Dutch regulations, table eggs are required to be derived from laying-hen flocks free of *Salmonella* spp., and consequently the Dutch control programme in the egg production chain aims to reduce *S. Enteritidis* and *S. Typhimurium* infections in

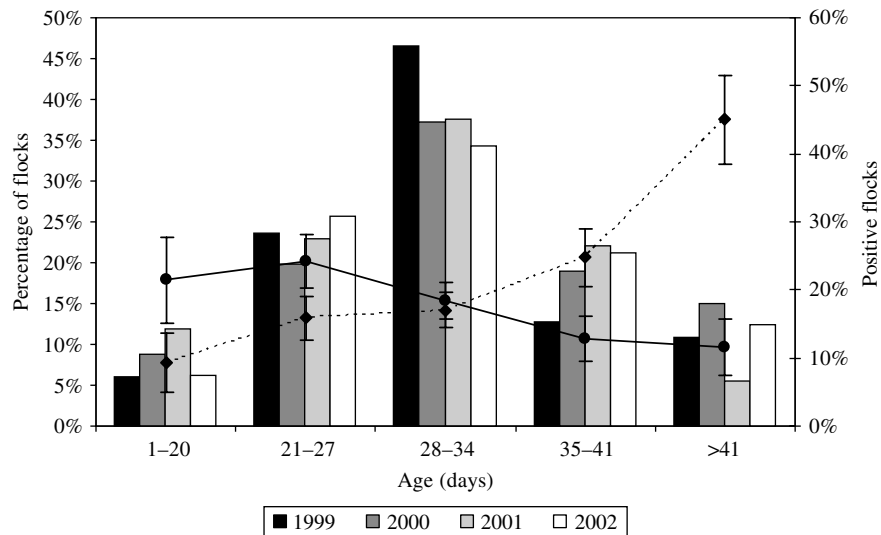


Fig. 5. Percentage of sampled broiler flocks and percentage (with standard errors) of *Salmonella*-positive (—●—) and *Campylobacter*-positive (—◆—) flocks according to age.

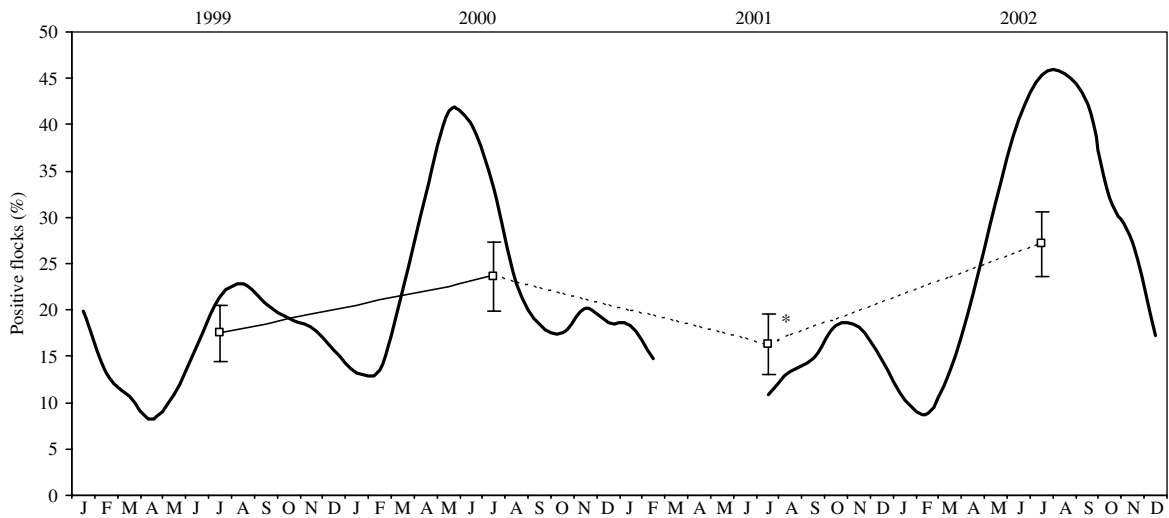


Fig. 6. Crude annual prevalence estimates (with standard errors) and seasonal variation of *Campylobacter* spp. in broiler flocks. * Inaccurate estimate caused by a non-sampling period from March to July 2001 due to an epidemic of foot-and-mouth disease. --□--, Per year; —, per month.

laying hens to zero level. In the United Kingdom in 2003, a survey of *Salmonella* contamination of table eggs revealed that 0.3% of the eggs were contaminated with *Salmonella* spp. This is a threefold reduction in the level of contamination since 1995/1996 and may well be attributable to the *Salmonella* control measures introduced by the UK egg industry, which included vaccination of laying hens [26].

In this study, PT4 was found to be the predominant *S. Enteritidis* phage type in laying hens and not surprisingly, in human *S. Enteritidis* infections during the study period as well. However, the contribution of

PT4 to human *S. Enteritidis* infections declined from 80% in 1997 to 50% in 2002 [6]. In contrast, other *S. Enteritidis* phage types such as PT21 and PT1 emerged in humans between 1997 and 2002, whereas these phage types were rarely isolated from laying hens in this study. The emergence of phage types uncommon in Dutch laying hens may be related to the import of table eggs from other European countries. In 2003, an exceptional increase of human *S. Enteritidis* infections in The Netherlands was associated with the import of Spanish table eggs due to the avian influenza crisis in the Dutch poultry industry [27]. Also, in a

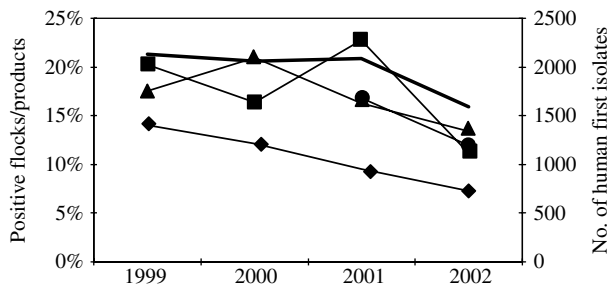


Fig. 7. Comparison of *Salmonella* prevalences as measured in (i) the RIVM monitoring programme in broiler flocks (—■—), (ii) the poultry industry's (PVE) monitoring programme in broiler flocks (—◆—) and breast skins (—●—) [6], (iii) the monitoring programme of the Inspectorate for Health Protection and Veterinary Public Health (KvW) in chicken end-products at retail (—▲—) [14] and (iv) the public health laboratory surveillance in human stool specimens (—) [6].

recent 2-year Health Protection Agency survey of eggs derived from food premises and suppliers implicated in outbreaks of *Salmonella* spp., 5.6% of Spanish eggs were found to be contaminated with *Salmonella* spp. compared to 1.1% of eggs derived from non-vaccinated laying-hen flocks in the United Kingdom [28]. This development highlights the urgent need for the control of *Salmonella* spp. in laying hens and eggs at the European level.

Trend-analysis on the *Salmonella* prevalences measured in broiler flocks in 1999–2002 did not yield a decreasing trend ($P=0.20$). However, the decrease in *Salmonella* prevalence observed in 2002 as well as the decreasing contamination percentages measured in the poultry meat production chain by the poultry industry [6] and in poultry products at retail by the KvW [14] suggest that the intervention measures implemented by the poultry industry are effective. A comparison of the results from the different Dutch monitoring programmes in the poultry meat production chain combined with the results of the public health laboratory surveillance is shown in Figure 7. The *Salmonella* prevalences in broiler flocks measured by the poultry industry are structurally lower than those measured in our surveillance programme, which may be due to differences in sample size, sampling methods and analytical performance.

During the study period, the emergence of *S. Paratyphi* B var. Java (*S. Java*) in broilers was observed, corresponding with an explosive increase of this particular serotype in poultry products at retail in The Netherlands [29]. Simultaneously, a similar emergence of *S. Java* in poultry was observed in

Germany [30]. Characterization of *S. Java* isolates from Dutch and German poultry revealed the clonal spread of a strain multi-resistant to antimicrobial drugs [29]. Farm intervention studies indicated a high level of persistence of *S. Java* in contaminated broiler houses (N. M. Bolder, unpublished observations). Moreover, the *S. Java* clone in broilers was found to rapidly develop resistance to quinolones, presumably due to veterinary treatment [31]. Since in The Netherlands fluoroquinolones are the antibiotics of first choice in cases of human salmonellosis, quinolone resistance might hamper effective medical treatment. Fortunately, up to now, *S. Java* infection in humans in The Netherlands has been rare, despite its abundance on poultry meat.

Prevalence estimates for *Campylobacter* spp. in broiler flocks averaged around 20% in the period 1999–2002. The absence of a decreasing (or increasing) trend corresponds with the results of the *Campylobacter* monitoring programme run by the poultry industry [6] and with the results of the KvW monitoring of poultry products at retail [14]. *Campylobacter* contamination of poultry products at retail in The Netherlands was 23.5% in 1998 and 31.3% in 2002. Although these contamination levels are relatively low compared to those measured in some other European countries like Denmark (41.7%) and France (88.7%) in 2002 [1], these results indicate that the Dutch control programme implemented in the poultry meat production chain was unsuccessful in reducing the *Campylobacter* contamination levels. Therefore, the application of alternative intervention measures including channeling of infected flocks in combination with germicidal treatment of poultry carcasses, such as decontamination, freezing or irradiation, should be seriously considered. To this end, in The Netherlands, a national *Campylobacter* risk management and assessment project was launched in 2001 and completed in 2004 [32].

In contrast to *Salmonella* spp. for which a decline in the percentage of positive broiler flocks was observed after 3 weeks of age, a steady increase in the percentage of *Campylobacter*-positive flocks was observed during the broiler period, which may be attributable to the transmission of *Campylobacter* from environmental sources at different ages followed by the characteristic rapid spread of the bacterium within the flock [33].

The seasonal pattern for *Campylobacter* occurrence in broiler flocks showed a peak in the period May–July, which corresponds with a peak in the

Campylobacter contamination of poultry products in the period June–August. Although a similar seasonal variation is observed in human campylobacteriosis in The Netherlands, the relation between the summer peaks in *Campylobacter* occurrence in humans and poultry is uncertain due to a large discrepancy between the levels of fluoroquinolone resistance in human and poultry isolates in the summer period [34]. The phenomenon of seasonal variation of *Campylobacter* in broilers is also observed in other countries and may be related to climatic conditions influencing the environmental infection pressure of *Campylobacter* spp. [35].

The majority of the *Campylobacter* isolates from broilers were *C. jejuni*, which corresponds with findings from other studies [21, 36]. Moreover, *C. jejuni* is the predominant species causing human campylobacteriosis in The Netherlands [2].

The surveillance approach presented in this report demonstrated its worth in monitoring and evaluating trends in the occurrence of multiple zoonotic agents in farm animal populations. This approach can serve as a blueprint for monitoring schemes in farm animals to be developed in the context of the EC Zoonoses Directive.

ACKNOWLEDGEMENTS

This study was conducted by order of the Dutch Food and Consumer Product Safety Authority (VWA). We thank our colleagues at the Inspectorate for Health Protection and Veterinary Public Health (KvW/VWA) for taking the samples and the Animal Health Service (GD) for selecting the farms and contacting the selected farmers. Thanks are also due to Dr A. Mensink for a critical review of the paper.

DECLARATION OF INTEREST

None.

REFERENCES

1. Anon. Trends and sources of zoonotic agents in animals, feedstuffs, food and man in the European Union and Norway in 2003. Brussels: European Commission, 2005. SANCO/339/2005.
2. De Wit MA, et al. Gastroenteritis in sentinel general practices, the Netherlands. *Emerging Infectious Diseases* 2001; **7**: 82–91.
3. De Wit MA, et al. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. *American Journal of Epidemiology* 2001; **154**: 666–674.
4. Pelt W van, et al. Laboratory surveillance of bacterial gastroenteric pathogens in The Netherlands, 1991–2001. *Epidemiology and Infection* 2003; **130**: 431–441.
5. Giessen AW van de, Leeuwen WJ van, Pelt W van. *Salmonella enterica* serovar Enteritidis in the Netherlands: epidemiology, prevention and control. In: Saeed AM, Gast RK, Potter ME, Wall PG, eds. *Salmonella enterica serovar Enteritidis in Humans and Animals: epidemiology, pathogenesis and control*. Ames, IA: Iowa State University Press, 1999: pp. 71–80.
6. Anon. Report of trends and sources of zoonotic agents. The Netherlands, 2003. The Hague: Dutch Food and Consumer Product Safety Authority, 2004.
7. Fisher IST. International trends in *Salmonella* serotypes 1998–2003 – a surveillance report from the Enter-net international surveillance network. *Eurosurveillance* 2004; **9**: 9–10.
8. Van Duynhoven YTHP, et al. A one-year intensified study of outbreaks of gastroenteritis in the Netherlands. *Epidemiology and Infection* 2005; **133**: 9–21.
9. Health Protection Agency. National increase in *Salmonella* Enteritidis outbreaks. *Communicable Disease Report Weekly* 2003; **13** (35): 4.
10. Skirrow MB. *Campylobacter*. *Lancet* 1990; **336**: 921–923.
11. Tauxe RV. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized nations. In: Nachamkin I, Blaser MJ, Tompkins LS, eds. *Campylobacter jejuni Current Status and Future Trends*. Washington, DC: American Society for Microbiology, 1992: pp. 9–19.
12. Altekruse SF, et al. *Campylobacter jejuni* – an emerging foodborne pathogen. *Emerging Infectious Diseases* 1999; **5**: 28–35.
13. Allos BM. *Campylobacter jejuni* infections: update on emerging issues and trends. *Clinical Infectious Diseases* 2001; **32**: 1201–1206.
14. Van der Zee H, Wit B. Monitoring pathogens in poultry and poultry products, 2002 [in Dutch]. Zutphen: Inspectorate for Health Protection and Veterinary Public Health, 2003.
15. Van der Zee H, Wit B, de Boer E. Survey of pathogenic micro-organisms in raw meats [in Dutch]. *De Ware(n)-Chemicus* 2000; **30**: 185–188.
16. Anon. Action plan *Salmonella* and *Campylobacter* in the poultry meat sector 2000+ [in Dutch]. Rijswijk: Product Boards for Livestock, Meat and Eggs, 2000.
17. Anon. Action plan *Salmonella* in the egg sector 2001+ [in Dutch]. Rijswijk: Product Boards for Livestock, Meat and Eggs, 2001.
18. Anon. Directive 2003/99/EC of the European parliament and of the Council of 17 November 2003 on the monitoring of zoonoses and zoonotic agents, amending Council Decision 90/424/EEC and repealing Council Directive 92/117/EEC. *Official Journal of the European Union*, 12 Dec. 2003, L325: pp. 31–40.
19. Frankena K, et al. EPISCOPE: computer programs in veterinary epidemiology. *Veterinary Record* 1990; **126**: 573–576.

20. **Anon.** *Microbiology – general guidance on methods for detection of Salmonella*, 3rd edn. Geneva: International Standard Organization, 1993; ISO 6579.
21. **Van de Giessen AW, et al.** Reduction of *Campylobacter* infections in broiler flocks by application of hygiene measures. *Epidemiology and Infection* 1998; **121**: 57–66.
22. **Thrusfield M.** *Veterinary Epidemiology*, 2nd edn. Oxford: Blackwell Science, 1995: p. 483.
23. **SAS Institute Inc.** SAS version 8.02. Cary, NC: SAS Institute Inc., 2001.
24. **Statistics Netherlands.** Voorburg, 2002. StatLine (<http://statline.cbs.nl/StatWeb>). 2003.
25. **De Boer E, Wit B.** *Salmonella* in eieren. *Tijdschrift Voor Diergeneeskunde* 2000; **124**: 126–127.
26. **Elson R, Little C.** First United Kingdom-wide study of raw shell eggs and their use in catering premises published. *Eurosurveillance Weekly* releases 2004; **8**(12): 040318 (www.eurosurveillance.org/ew/2004/040318.asp).
27. **Pelt W van, et al.** An explosion of *Salmonella* infections in 2003 in the Netherlands: hot summer or side effect of the avian influenza outbreak? *Eurosurveillance* 2004; **9**: 3–4.
28. **O'Brien S, et al.** Increase in *Salmonella* Enteritidis outbreaks in England and Wales. *Eurosurveillance Weekly* 2003; **7**: 28 August 2005.
29. **Pelt W van, et al.** Explosive increase of *Salmonella* Java in poultry in the Netherlands: consequences for public health. *Eurosurveillance* 2003; **8**: 31–35.
30. **Dorn C, et al.** Increasing number of *Salmonella* paratyphi B isolates from slaughtered poultry sent in to the National *Salmonella* Reference Laboratory [in German]. *Berliner und Munchener Tierarztliche Wochenschrift* 2001; **114**: 179–183.
31. **Mevius DJ, Pelt W van (eds.)** MARAN 2002 – monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2002. The Hague: Dutch Food and Consumer Product Safety Authority, 2003.
32. **Havelaar AH (ed.)** *Campylobacteriosis in the Netherlands* [in Dutch]. Bilthoven, The Netherlands: National Institute for Public Health and the Environment, 2001. Report ID 250911001/2001.
33. **Newell DG, Fearnley C.** Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology* 2003; **69**: 4343–4351.
34. **Pelt W van, et al.** Resistance to fluoroquinolones marking the origin of human *Campylobacter* infections in the Netherlands. *Clinical Microbiology and Infection* 2001; **7**: 308.
35. **Wallace JS, et al.** Seasonality of thermophilic *Campylobacter* populations in chickens. *Journal of Applied Microbiology* 1997; **82**: 219–224.
36. **Refrégier-Petton J, et al.** Risk factors for *Campylobacter* spp. contamination in French broiler-chicken flocks at the end of the rearing period. *Preventive Veterinary Medicine* 2001; **50**: 89–100.