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Surveillance of zoonotic bacteria in farm animals in The Netherlands
Results from January 1998 until December 2000

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Abstract

To obtain reliable quantitative data on the occurrence of zoonotic bacteria in farm animals in The Netherlands, a surveillance programme was implemented in April 1997. Results for January 1998 through December 2000 are presented in this report. In this period, faecal samples from in total 2,378 flocks/herds of layers, broilers, finishing pigs, dairy cattle and veal calves were examined for the presence of *Salmonella* spp., *Campylobacter* spp. and/or verocytotoxin-producing *E. coli* O157. Questionnaires were used to obtain data for risk factor analyses. For layers, prevalences of salmonella positive flocks were 12% (1998, using Rappaport-Vassiliadis (RV) as selective enrichment medium) and averaged around 20% in 1999 and 2000 (using both RV and modified semisolid RV (MSRV)); for broilers, the salmonella prevalence declined from 28% (1998, RV) to 16% (2000, RV & MSRV). For finishing pig, 34% (1998; 4th quarter only), 13% (1999) and 16% (2000) positive herds were identified, while for dairy cattle and veal calves, salmonella prevalences were around 3% (based on the use of RV only). Serotype discrimination showed the predominance of *S.* Enteritidis (mainly phagetype PT4) in layers in all years; for broilers this serotype prevailed until 1999, whereas *S.* Paratyphi B var. Java prevailed in 2000. In finishing pigs, *S.* Typhimurium predominated, with an increase of phagetype DT104 during the study period. The campylobacter prevalence in broilers decreased from 31% (1998) to 18% (1999), reaching 24% in 2000. Finishing pigs, dairy cattle and veal calves showed lower campylobacter prevalences for 1999 compared to 1998. *C. jejuni* was the dominating species in broilers and dairy cattle, whereas *C. coli* predominated in pigs; both species prevailed equally in veal calves. Prevalence estimates for *E. coli* O157 in dairy cattle were 5% (1998), 8% (1999) and 6% (2000; 8% with an adjusted processing of samples); for veal calves these were 5% (1998), 9% (1999) and 11% (2000; 17% with the adjusted method). PCR-test results revealed the presence of the virulence associated SLT- and/or *eae*-genes in all isolates examined. Potential risk factors were identified for *E. coli* O157 in dairy cattle and for *Campylobacter* spp. in broilers.

Preface

For the successful realisation of this surveillance program, the input of the inspectors and colleagues of the Inspectorate for Health Protection and Veterinary Public Health (KvW) was essential and indispensable. Many samples were collected routinely and questionnaires with numerous questions were completed, enabling the analyses presented in this report and in other publications. Also, the comments and suggestions received from Rob van Oosterom (KvW) were valuable for the preparation of this report.
Secondly, we would like to acknowledge the contributions of the Animal Health Service (GD) and the Foundation for Quality Guarantee of Veal (SKV) performing the selection of flocks/herds to be sampled and for contacting the selected farm holders.
Furthermore, we would like to acknowledge Henny Maas of the Diagnostic Laboratory Infectious Diseases and Perinatal Screening for serotyping the isolates, and thank Eric Evers for his review of the mathematical aspects of this report. Also, the contributions of Cor de Lezenne Coulander for assistance on the geographical figures is greatly acknowledged.
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The authors.

Contents

Samenvatting

Sinds april 1997 vindt bij het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) een gestructureerde surveillance plaats van zoönosenverwekkers bij landbouwhuisdieren in Nederland. Dit in opdracht van en in samenwerking met de Keuringsdienst van Waren (KvW) en in samenwerking met de Gezondheidsdienst voor Dieren (GD) en de Stichting Kwaliteitsgarantie Vleeskalveren (SKV). De resultaten van dit programma in de periode 1998-2000 zijn beschreven in dit rapport.

Wekelijks zijn mestmonsters genomen van koppels leghennen, vleeskuikens, vleesvarkens, melkkoeien en vleeskalveren en microbiologisch onderzocht op de aanwezigheid van *Salmonella* spp.. Daarnaast zijn de monsters van een aantal diersoorten onderzocht op het vóórkomen van *Campylobacter* spp. (alle diersoorten exclusief leghennen in 1998 en 1999 en alléén vleeskuikens in 2000) en *E. coli* O157 (alle diersoorten in 1998 en 1999 en alléén melkkoeien en vleeskalveren in 2000). De hoofddoelstelling van de surveillance is inzicht te verkrijgen in het vóórkomen, en de trends hierin, van deze zoönotische bacteriën bij landbouwhuisdieren. Deze informatie is onder meer van belang om effecten van genomen maatregelen in productiesectoren te kunnen evalueren. Daarnaast worden risicofactoren voor besmetting van landbouwhuisdieren met deze zoönosenverwekkers geïdentificeerd en gekwantificeerd, om daarmee een mogelijke basis te verschaffen voor gerichte interventiestrategieën. Tevens worden de resultaten van de surveillance gebruikt om epidemiologische verbanden te onderzoeken tussen het vóórkomen van deze pathogene micro-organismen bij deze landbouwhuisdieren en het optreden van infecties bij de mens, middels typeringen van isolaten.

In 1998, 1999 en 2000 zijn in het totaal respectievelijk 859, 741 en 778 koppels dieren bemonsterd. Opgesplitst naar diersoort resulteerde dit in 1998 in 207 koppels leghennen, 192 koppels vleeskuikens, 41 koppels vleesvarkens, 267 koppels melkkoeien en 152 koppels vleeskalveren. In 1999 was deze onderverdeling: 168 koppels leghennen, 155 koppels vleeskuikens, 189 koppels vleesvarkens, 169 koppels melkkoeien en 60 koppels vleeskalveren. In 2000 zijn 166 koppels leghennen, 127 koppels vleeskuikens, 194 koppels vleesvarkens, 158 koppels melkkoeien en 133 koppels vleeskalveren bemonsterd.

In de drie opeenvolgende jaren van onderzoek werd *Salmonella* spp. aangetoond in respectievelijk 12%, 21% en 19% van de koppels leghennen. Bij vleeskuikens waren deze percentages 28%, 20% en 16%. Bij vleesvarkens werden 34% positieve koppels in het vierde kwartaal van 1998 gevonden, 13% in 1999 en 16% (36% bij additioneel gebruik van een *semi-solid* medium) in 2000. Bij melkkoeien werd in respectievelijk 3%, 2% en 1% van de koppels *Salmonella* spp. aangetroffen, bij vleeskalveren in respectievelijk 1%, 5% en 1% van de koppels. Serotypering van de isolaten liet zien dat *S.* Enteritidis het meest vóórkomende serotype bij leghennen was (respectievelijk 7%, 10% en 9% van de koppels in 1998, 1999 en 2000). Bij vleeskuikens domineerde in 1998 en 1999 eveneens *S.* Enteritidis, maar dit serotype werd in 2000 voorbijgestreefd door het opkomende serotype *S.* Paratyphi B var. Java. Het grootste deel van de *S.* Enteritidis isolaten van beide diersoorten betreft faagtype

PT4, welke bij de mens eveneens het meest voorkomt. Het meest prevalente serotype in varkens in alle onderzoeksjaren was *S.* Typhimurium, met een toenemende rol voor *S.* Typhimurium faagtype DT104 van 13% naar 19% van de Typhimurium isolaten.

Het onderzoek op *Campylobacter* spp. bij vleeskuikens resulteerde in prevalentieschattingen van 31%, 18% en 24% in respectievelijk 1998, 1999 en 2000. Voor vleesvarkens zijn de jaarschattingen voor 1998 en 1999 respectievelijk 97% en 46%, voor melkkoeien respectievelijk 32% en 7% en voor vleeskalveren respectievelijk 84% en 58%. Uit de species-identificatie van campylobacter isolaten bleek een dominantie van *C. jejuni* in vleeskuikens (ruim 80%) en melkkoeien (>90%), tegenover een prominente aanwezigheid van *C. coli* in vleesvarkens. Bij vleeskalveren werden *C. coli* en *C. jejuni* beide even frequent aangetroffen, voor een deel in dezelfde koppels.

E. coli O157 is in 1998 niet in koppels leghennen aangetoond en slechts incidenteel in koppels vleeskuikens (1%, n=186) en vleesvarkens (2%, n=41). In 1999 zijn respectievelijk 1% (n=100), 0% (n=189) en 4% (n=113) van de koppels leghennen, vleesvarkens en vleeskuikens positief bevonden. Bij melkkoeien is in 1998, 1999 en 2000 in respectievelijk 5%, 9% en 6% (9% bij een gewijzigde methodiek) van de koppels *E. coli* O157 aangetoond. Voor vleeskalveren zijn deze percentages respectievelijk 5%, 13% en 11% (17% bij een gewijzigde methodiek), waarbij zich mogelijk een toenemende trend ontwikkelt in het voorkomen van *E. coli* O157 (P=0.0712). Uit DNA-onderzoek van de *E. coli* O157-isolaten is gebleken dat alle isolaten tenminste één virulentie-gen bevatten en dat met name de combinaties tussen het *eae* gen en één of beide SLT genen voorkomen.

Risicofactor-analyse voor *E. coli* O157 in melkkoeien heeft geleid tot de identificatie van de volgende significante (P<0.10) associaties: de aanwezigheid van minstens één varken op het bedrijf, de aankoop van dieren binnen twee jaar voor de bemonstering, het verstrekken van maïs aan de koeien, het verstrekken van bietenpulp aan de koeien en bemonstering in 2000 in vergelijking met bemonstering in 1998.

Risicofactor-analyse voor *Campylobacter* spp. in de vleeskuikens heeft geresulteerd in de volgende potentiële risicofactoren: toenemende leeftijd, de aanwezigheid van vijf of meer stallen, aanwezigheid van andere landbouwhuisdieren op het bedrijf, aanwezigheid van andere bedrijven binnen een straal van één km, de komst van kinderen in de stal, het gebruik van aparte werkkleding voor de vleeskuikens en de seizoenen zomer en herfst. Daarnaast vertonen verschillende broederijen uiteenlopende effecten, maar deze variabele was gecorreleerd met de voerleverancier en integratie. Effect modificatie was aanwezig tussen de komst van kinderen in de stal en het gebruik van aparte werkkleding voor de vleeskuikens.

Op basis van de resultaten beschreven in dit rapport kan worden geconcludeerd dat met name leghennen, vleeskuikens en vleesvarkens nog steeds belangrijke dierlijke reservoirs zijn voor *Salmonella* spp.. De consistentie in de gemeten salmonella-prevalenties bij leghennen in de drie onderzoeksjaren en daarbinnen de consistente bijdrage van *S.* Enteritidis geven aan dat de in de eiersector getroffen maatregelen in de periode 1998-2000 niet hebben geleid tot de gewenste reductie in de besmetting met *S.* Enteritidis. In de pluimveevleesketen daarentegen lijken de maatregelen een effect te hebben gezien de dalende trend in de jaarlijkse prevalentieschattingen voor *Salmonella* spp. bij vleeskuikens. Deze trend komt

overeen met de monitoringsresultaten van de pluimvee-industrie, terwijl de resultaten van de door de KvW uitgevoerde monitoring van *Salmonella* spp. op kipproducten in de detailhandel in 2000 een toename in besmetting laten zien. De daling van *S.* Enteritidis bij vleeskuikens hangt mogelijk samen met de opmars van *S.* Paratyphi B var. Java, een serotype dat vooralsnog minder gevaarlijk lijkt voor de humane populatie maar op bedrijfsniveau zeer persistent blijkt te zijn. Bij vleesvarkens is vooral de toename van *S.* Typhimurium DT104 (Nederlandse faagtypen 401 en 506) van belang, hetgeen mogelijk een toenemend gevaar vormt voor de volksgezondheid.

Campylobacter spp. komt frequent voor in vleeskuikens, maar ook varkens en runderen vormen reservoirs voor deze voor de mens pathogene bacterie. Echter, onderzoek in de detailhandel heeft uitgewezen, dat eindproducten van deze laatste twee diersoorten niet of nauwelijks met *Campylobacter* spp. zijn besmet. Monitoring van campylobacter bij deze diersoorten wordt daarom sinds 2000 niet meer jaarlijks uitgevoerd. Bij vleeskuikens is nog geen dalende trend in campylobacter prevalentie zichtbaar, ondanks de aandacht van de pluimveesector voor dit micro-organisme sinds 1997. De trend in het voorkomen van *Campylobacter* spp. in dit onderzoek wijkt af van de trend gemeten door de pluimveesector (PVE), maar komt overeen met de trend gemeten door de KvW in de detailhandel.

Voor *E. coli* O157 vormen vleeskalveren en melkvee belangrijke reservoirs. Trendanalyse liet een indicatie zien voor een mogelijke toenemende trend bij vleeskalveren. Één isolaat van kalveren uitgezonderd, bevatten alle geïsoleerde stammen één of meerdere genen die coderen voor de productie van het shiga-like toxine (SLT-I en/of SLT-II). Nader onderzoek is gewenst naar de mogelijk stijgende trend in *E. coli* O157-prevalentie bij vleeskalveren, evenals naar de risicofactoren voor *E. coli* O157-besmetting bij runderen.

Summary

Since April 1997, a structural surveillance programme is implemented in The Netherlands, under authority of the Inspectorate for Health Protection and Veterinary Public Health (KvW) and the National Institute for Public Health and the Environment (RIVM), and in collaboration with the Animal Health Service (GD) and the Quality Guarantee of Veal (SKV). This report describes the results from January 1998 until December 2000.

Faecal samples were collected weekly from layers, broilers, finishing pigs, dairy cattle and veal calves and examined for contamination with *Salmonella* spp.. Furthermore, a selection of animal species was subjected to the examination for *Campylobacter* spp. (not for layers in 1998 and 1999 and only for broilers in 2000) and for *E. coli* O157 (all animal species in 1998 and 1999 and only for dairy cattle and veal calves in 2000). The programme is focused on monitoring the prevalences, and their trends, of these zoonotic bacteria in farm animals. This information is amongst others useful in observing possible effects from intervention efforts implemented by the industry. Furthermore, risk factors for contamination of farm animals with these bacteria are identified and quantified to provide a basis for intervention strategies. Additionally, the occurrence of certain types of bacteria prevailing in animals is related to those causing disease in humans by means of serotyping isolates.

In 1998, 1999 and 2000, a total of respectively 859, 741 and 778 flocks/herds were examined for the presence of one or more bacteria species. Per animal species, these figures for 1998 were: 207 layer flocks, 192 broiler flocks, 41 finishing pig herds, 267 dairy cattle herds and 152 veal calf herds. For 1999, this was: 168 layer flocks, 155 broiler flocks, 189 finishing pig herds, 169 dairy cattle herds and 60 veal calf herds. The numbers for 2000 were: 166 layer flocks, 127 broiler flocks, 194 finishing pig herds, 158 dairy cattle herds and 133 veal calf herds.

Prevalence estimates for *Salmonella* spp. in 1998, 1999 and 2000 were 12%, 21% and 19%, respectively. For broilers, prevalence estimates were 28%, 20% and 16%, respectively. For finishing pigs, 34% of the herds were found positive in the fourth quarter of 1998, 13% in 1999 and 16% (36% when using the additional semi-solid medium) in 2000. In dairy cattle, 3%, 2% and 1% of the sampled herds were found positive in the consecutive years. For veal calves, 1%, 5% and 1% of the herds were salmonella positive in this period. Serotyping the isolates showed a predominant isolation of *S.* Enteritidis in layers. At flock level, 7%, 10% and 9% of the flocks were positive for *S.* Enteritidis in consecutive years. Likewise, this serotype was the most predominant one for broilers in 1998 and 1999, but the emerging *S.* Paratyphi B var. Java surpassed this serotype in 2000. In both poultry species, *S.* Enteritidis phagetype PT4 dominated within the Enteritidis isolates, which coincides with the observation in humans. In finishing pigs, the leading serotype was *S.* Typhimurium, with an increasing role within this type for definitive type (DT) 104 (from 13% of the *S.* Typhimurium isolations in 1998 to 19% in 2000).

Examination of the samples for *Campylobacter* spp. resulted in prevalence estimates of 31%, 18% and 24% for broiler flocks in 1998, 1999 and 2000, respectively. For finishing

pigs, 97% and 46% of the herds were found positive for *Campylobacter* spp. in 1998 and 1999, respectively. For dairy cattle, the two annual estimates were 32% and 7%, respectively, for veal calves 84% and 58%, respectively. Discrimination of campylobacter species into *C. jejuni* and *C. coli* showed a leading prevalence of *C. jejuni* in samples obtained from broilers (over 80%) and dairy cattle (over 90%), whereas finishing pigs were predominantly contaminated with *C. coli.* For veal calves, both serotypes were isolated equally, partly in the same herds.

E. coli O157 was not isolated from samples from layers and occasionally from broiler flocks (1%, n=186) and finishing pig herds (2%, n=41) in 1998. In 1999, these percentages were 1% (n=100), 0% (n=189) and 4% (n=113) for layers, finishing pigs and broilers, respectively. For dairy cattle, the microbiological isolation gave 5%, 9% and 6% (9% with an altered examination method) positive herds for 1998, 1999 and 2000, respectively. For veal calves, these percentages were 5%, 13% and 11% (17% with an altered examination method) in respectively 1998, 1999 and 2000. Trend analysis indicated a possible development of an increasing trend (P=0.0712). Molecular examination of the *E. coli* isolates showed that isolates contained at least one virulence gene and most gene combinations existed of the *eae* gene and either one or both SLT genes.

Risk factor analysis on *E. coli* O157 occurrence in dairy cattle revealed the following potential risk factors: presence of at least one pig at the farm, purchase of animals within the last two years before sampling, supply of maize to the cows, supply of beet pulp to the cows, and sampling of a herd in 2000 compared to sampling in 1998.

Risk factor analysis for *Campylobacter* spp. in broiler flocks gave the following potential risk factors: increasing age, the presence of five or more broiler houses on the premises, the presence of other farm animals on the farm, the presence of animals on farms within a 1-km range, children entering the broiler house, the use of broiler-specific workclothes and the seasons summer and fall. Furthermore, different hatcheries show diverse effects, but this variable showed strong collinearity with the integrated poultry operation to which a farm was linked and the feed mill that supplied the broiler feed. Effect modification was present between children entering the broiler house and the use of separate workclothes for broilers.

Based on the results described in this report, it can be concluded that animal reservoirs for *Salmonella* spp. are layers, broilers and finishing pigs. The consistency in measured salmonella prevalences in layers in the three years of study, and the consistent contribution of *S.* Enteritidis within the salmonella occurrence, showed that the implemented intervention efforts by the industry have not led to the intended prevalence reduction in 2000. In broilers, these measures presumably have their effect, considering the decreasing trend in annual prevalence estimates. This observation coincides with data from the monitoring programme run by the industry, while data from the KvW on poultry meat in retail stores in 2000 shows an increase in contamination percentage. The decrease of *S.* Enteritidis in broilers may be related to the emergence of *S.* Paratyphi B var. Java, a serotype with apparently less impact on humans, but more persistent on farms once it is present. In

finishing pigs, *S.* Typhimurium DT104 showed an increase during the study period, possibly indicating an increased risk for public health.

Campylobacter spp. was frequently isolated from broiler samples, but also finishing pigs, dairy cattle and veal calves act as reservoirs for this zoonotic bacterium. However, research on products from the latter three from retail stores has shown low campylobacter contamination percentages. Therefore, monitoring of campylobacter occurrence in these animal species was not continued after 1999. For broilers, no decrease was measured in the prevalences during the study years, despite efforts made by the industry since 1997 to reduce the campylobacter prevalence. The pattern in prevalences measured in this programme deviates from figures produced by the industry, but coincides with data on contamination of products from retail stores.

E. coli O157 was frequently isolated from samples from dairy cattle and veal calves. Trend analysis showed an indication for an increasing trend for veal calves. One isolate excluded, all isolates possessed one or both genes that enable shiga-like toxin production (SLT-I and/or SLT-II). Further research is needed on the potential increase in prevalence for veal calves and on risk factors for *E. coli* O157 in veal calves.

1. Introduction

Zoonotic micro-organisms transmitted to humans through food of animal origin pose a continuous problem to veterinary public health authorities. An important group of zoonotic agents are the bacteria causing gastro-enteritic symptoms, such as *Salmonella* spp., *Campylobacter* spp. and verocytotoxin producing *E. coli* O157. World-wide, gastro-enteritis is considered a serious public health problem with a significant impact on both economy and society (9). A recent sentinel study on human gastro-enteritis in general practices in The Netherlands has yielded an estimated incidence of 79.7 per 10,000 person years in the period 1996–1999 (13). The majority of these infections were caused by *Campylobacter* spp., being responsible for 10% of the cases. *Salmonella* spp. was associated with 4% of the cases. In 1999-2000, a study was conducted in the Dutch general population indicating a total number of cases of campylobacteriosis and salmonellosis of approximately 100,000 and 50,000, respectively (14).

The main campylobacter species causing disease in humans are *C. jejuni* (accounting for the majority of the cases) and *C. coli* (41). In developed countries world-wide, campylobacter infections in humans have been mainly associated with the consumption of undercooked poultry meat (46; 2), consumption of raw milk (51) or untreated drinking water (3), direct contact with pets (3), foreign travel (13) and, to a lesser extent, with pork (47). In The Netherlands, 30.5% of chicken products sampled at retail in 2000 and 32.5% in 2001 were contaminated with campylobacters, whereas pork samples were not found to be contaminated (60; 62).

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 phagetype. In 1995 this serotype accounted for approximately 50% of the salmonella infections in humans, but the contribution of this serotype has slightly declined in the last few years (66). *S.* Enteritidis infections have predominantly been associated with the consumption of raw eggs and egg-containing foods (50). Over the past years, in many countries, including The Netherlands, *S.* Typhimurium phagetype DT104 has emerged in humans as well as farm animals (7; 66; 71). *S.* Typhimurium DT104 is multi-resistant to antibiotics and there are indications that the clinical course of infections with this specific phagetype in humans is more severe in comparison with other salmonella infections (67). In The Netherlands, pigs and cattle appear to be main reservoirs of *S.* Typhimurium DT104 (65). Salmonella infections in humans have mainly been associated with consumption of (undercooked) foods of animal origin.

Shiga-toxin-producing *Escherichia coli* (STEC), and especially *E. coli* O157, has been shown in the past 10-15 years to be an important zoonotic agent causing haemorrhagic colitis (HC),

with potentially further complications, such as the haemolytic uraemic syndrome (HUS) (33; 52). HUS is characterised by acute renal failure. Young children (0–4 years of age) and the elderly are particular risk groups. In The Netherlands, approximately 20 children are seriously affected per year (25).
Cattle acts as the main reservoir for this particular serotype of *E. coli* (28) and contaminated beef products, raw milk and direct contact with farm animals are considered the main routes of transmission (64; 29). The virulence of STEC has been associated with the ability of toxin production, mediated through the Shiga-like toxin genes (SLT-I and SLT-II). Also, the presence of the *E. coli* attaching and effacing gene (eae-gene) has been associated with pathogenicity. This gene enables the pathogen to attach to the intestine wall and efface the lumen.

In 1997, the Dutch Product Boards for Livestock, Meat and Eggs (Productschap voor Vee, Vlees en Eieren (PVE)) have 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 layers (39; 38). 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.

Adequate control of the above mentioned bacteria and other zoonotic agents in the food production chain largely depends on the availability of reliable data on the occurrence of these agents both at farm animal level and at retail level. In fulfilling this need, the Zoonosis Directive (92/117/EEC) issued by the European Community in 1992, obliges all Member States to report zoonoses-data of diverse origin annually. In this context, the Inspectorate for Health Protection and Veterinary Public Health (Keuringsdienst van Waren (KvW)) monitors, for instance, chicken products in retail stores on *Salmonella* spp. and *Campylobacter* spp.. The same Inspectorate commissioned the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu (RIVM)) to initiate an integrated monitoring program in The Netherlands. The programme started in 1997 and is continuously running, with the main focus on monitoring prevalences, and their trends, of *Salmonella* spp., *Campylobacter* spp. and *E. coli* O157 in layers, broilers, finishing pigs, dairy herds and veal calves. Additionally, the occurrence of certain types of bacteria prevailing in animals is related to those causing disease in humans. Also essential data on farm and flock/herd characteristics are gathered to perform risk factor analyses (RFAs). Results can be used in the developmental stage of intervention strategies to reduce the prevalence.
The results of the monitoring programme obtained in 1997 are described in a previous report (in Dutch) by Heuvelink *et al* (26). The current report describes the monitoring programme as it is to date and presents the results obtained from January 1998 through December 2000. First, the programme design is described, followed by brief descriptions of the microbiological isolation techniques used. Then logistic aspects of the sampling procedure

are described, and prevalence estimates and observed trends are presented per pathogen. Additionally, two summaries of performed risk factor analyses (*E. coli* O157 occurrence in dairy cattle and *Campylobacter* spp. occurrence in broiler flocks) are presented. Conclusively, a chapter is dedicated to discussion and conclusions.

2. Materials and Methods

2.1 Programme design

The unit of observation and analysis throughout the entire programme is a flock/herd of animals. For layers, broilers and veal calves, a flock/herd includes all animals of similar age housed within one building. A dairy cattle herd is similar to all cows except of dry ones and young stock. A herd of finishing pigs equals all animals housed in one building, usually existing of several divisions and therefore including animals in various production stages and of diverse ages.

A two-stage sampling scheme (i.e. using a primary and secondary sample size) was used to accurately estimate the national prevalence of positive flocks/herds in a certain year for each target bacterium. The secondary sample size was calculated to allow detection of an organism in a flock/herd at a minimal contamination level of 5% with a reliability of 95%. Results of the calculations made with the epidemiological computer programme WinEpiscope (15) are presented in Table 2.1. To minimise laboratory efforts, studies have been conducted on the effect of pooling samples, and data indicate that pooling of individual samples allows detection of the organism without unacceptable loss of performance for *Salmonella* spp. (54). Therefore, a number of individual samples was aggregated into pooled samples according to Table 2.1 and examined for the presence of the target micro-organisms.

The primary sample size specifies the number of flocks/herds to be sampled to estimate the national prevalence with the *a priori* defined accuracy and precision. Input parameters include the predicted prevalences for the target bacteria in the coming year, the total number of flocks/herds, and the confidence and accuracy levels. The prevalence estimates are based on a 90% confidence level and an accuracy level ranging between 3% – 5% (depending on the prevalence, where a lower predicted prevalence resulted in a higher desired accuracy). The parameters used and the results of the calculations are presented in Appendix 1A – E.

The Animal Health Service (Gezondheidsdienst voor Dieren (GD)) selected a random number of layer, broiler, finishing pig and dairy cattle farms in The Netherlands. Stratification was applied according to five regions for all species and according to farm size for finishing pigs and dairy cattle. The Quality Guarantee of Veal (Stichting Kwaliteitsgarantie Vleeskalveren (SKV)) provided a sampling population for veal calves,

Table 2.1 Number of individual and pooled samples per flock, depending on flock size.

Flock size	Individual samples	Pooled samples
1 – 24	Equal to flock size, with a max. of 20	2
25 – 29	20	2
30 – 39	25	2
40 – 49	30	3
50 – 59	35	3
60 – 89	40	4
90 – 199	50	4
200 – 499	55	5
≥ 500	60	5

stratified according to region, farm size and age. Farm holders were contacted through mail and asked for voluntary participation in the programme. After the response period, a list of potential sampling units was made and sent to the KvW. Subsequently, a number of farms corresponding to the primary sample size was selected from this list and sampled by KvW-employees according to a strict protocol. If more than one flock/herd was present on the farm, one was randomly selected. Subsequently, the appropriate number of faecal droppings was collected from the floor or – in case of laying hens - manure conveyer and transported to the RIVM in cooled transport boxes by a professional delivery service. Microbiological examination started within 48 hours.
In addition to the faecal samples, a questionnaire addressing farm- and flock/herd-specific information was completed in co-operation with the farm manager. Questionnaires were sent to the RIVM for use in analyses on risk factors and on representativeness of the sampled flocks/herd for the respective populations.

2.2 Microbiological examination

2.2.1 *Salmonella* spp.

For the detection of *Salmonella* spp. in the faecal samples, a RIVM standard operating procedure (MGB/M124) was used based on ISO 6579 (32; 42). In this procedure, several modifications of the ISO-method are included. No selenite/cystine medium for selective enrichment and no bismuth sulfite agar for selective isolation were used. These media are particularly useful for the detection of *S.* Typhi, a serotype that is not found in farm animals. For the selective enrichment of samples from broilers, layers and finishing pigs, a semisolid medium was used in addition to the prescribed Rappaport-Vassiliadis (RV; Oxoid, Haarlem, The Netherlands; catalogue number cm866) as part of a comparison study (69). Samples from layers and broilers were subjected to this medium from October 1998 onwards, samples from finishing pigs in 2000 only. Samples from veal calves and dairy cattle were examined by using RV only.
From each pooled sample, 25 g was added to 225 ml of buffered peptone water (NVI, Bilthoven, The Netherlands; E4900z) and incubated for 16 to 20 hours at 37 ± 1 ^{o}C.
Of the pre-enrichment culture, 0.1 ml was inoculated in 10 ml RV broth and incubated for 2 x 24 ± 1 h at 42 ± 0.5 ^{o}C. After 24 h the culture was plated on brilliant green agar (BGA; Oxoid; cm329) and incubated for 24 ± 2 h at 37 ± 1 ^{o}C. If no suspect colonies were obtained, the RV-culture – after 48 h of incubation – was plated-out on BGA again and incubated under similar conditions. In addition, for selective enrichment of samples from layers, broilers, and finishing pigs, the modified semi-solid RV medium (MSRV; Difco; 1868-17) was used. Preparation of the medium, containing 0.01 g l^{-1} novobiocine (Sigma-Aldrich Chemie, Wyndrecut, The Netherlands; n1628), was done as described by the manufacturer. Three drops of the pre-enrichment culture were inoculated on this medium, followed by an incubation for 2 × 24 ± 3 h at 41.5 ± 1 ^{o}C. White colonies were transferred to 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; E6025z) and lysine-decarboxylase broth (NVI; E4000z). Positive isolates were sent to the National Salmonella Centre (Diagnostic Laboratory for Infectious Diseases and Perinatal Screening at the RIVM, Bilthoven, The Netherlands) for sero- and phagetyping.

2.2.2 *Campylobacter* spp.

For the detection of thermophilic *Campylobacter* spp. a RIVM standard operating procedure (43) was used. For this, each pooled faecal sample was directly plated on Campylobacter blood-free selective agar (CCDA; Oxoid; cm739) using sterile swabs, followed by incubation for 44–52 hours at 42 ± 1 ^{o}C using the GENbox Microaër system (Biomerieux, Marcy, l'Etoile, France; 96125). 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/herd on the same plate. The microaerobic incubation procedure was repeated and characteristic campylobacter colonies were examined under a microscope for typical spiral-shaped cells and rapid motility. If the microscopic results were ambiguous, the Indx Campy agglutination test (Bipharma, Weesp, The Netherlands; 22000105) was performed additionally, as prescribed by the manufacturer. One campylobacter isolate per positive pooled sample was stored in 2 ml peptone glycerol and subjected to the mixed *polymerase chain reaction* (PCR) method described by Van de Giessen *et al.* (55) at a later stage to discriminate *C. coli* from *C. jejuni*.

2.2.3 *E. coli* O157

Until January 2000, the pooled samples were aggregated further into one pooled sample per flock/herd and examined for the presence of *E. coli* O157. Since January 2000, all pooled samples were examined individually for the presence of the bacteria. For this examination, a RIVM standard operating procedure MGB/M517 was used (44).

A portion of 10 g of the faecal sample was added to 90 ml of modified Trypton Soya Broth (mTSB; Oxoid; cm129) with 5 ml l^{-1} acriflavine and incubated in a glass jar for 7 ± 1 h at 37 ± 1 ^{o}C. One ml was subsequently added to a 1.5 ml vial with 20 µl anti-*E coli* O157 dynabeads (Dynal, Oslo, Norway; 71004) and incubated for 0.5 h at 20 ± 2 ^{o}C on a rotary shaker at 150 rotations per minute. Immuno magnetic separation (IMS) was performed by placing the substance in a Magnetic Partial Concentrator (Dynal) and aspirating the supernatant. The dynabeads were resuspended by adding 1 ml of wash buffer and again subjected to IMS. This was repeated twice, after which the dynabeads were resuspended in 100 µl wash buffer and mixed on a rotary shaker briefly. This solution was subsequently streaked onto sorbitol MacConkey agar (SMAC; Oxoid; cm813) enriched with 200 µl cefixime-solution and 200 µl tellurite-solution per 200 ml SMAC (CT-SMAC). These plates were incubated for 18 to 20 hours at 37 ± 1 ^{o}C, enabling screening for the presence of sorbitol-negative colonies. If present, 12 colonies were confirmed on SMAC supplemented with 0.1 g 4-methylbelliferyl-β-D-glucuronide (MUG; Sigma Chemical Co., St. Louis, MO; m9130) and on SMAC supplemented with 37.5 g l^{-1} eosin methylene blue agar (EMB; Oxoid;

cm69). These were incubated at 37 ± 1 °C for 18 to 20 hours, after which screening for pink mauve metallic colonies on EMB and for achromatic colonies being non-fluoresecent to UV-light (302 nm) on SMAC+MUG was performed. These colonies were suspected to be *E. coli* O157 and subjected to an agglutination test (Murex, Kent, UK; zc60) to confirm the authenticity of the colonies. In addition, one isolate per flock/herd was serotyped at the Diagnostic Laboratory for Infectious Diseases and Perinatal Screening.
If the confirmation resulted in the identification of *E. coli* O157, the isolates were stored in 1 ml peptone glycerol and stored at -70 °C, and at a later stage subjected to PCR (44) for detection of the SLT-I, SLT-II and *eae* genes.

2.3 Questionnaires

Questionnaires are divided into three major sections addressing either farm specific information, flock/herd specific information or hygiene-related measures. Questionnaires are in Dutch and therefore not included in the appendices, but can be obtained from the first author upon request. Differing per animal species, the number of variables derived from the questionnaires ranged between 36 and 56.
In 1999, the questionnaires were structurally revised. Several questions were added, others removed, several open-ended questions were replaced by multiple choice questions and some questions were more elaborated, since the earlier questions did not yield the information aimed at. These changes influenced the initial structure of collected data for some variables, but in general improved the data quality.

2.4 Data analyses

Prevalence estimates were obtained by dividing the total number of positive flocks/herds by the number of examined flocks/herds per year. Standard errors for this estimate were obtained through eqn. (1)

$$sem = \sqrt{\frac{\hat{P}(1-\hat{P})}{N}} \qquad (1)$$

where $\hat{P}$ equals the estimated prevalence and N indicates the sample size (53). Confidence limits were calculated by multiplying the standard error of the mean (*sem*) with 1.64 (90% confidence level) and either subtract (lower confidence limit) or add (upper confidence limit) this product to the estimated prevalence. This method uses a normal approach to the binomial distribution of the data and is valid when the estimated prevalence equals or exceeds 0.05, and both *NP* and *N(1-P)* are bigger than five (53). In case of a $P<5\%$, the normal approach does not hold and a Beta distribution was used for calculating the modi (i.e. prevalences) and confidence intervals (70).
The qualitative geographical analyses were performed with the SAS/GIS procedure in the statistical software package SAS, version 8.2 (40). Statistics Netherlands (45) provided the regional spread of farms in The Netherlands, that were used for comparison with the regional spread of sampled farms. The statistical tests for possible regional differences between farms

in the database and the actual spread of farms in The Netherlands were based on the χ^2-distribution.

Trend analyses were performed according to the principles described by Mantel (35). The method to assess the significance of differences in annual prevalence estimates is provided by Thrusfield (53).

Monthly data were examined for cyclic trends to detect possible seasonal fluctuations. A useful tool in that process is the smoothing of data, which is described by *a.o.* Diggle (16). There are numerous types of smoothing available and the method used in this report is based on the simple moving average of order 3, represented in Eqn. (2):

$$s_t = \frac{1}{9}(y_{t-2} + 2y_{t-1} + 3y_t + 2y_{t+1} + y_{t+2}) \tag{2}$$

where y_t is the percentage of positive flocks/herds at time t and s_t the smoothed estimated percentage of positive flocks/herds at time t. As the equation indicates, s_t is based on the two previous and subsequent measurements for time t, with a separate weight factor for each measurement. The estimated prevalence for the time-point of interest receives most weight. However, when applying this equation to the data from the surveillance programme, it does not adjust for the sample size and thus assumes its equalness per time unit. This is not correct, since sample sizes differ monthly. An additional weight factor should therefore be incorporated in the equation. However, adding this into Eqn. (2) was not easily accomplished, since the weight factor for sample size is based in a three-time-step interval, as is explained in Eqn. (3) (16).

$$s_t = \frac{1}{9}\left[(y_{t-2} + y_{t-1} + y_t) + (y_{t-1} + y_t + y_{t+1}) + (y_t + y_{t+1} + y_{t+2})\right] \tag{3}$$

For instance, the weight for y_{t-1} should be based on the ratio between its sample size and the average sample size for y_{t-2}, y_{t-1} and y_t, but also on the ratio between its sample size and the average of y_{t-1}, y_t and y_{t+1}, etc.. This was not easily incorporated into Eqn. (2), but more easy incorporated in Eqn. (3), resulting in Eqn (4),

$$s_t = \frac{1}{9}\left[\left(\frac{y_{t-2}\times n_{t-2} + y_{t-1}\times n_{t-1} + y_t \times n_t}{\sum_{i=t-2}^{t} n_i}\right) + \left(\frac{y_{t-1}\times n_{t-1} + y_t \times n_t + y_{t+1}\times n_{t+1}}{\sum_{i=t-1}^{t+1} n_i}\right) + \left(\frac{y_t \times n_t + y_{t+1}\times n_{t+1} + y_{t+2}\times n_{t+2}}{\sum_{i=t}^{t+2} n_i}\right)\right] \tag{4}$$

where n_t is the monthly sample size at time t. A shorter, general notation is given in Eqn. (5).

$$s_t = \frac{1}{9}\sum_{a=0}^{2}\left(\frac{y_{t-a}\times n_{t-a} + y_{t-a+1}\times n_{t-a+1} + y_{t-a+2}\times n_{t-a+2}}{\sum_{i=t-a}^{t-a+2} n_i}\right) \quad (5)$$

For the smoothed estimates of the first two months of 1998, data from late 1997 were used. A similar approach was used for data on November and December 2000, where data from January and February 2001 were used for smoothing.
Smoothing is in essence an exploratory tool, but can be useful in observing cyclic trends (16).

2.5 Available data

Not all target bacteria were monitored in all species continuously and also alterations to the sampling scheme occurred. These facets hamper certain interpretations of the data presented later on.
Finishing pig samples were not taken at the beginning of the programme, but were planned to be collected from 1998 onwards. However, a classical swine fever outbreak in The Netherlands further delayed these plans until the fourth quarter of 1998. Therefore, results from 1998 for this animal species have to be carefully related to later findings.
In 1998 and 1999, *Campylobacter* spp. were monitored in all farm animal categories excluding layers. However, samples from veal calves and dairy cattle were examined for campylobacter contamination from April through May 1998 and from September 1998 through December 1999. In 2000, this micro-organism was monitored in broilers only. Furthermore, *E. coli* O157 was monitored in all animal species in 1998 and 1999, but only in dairy cattle and veal calves in 2000.

3. Results

3.1 Logistics

3.1.1 Primary sample sizes

In the period January 1998 and December 2000, approximately 11,200 pooled samples from 2,378 flocks/herds were examined for the target bacteria. To adequately estimate the annual prevalences in terms of the initial parameter inputs (confidence level, accuracy and expected prevalences), a total of 3,061 flocks/herds needed to be sampled. Table 3.1 shows the calculated sample sizes compared to the number of sampled flocks/herds per year.
The target sample sizes were not reached for poultry and finishing pigs in most years, and for veal calves in 1999. Sufficient sample sizes were obtained for dairy cattle in all years, for veal farms in 1998 and 2000, and for layers in 1998.

3.1.2 Geographical distribution of sampled farms

The geographical distribution of sampled flocks/herds per species per year is presented in Appendices 2A – E. Not all collected samples could be related to a zip code (e.g. due to a missing questionnaire, a missing zip code on a questionnaire, or a zip code being unrecognisable for the spatial databases), for which a number of flocks/herds are missing in the figures.
An interesting notice from the figures is the clustering of the sampled farms with laying hens and veal calves that is consistently observed in The Central Netherlands. Also, the sampling of finishing pig herds was mainly concentrated in the southern part of The Netherlands. For dairy cattle, a clustering is present in the western part of the South in 2000.
Statistical analyses on the difference in geographical distribution between the sampled farms in the database and all Dutch farms divided over the five KvW regions, shows that a significant difference was present for layers, broilers, finishing pigs and dairy cattle in 1998 and 1999 (all significant $P<0.005$). In 2000, a statistical difference was observed for all animal species ($P=0.048$ for veal calves, all other $P<0.0001$). An adjustment for these discordancies in regional spread on the annual prevalence estimates is shown in Appendix 3. Differences between the measured and adjusted prevalences were relatively small, with the majority of differences varying between two and five per cent.

Table 3.1 Comparison of the calculated sample sizes (N_c) with the actual sample sizes (N_s) and the percentage sampled from N_c.

	1998			1999			2000		
Species	N_c	N_s	%	N_c	N_s	%	N_c	N_s	%
Layers	174	207	119	216	167	77	216	166	77
Broilers	260	192	74	260	154	59	216	128	59
Finishing pigs	271	41	15	271	189	70	216	194	90
Dairy cattle	271	267	99	143	169	118	143	158	110
Veal calves	135	152	113	135	60	44	135	133	99
TOTAL	1110	859	77	1025	741	72	926	778	84

3.1.3 Age distribution

In Figure 3.1A – D, the relative age distribution of the sampled flocks/herds is given.

The distribution for layers shows a similar pattern throughout the years, with a minor contribution of flocks that are producing eggs for over 60 weeks. The moment of sampling in the production period varied from 4 days to 104 weeks with a median of 30 weeks.

For broiler flocks, a cut-off value was set at 60 days of age to exclude unrealistic ages. Despite the emphasis that was put on sampling flocks ageing 4 – 6 weeks, younger flocks were frequently sampled. A similar pattern in age distribution is observed over the years. The age of the broilers ranged between 4 and 59 days with the median at 31 days of age.

For finishing pigs, the majority of the herds sampled in 1998 and 1999 consisted of relatively young animals, whereas in 2000, most herds harboured pigs of various production-ages. The occurrence of this category in 2000 only is caused by a change in the questionnaire (from open-ended question to multiple choice). Furthermore, one herd was reported to be in production for 365 days. Since this is unlikely, this record was omitted from the age-analysis. The median age for pigs in 1998 and 1999 was 73 days within a range between 4 and 194 days. For 2000, no median age and range can be specified.

The distribution of age in veal calves shows a main tendency of sampling herds in the range of 0 – 30 weeks of age for all years. Age of the calves ranged between 4 days and 53 weeks with a median age of 13 weeks.

For dairy cattle, no data on age were collected.

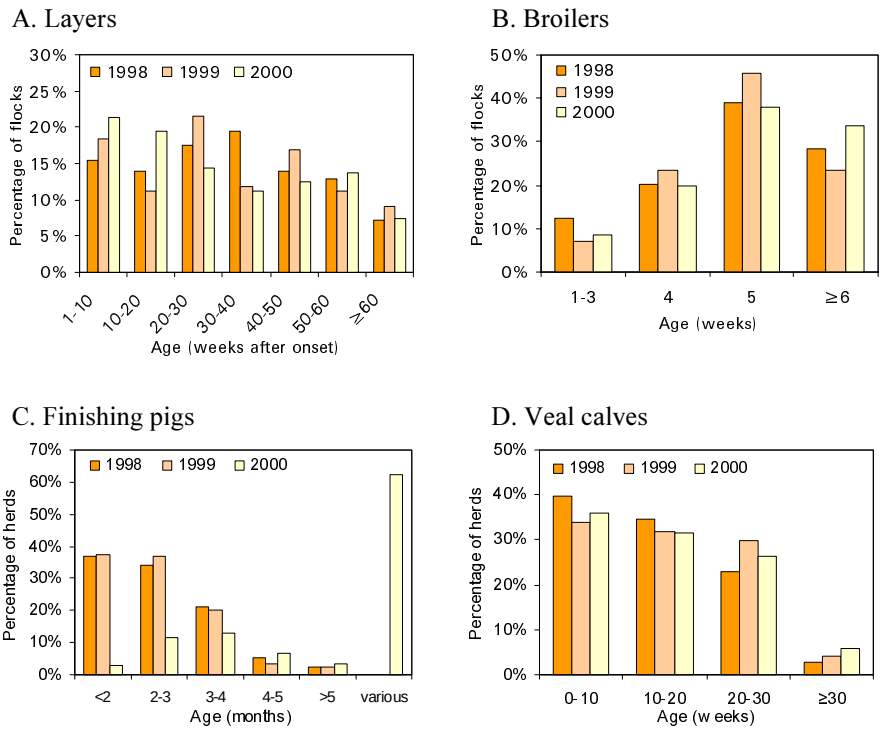

Figure 3.1 Age (or production age) distribution per year as percentages of sampled flocks for layers, broilers, finishing pigs and veal calves.

3.1.4 Flock-/herdsize distribution

The relative distribution of flock-/herdsizes for the sampled flocks/herds per year is shown in Figure 3.2A – E.

For layers, a shift from the category with 2,000 – 5,000 birds to ≥20,000 birds was present during the study period. Flocksize ranged from 5 – 160,000 with a median of 9,000 birds per flock.

For broiler flocks, an approximate 10% decrease of flocks with a flocksize of 10,000 – 20,000 birds in 2000 compared to 1998 is contrasted by an approximate 10% increase in flocks with more than 30,000 birds. In each year, flocks with less than 10,000 birds were less frequently sampled. Flock sizes in broilers ranged from 100 to 87,000 birds, with a median of 20,000 birds per flock.

For finishing pigs, the relative distribution changed due to an increase in the contribution of larger herds in 2000. Consequently, smaller herds were less frequently sampled. The overall range for herd sizes in pigs was 18 – 2,100 with a median of 120 animals.

In dairy cattle, a relatively similar distribution was observed for the three years. Herds with

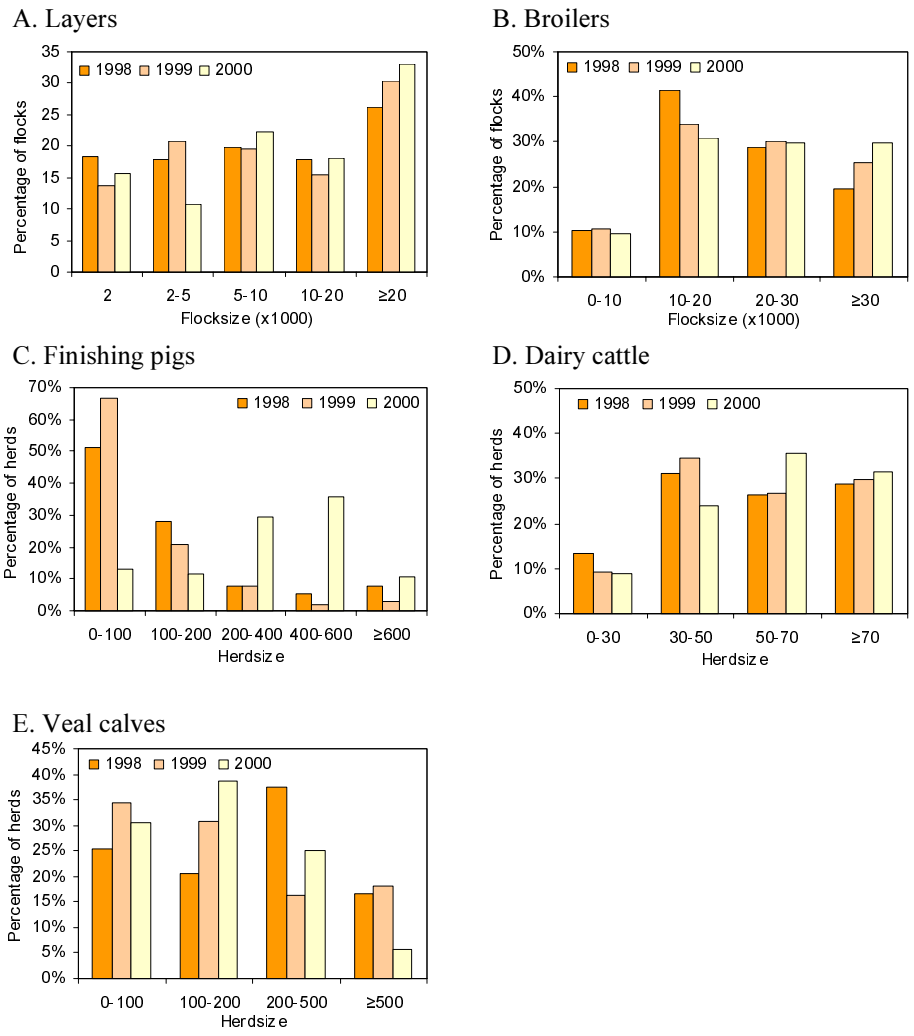

Figure 3.2 Flock-/herdsize distribution per year as percentages of sampled flocks/herds for layers, broilers, finishing pigs, dairy cattle and veal calves.

less than 30 animals were sampled less frequently than larger herds. Herd sizes ranged between 6 – 220 animals, with a median of 55.
For veal calves, a relatively low number of larger herds was sampled in all years, but in 2000 the contrast with the other categories of herd size had increased. This reduction in sampling larger herds coincided with a simultaneous increase in sampling herds with 100 – 200 animals. Herd sizes for veal calves ranged between 5 – 1,220 animals with a median of 156.

3.2 *Salmonella* spp.

In Figure 3.3, the observed prevalence of *Salmonella* spp. is shown for all animal species. For layers and broilers in all three years and for finishing pigs in 2000, two annual estimates were obtained due to the change in detection method (see chapter 2.2.1). In Table 3.2, the values for the prevalence estimates and the 90% confidence intervals are given.
A significant decrease in prevalence was observed in broilers between 1998 and 1999. All other estimates did not differ significantly from the previous prevalence estimates. Trend analysis over the three years showed a significant decreasing trend for broilers (P<0.0001; RV-data); no significant trends were found for the other animal species. In the three animal

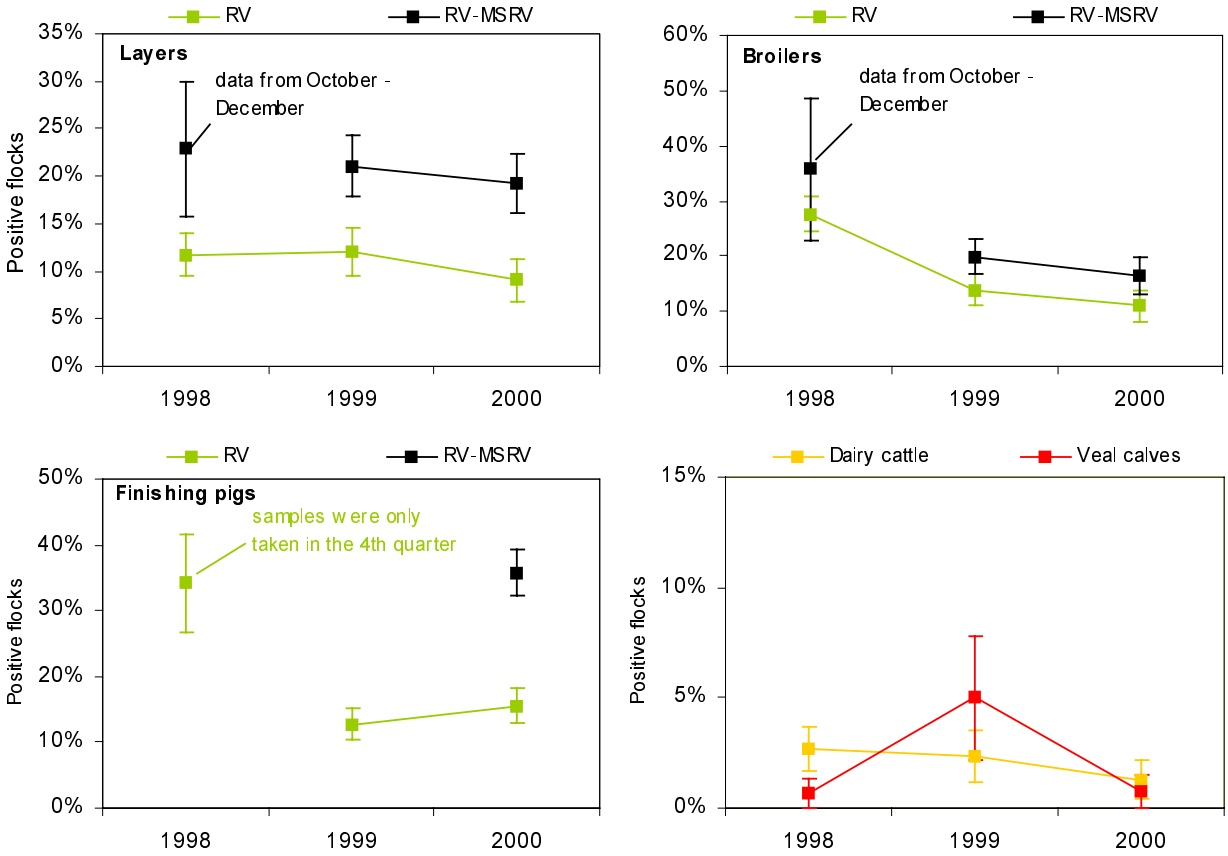

Figure 3.3 Estimated Salmonella *spp. prevalence per year (± standard error) for layers, broilers and finishing pigs using the selective enrichment medium Rappaport-Vassiliadis (RV) and the additional modified semi-solid RV (MSRV) and the estimated* Salmonella *spp. prevalence (± standard error) for dairy cattle and veal calves using RV. Marked estimates (*) indicate a significant difference with the previous estimate.*

Table 3.2 Salmonella prevalences (P) and 90% confidence intervals (90% CI). N gives the number of flocks examined that year.

	1998			1999			2000		
	N	P	90% CI	N	P	90% CI	N	P	90% CI
Layers*	207	11.6	8.0 – 15.2	166	21.1	16.0 – 26.2	166	19.3	14.4 – 24.2
Broilers*	192	27.6	22.3 – 32.9	151	19.9	14.6 – 25.2	128	16.4	11.0 – 21.8
Finishing pigs$	41	34.1	21.9 – 46.3	189	12.7	8.7 – 16.7	194	35.8	29.9 – 41.7
Dairy cattle†	263	2.7	1.5 – 4.9	169	2.4	1.2 – 5.3	156	1.3	0.5 – 4.0
Veal calves†	148	0.7	0.2 – 3.1	60	5.0	0.4 – 9.6	132	0.8	0.3 – 3.5

* Estimate of 1998 is based on the use of RV medium, others on the use of RV and MSRV.
$ Estimates of 1998 and 1999 are based on the use of RV medium, the estimate of 2000 on the use of RV and MSRV.
† All estimates based on the use of RV medium.

species where the additional semi-solid selective enrichment medium was used, a higher number of positive flocks/herds was found. For layers and finishing pigs, differences reached up to a more than two-fold increase in the prevalence estimates. For broilers, this difference was smaller, but still evident.

In Appendix 4A, smoothed graphs of the percentages of positive flocks/herds per month are shown for layers, broilers and finishing pigs. The prevalences for *Salmonella* spp. in dairy cattle and veal calves are too low to produce meaningful graphs. None of the three graphs shows signs of a distinct seasonal pattern.

The most frequently isolated serotypes from layers, broilers and finishing pigs per year are given in Table 3.3A – C. All isolated serotypes for all five species, classified per year, can be

Table 3.3 The most prevalent salmonella serotypes per year in layers, broilers and finishing pigs. N gives the number of Salmonella *spp. positive flocks.*

A. Layers

1998 (N=24)*			1999 (N=37)$			2000 (N=32)$		
Serotype	*n*	*%*	*serotype*	*n*	*%*	*serotype*	*n*	*%*
Enteritidis	14	58	Enteritidis	16	43	Enteritidis	15	47
Infantis	4	17	Braenderup	5	14	Infantis	6	19
Virchow	3	13	Infantis	5	14	Braenderup	4	13
Braenderup	2	8	Livingstone	5	14	Mbandaka	2	6
						Montevideo	2	6

B. Broilers

1998 (N=54)*			1999 (N=32)$			2000 (N=21)$		
serotype	*n*	*%*	*serotype*	*n*	*%*	*serotype*	*n*	*%*
Infantis	16	30	Enteritidis	8	25	Paratyphi B var. Java	8	38
Enteritidis	7	13	Infantis	5	16	Virchow	4	19
Heidelberg	6	11	Paratyphi B var. Java	4	13	Mbandaka	3	14
Paratyphi B var. Java	5	9	Mbandaka	3	9	Enteritidis	2	10
Mbandaka	5	9	Uganda	3	9	Manhattan	2	10

C. Finishing pigs

1998 (N=14)*			1999 (N=24)*			2000 (N=63)$		
serotype	*n*	*%*	*serotype*	*n*	*%*	*serotype*	*n*	*%*
Typhimurium	8	57	Typhimurium	12	50	Typhimurium	32	51
Derby	2	14	Derby	5	21	Derby	8	13
Infantis	2	14	Livingstone	3	13	Livingstone	7	11
			Give	2	8	Brandenburg	6	10
			London	2	8	Infantis	3	5

* Based on the use of Rappaport Vassiliadis (RV) medium.
$ Based on the use of RV medium and Modified semi-solid RV (MSRV) medium.

found in Appendices 5A – C. In layers, *S.* Enteritidis was the most prevalent serotype in the study period, with 7%, 10% and 9% positive flocks in the consecutive years. Phagetype PT4 was the leading serotype within these Enteritidis isolations, with 64%, 81% and 93% (9, 13 and 14 flocks) in 1998, 1999 and 2000, respectively. Other serotypes frequently isolated from layers in all years were *S.* Infantis and *S.* Braenderup.

In broilers, *S.* Enteritidis was one of the leading serotypes in 1998 and 1999, with approximately 5% of positive flocks in these two years. In 2000, *S.* Enteritidis and *S.* Infantis showed a strong decrease in occurrence, while *S.* Paratyphi B var. Java emerged. Phagetype PT4 was identified in 57% and 100% of the *S.* Enteritidis positive flocks in 1998 and 1999, respectively, and in one of the two flocks in 2000.

In finishing pigs, a predominance of *S.* Typhimurium was observed, representing roughly 50% of the isolates in all years. At herd level, 20%, 6% and 8% (16% when using semi-solid medium in addition) were positive for *S.* Typhimurium in 1998, 1999 and 2000, respectively. *S.* Typhimurium phagetype DT104 was isolated from one (13% of the *S.* Typhimurium positive herds), two (17%) and three (six when using the additional semi-solid medium) herds (19%) in consecutive years. *S.* Derby was also isolated relatively frequent in all three years, accounting for roughly 15% of the salmonella positive herds.

Prevalences in dairy cattle and veal calves are low. An overview of the most prevalent serotypes for these farm animals can be found in the Appendices 5A – C.

A comparison between the estimated prevalences of *Salmonella* spp. in this surveillance program with those found in the monitoring programme run by the industry (PVE) and the results from monitoring poultry end-products in retail stores (KvW) is shown in Appendix 6. Although in both programmes focussing on broiler farms the prevalence measurements indicate a decrease in percentage of salmonella positive flocks over time, the monitoring at retail shows an increase in positive end-products in 2000. Furthermore, the data from the RIVM surveillance programme show a steeper decrease in salmonella prevalence than the PVE data.

3.3 *Campylobacter* spp.

Figure 3.4 shows the observed annual prevalences of *Campylobacter* spp. for broilers, dairy cattle, finishing pigs and veal calves. The results in percentages with the 90% confidence intervals are presented in Table 3.4.

A significant decrease in campylobacter occurrence was observed for broilers between 1998 and 1999, followed by a non-significant increase between 1999 and 2000. Trend analysis indicates the

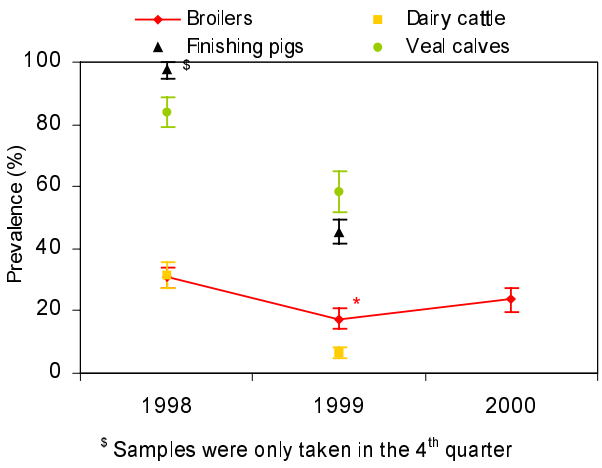

Figure 3.4 Estimated prevalence (± standard error) for broiler, finishing pigs, dairy cattle and veal calves. Marked estimates (*) indicate a significant difference with the previous estimate.

Table 3.4 Campylobacter prevalences (P) and 90% confidence intervals (90% CI). N gives the number of flocks examined that year.

	1998			1999			2000		
	N	P	90% CI	N	P	90% CI	N	P	90% CI
Broilers	189	30.7	25.2 – 36.2	154	17.5	12.5 – 22.5	127	23.6	17.4 – 29.8
Finishing pigs	38	97.4	93.1 – 100	189	45.5	39.6 – 51.5		n.d.*	
Dairy cattle	130	31.5	24.8 – 38.2	169	6.5	3.4 – 9.6		n.d.	
Veal calves	62	83.9	76.2 – 91.6	60	58.3	47.9 – 68.8		n.d.	

* n.d.: not determined

absence of a significant decreasing trend (P=0.0893). For dairy cattle, veal calves and finishing pigs, the prevalence estimate for 1999 was lower than for 1998. However, the limitations in sampling discussed in chapter 2.5 cannot be excluded as cause of these differences.

Appendix 4B contains a graph with smoothed data on the percentages of campylobacter positive broiler flocks. A seasonal fluctuation was present, with the percentage of positive flocks peaking mainly around May through July.

The results of the PCR method used to discriminate between *C. jejuni* and *C. coli* are presented in Table 3.5. In flocks of broilers and dairy cattle *C. jejuni* was the predominating species, whereas in finishing pigs *C. coli* predominantly occurred. The isolates obtained from veal calf samples mainly belonged to *C. coli*, but also *C. jejuni* was frequently observed.

The comparison between data on campylobacter prevalence in broilers derived from this programme, the monitoring programme run by the industry and the monitoring of poultry meat end-products in retail stores is presented in Appendix 6. Data from all three programmes show a decrease between 1998 and 1999, although the slopes of the three trends differ. The slope of the RIVM line shows the largest decrease, the KvW-slope the smallest. Between 1999 and 2000, both the RIVM data and the KvW data show an increase in campylobacter contamination, whereas PVE data suggests a further decrease.

Table 3.5 The differentiation of campylobacter isolates in C. jejuni *and* C. coli *per animal species per year.*

Year	Species	# flocks examined*	*% C. coli*	*% C. jejuni*	% Both
1998	Broilers	41	14.6	85.4	0
	Pigs	21	90.5	0	9.5
	Cattle	35	2.9	94.2	2.9
	Calves	71	53.5	31.0	15.5
1999	Broilers	20	15.0	80.0	5.0
	Pigs	35	97.1	2.9	0
	Cattle	8	0	100	0
	Calves	22	45.5	13.6	40.9
2000	Broilers	30	6.7	86.6	6.7

* Not all positive flocks could be taken into examination.

3.4 *E. coli* O157

Figure 3.5 shows the annual *E. coli* O157 prevalence for dairy cattle and veal calves. The results of the examination of each pooled sample individually are also presented in the figure. The prevalence estimates and 90% confidence interval are shown in Table 3.6, including data on the occurrence of *E. coli* O157 in layers, broilers and finishing pigs. No statistically significant differences in *E. coli* O157 prevalences were observed between the years of study for dairy cattle and veal calves. Trend analyses for a significant increase of the prevalence estimates over time failed to reach significance for veal calves (P=0.0712), although this figure might indicate a tendency towards a possible trend. For dairy cattle, no increasing or decreasing trend in annual prevalence estimates was observed.

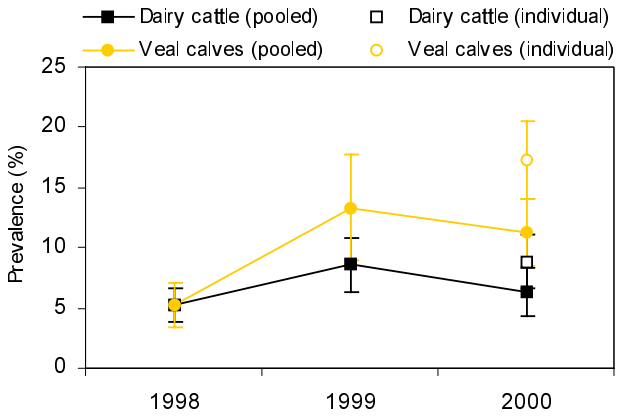

Figure 3.5 Estimated E. coli *O157 prevalence (± standard error) per year for dairy cattle and veal calves. Until 2000, pooled samples were further aggregated into one pooled sample at the laboratory, whereas in 2000 pooled samples were also examined individually.*

In layers, none of the 202 samples were *E. coli* O157 positive in 1998, while in 1999 one positive flock was found (n=113). In broilers, prevalences of 1.1% (n=186) and 4% (n=100) were observed in 1998 and 1999, respectively. For finishing pigs, one out of 41 samples was found positive in 1998 (2.4%) and none in 1999 (N=189).

Appendix 4C contains graphs showing the seasonal variation of percentages of *E. coli* O157 positive dairy and veal calf herds. In both figures, a clear seasonal pattern is observed with the number of *E. coli* O157 positive herds peaking between September and November. The percentage of positive herds rises during spring and steeply decays after November. The indications for a possible increasing trend for *E. coli* O157 in veal calves is less evidently observed in the figure.

The occurrence of strains with SLT-I, SLT-II and / or the eae-gene is presented in Table 3.7. It was not possible to isolate DNA from all positive pooled samples, therefore the number of

Table 3.6 E. coli O157 prevalence (P (%)) and 90% confidence interval (90% CI) in layers, broilers, finishing pigs, dairy cattle and veal calves. N gives the number of flocks examined that year.

	1998			1999			2000		
	N	P	90% CI	N	P	90% CI	N	P	90% CI
Layers	202	0	0 – 1.5	113	0.9	0.3 – 4.1		n.d.*	
Broilers	186	1.1	0.4 – 3.3	100	4.0	2.0 – 8.8		n.d.	
Finishing pigs	41	2.4	0.9 – 10.8	189	0	0 – 1.6		n.d.	
Dairy cattle$	267	5.2	3.0 – 7.4	163	8.6	5.0 – 12.2	158	8.9	5.2 – 12.6
Veal calves$	152	5.3	2.3 – 8.3	60	13.3	6.1 – 20.5	133	17.3	11.9 – 22.7

* n.d.: not determined

$ Until 2000, pooled samples were further aggregated into one pooled sample at the laboratory, whereas in 2000 pooled samples were examined individually.

Table 3.7 Results from the PCR for the detection of SLT-I, SLT-II and eae genes in E. coli *O157 strains from dairy cattle and veal calves.*

	Daiy cattle			Veal calves		
	1998	1999	2000	1998	1999	2000
SLT-II	0	0	0	0	0	1
Eae	0	1	0	0	0	0
SLT-I + SLT-II	0	0	0	1	0	0
SLT-II + eae	8	4	11	2	2	16
SLT-I + SLT-II + eae	3	5	1	3	4	6

herds on which these data are based is lower than the total number of *E. coli* O157 positive herds. In the majority of *E. coli* O157 isolates from both animal species, a combination of the *eae* gene with one or both SLT genes was found.

3.5 Risk factors for *E. coli* O157 in dairy cattle

(Schouten JM, Bouwknegt M, Van de Giessen AW, Frankena K, De Jong MCM and Graat EAM. Risk factor analysis of Escherichia coli *O157 on Dutch dairy farms. Submitted)*.

In total, 49 of 678 herds were found *E. coli* O157 positive (7.2%). Univariate logistic regression of variables obtained from the questionnaire, whereby *E. coli* O157 positive herds were compared to *E. coli* O157 negative herds, was performed. Seventeen variables with a P<0.30 entered multivariate logistic regression. Four variables could not be used in the multivariate models, due to a prevalence of zero in one of the categories. Because of missing values, 506 herds were left in the final model of this study, of which 38 were cases.
In the final model, the following factors were significantly (P<0.10) associated with the occurrence of *E. coli* O157 on farms (followed by a '+' to indicate a positively associated factor and a '-' to indicate a negatively associated factor): presence of at least one pig at the farm (+), purchase of animals within the last two years before sampling (+), supply of maize (-) or beet pulp (+) to the cows, and sampling of a herd in 2000 compared to sampling in 1998 (+). Three variables had P-values exceeding 0.10, but were retained in the model as potential confounders.
The majority of interactions could not be tested in the final model due to the low prevalence. Interactions that could be calculated did not show statistical significance.

3.6 Risk factors for *Campylobacter* spp. in broilers

(Bouwknegt M, Van de Giessen AW, Dam-Deisz WDC, Havelaar AH, Nagelkerke NJD, and Henken AM. Potential risk factors for Campylobacter *spp. occurrence in Dutch broiler flocks. Submitted*).

Data derived from this surveillance programme on *Campylobacter* spp. in broiler flocks were used in univariate and multivariate logistic regression. In total, faecal samples and questionnaires from 495 broiler farms collected between April 1997 and December 2000 were analysed. Processing the questionnaires resulted in 41 variables to be examined for association with *Campylobacter* spp. occurrence. Final results show a multivariate model

based on 457 broiler flocks with a campylobacter prevalence of 24.5% (n_{pos}=109). Factors that were significantly associated with the occurrence of the bacterium were increasing age (+), the presence of more than four broiler houses on the premises (+), the presence of other farm-animals on the farm (+), the presence of farm-animals on farms within a 1km range (+), the use of broiler specific clothing (-), the admittance of children in the broiler house (+) and the seasons summer (+) and fall (+). Furthermore, different hatcheries show diverse effects, but this variable showed strong collinearity with the integrated poultry operation company to which a farm was linked and the feed mill that supplied the broiler feed. Analyses on effect modifications could be performed on most combinations of variables and resulted in a significant interaction term, where the risk effect of children entering the broiler house was diminished by the use of broiler-specific workclothes.

4. Discussion

In this report, results from the national surveillance programme on zoonotic bacteria in farm animals from the period January 1998 until December 2000 are presented. The examination of samples from 2,378 flocks/herds resulted in prevalence estimates and trend observations for *Salmonella* spp., *Campylobacter* spp. and *E. coli* O157. Furthermore, two risk factor analyses were performed on questionnaire data, indicating which elements in farm management and farm characteristics should be further investigated on intervention possibilities.

Evaluation of the programme design

The voluntary basis of this study can have an effect on the obtained estimates in this report. The willingness to participate in the programme can be related to the awareness of certain problems on a farm. This, in turn, might be related to the farm or flock/herd status for the target bacterium (bacteria), but also to general management factors, disease awareness and the farmer's perceptions on animal health and welfare. Moreover, due to the obligatory participation of *e.g.* poultry farmers in the salmonella monitoring and control programme of the poultry industry, the willingness to participate in this voluntary programme decreases. Each mentioned item potentially results in the absence or overrepresentation of certain (categories of) farms in this study, interferes with the randomisation of the sample, and affects sample sizes and thus the accuracy of presented estimates. The amount and direction of these effects, however, mostly are difficult to assess (53).

The collection of faecal samples from the ground might result in a lower detection probability caused by a die-off of bacteria due to exposure to non-favourable circumstances. *Campylobacter* spp., for instance, is known for its sensitivity to dry conditions (17). On the contrary, several experimental studies showed the possibility of survival of *Salmonella* spp., *Campylobacter* spp. and *E. coli* O157 under non-favourable circumstance (10; 19; 24; 30; 31; 34; 37). Also, sample-collectors are instructed to collect optically fresh samples, which presumably reduces the probability of false negative results due to die-off of bacteria.

The geographical distribution of farms showed clustering in sampled flocks for layers in all years, for veal calves in 1998 and 2000 and for dairy cattle in 2000. Furthermore, the sampling of herds with finishing pigs is mainly concentrated in the Southern region.

Clustering might be the result of a higher density of certain farms in an area and thus a required scenario within the monitoring programme, or due to a convenient selection of farms from the initial list of primary sampling units. For layers the clustering is observed in all years, which could imply a structural contingency. Statistics Netherlands reports that most layer farms are present in that region (45). Whether the distribution of farms within that region resembles the spread of farms in the surveillance database, however, remains unknown. For veal calves, the clustering is observed in two years. Statistics Netherlands reports a greater concentration of farms in that particular cluster-region, representing over 40% of all Dutch veal farms (45). Again, the distribution of farms within this region remains

unknown, but the likelihood of a higher concentration of farms in this region is large. The same applies to the clustering of pig farms in the south of The Netherlands in 2000. This province is known for its high concentration in porcine rearing and therefore more pig herds need to be sampled in this region. However, Statistics Netherlands reports an apparent equal amount of farms in the province of Gelderland (45), but this equality is not shown in the distribution of sampled herds. A result of clustering can be an overestimation of the annual prevalences due to for instance an increased contamination risk of neighbouring farms. Neighbouring farms are identified as a potential risk factor for *Campylobacter* spp. occurrence on broiler farms (8). However, the direction of the possible spatial bias and its relevance are difficult to assess.
The prevalences presented in this report are not adjusted for misclassification of flocks/herds due to imperfect diagnostic testing, and therefore resemble 'crude' prevalences. Ideally, crude prevalences are corrected to 'true' prevalences by taking into account the sensitivity and specificity of the tests. Since salmonella and *E. coli* isolates are subjected to serotyping, and campylobacter isolates are confirmed using a combination of an agglutination test and PCR-test, false-positives do not occur. The amount of false-negatives, however, is unknown, thus it is likely that the presented prevalences underestimate the true prevalences.

Salmonella in layers
No differences were found between the annual prevalence estimates for *Salmonella* spp. in layers in the three years of study. The use of additional semi-solid medium has proven to increase the detection probability of positive flocks, with an approximate two-fold increase in the annual prevalence estimates. The main serotype isolated from layers was *S*. Enteritidis, with 7%, 10% and 9% positive flocks in 1998, 1999 and 2000, respectively. In the programme of the poultry industry serological examination of laying hen flocks resulted in similar results, with 11% of the examined flocks found positive for *S*. Enteritidis in 2000 (66).
It has been described that human cases of salmonellosis due to *S.* Enteritidis often result from consumption of insufficiently heated eggs and egg-containing foods (22; 50). In 1999, *S.* Enteritidis was isolated from 0.03% of consumption-eggs in retail stores in The Netherlands (66). In our study, phagetype 4 dominated all other *S.* Enteritidis phagetypes, with 64%, 81% and 93% of the Enteritidis serotypes in 1998, 1999 and 2000, respectively. In the period 1998-2000, the majority of human salmonella infections were caused by this phagetype, accounting for approximately 30% of all reported cases from 16 Public Health Laboratories (covering 64% of the Dutch population) (66).
Efforts have been made by the PVE to decrease *S*. Enteritidis and *S*. Typhimurium prevalence to a maximum of 5% contaminated layer flocks in 2000 (38). The data presented in this report indicates that this level has been reached for *S.* Typhimurium, but not for *S.* Enteritidis.

Salmonella in broilers
A significantly decreasing trend for salmonella occurrence in broiler flocks was observed, with an emergence of *S*. Paratyphi B var. Java (PBvJ) as important serotype in broilers in this

period. A similar pattern for the emergence of *S.* PBvJ was found in the monitoring of end-products in retail stores, with 11% of the salmonella isolates obtained from raw chicken meat in 1998, 14% in 1999 and 33% in 2000 (66). In this study, we found 9% of the positive broiler flocks to be contaminated with *S.* PBvJ in 1998, 13% in 1999 and 38% in 2000. The increasing pattern in broilers, however, does not seem to affect the incidences of human salmonellosis (66). The percentage of human cases of illness caused by *S.* PBvJ was estimated at 0.2% of human isolates in 2000, which is similar to the findings for previous years. A greater concern may be the rapid increase in resistance of the Java clone to antibiotics (68).

Salmonella in finishing pigs

Prevalences for *Salmonella* spp. in finishing pigs were 34%, 13% and 16% in 1998, 1999 and 2000, respectively. However, since in 1998 the samples were collected only in October – December, the prevalence estimate for this year cannot be compared to those for later years. The graph in Appendix 4A on seasonal trends for *Salmonella* spp. in finishing pigs shows that occurrence of the bacterium as a seasonal event in The Netherlands is unlikely, which hampers suggestions on the over- or underestimation of the annual prevalence for 1998.

Furthermore, the use of the additional, more sensitive selective enrichment medium on finishing pig samples in 2000 resulted in a two-fold higher prevalence estimate (16% versus 36%). Therefore, the prevalences in 1998 and 1999 have presumably been underestimated. However, these changes are unavoidable as knowledge on microbiological diagnostic methods progresses. The results of a comparison study on salmonella detection in pig samples (12), similar to that conducted on poultry samples (69) showed that the use of the semi-solid medium in addition to RV significantly increased the sensitivity of the detection method. An estimated relative sensitivity of the RV medium compared to the use of all three media together was approximately 44% (12).

Data from the GD indicate a salmonella seroprevalence of approximately 25% in pig herds in The Netherlands in 1999 and 24% in 1996 (56). The difference with findings described in this report may be due to the difference in methodology, since serology usually results in a higher prevalence estimate due to the prolonged circulation of antibodies in the blood.

The main serotype isolated from pigs was *S.* Typhimurium, accounting for approximately 50% of the salmonella isolates. For many years, *S.* Typhimurium has been one of the most prevalent serotypes in pigs, both in The Netherlands and in other countries (20; 48; 57; 58). Within this serovar, an increasing role for phagetype DT104 was observed between 1998 and 2000, and hence shows a similar pattern as observed in human cases of salmonellosis (7; 66; 71). *S.* Derby was the second most isolated serotype in this study, accounting for approximately 15% of the salmonella isolates. In a recent study conducted at two Dutch abattoirs the occurrence of cross-contamination at slaughter was indicated (49), with serotype Typhimurium once more dominating in pig-samples. Another microbiological study on retail products in The Netherlands from 1999 indicates that *S.* Typhimurium was the leading serotype on raw pork (42% of the salmonella isolates) and mixed pork/beef (27%) (60). *S.*

Derby was isolated twice from raw pork in that study and with that the second most frequently isolated serotype (6%), together with *S.* Brandenburg and *S.* Bovismorbificans.
Combining data from the farms, abattoirs, retail stores and humans suggests that transmission of salmonellas from finishing pigs through the abattoir into the human food chain is likely to occur. Risk factor data on the occurrence of this bacterium on farms can be useful in reducing the prevalence at farm level, but potential positive effects are possibly counterbalanced by cross-contamination in the abattoirs if no efforts are made to minimise or prevent this. Therefore, control of salmonella in the pig sector should be based on an integrated chain approach, following the example of the monitoring and control programme in the poultry sector.

Salmonella in dairy cattle and veal calves
Dairy cattle and veal calves were contaminated with *Salmonella* spp. at low levels in the study years. The use of only the RV medium might have resulted in underestimation of the annual prevalences, although no significant increase in sensitivity was found when using the the semi-solid medium in addition to RV (12). The latter finding, however, might have been influenced by a low power in that study due to a low number of positive samples. The main serotypes isolated from cattle in The Netherlands concerned *S*. Typhimurium and *S*. Dublin (63), and the lack of performance of the RV medium for *S*. Typhimurium suggests an underestimation of the annual prevalences.

Campylobacter
Differences in campylobacter occurrence were found for the first two years in the study period for all species. However, results from finishing pigs, dairy cattle and veal calves in 1998 are biased by the prior mentioned seasonal influences due to temporal restrictions in sampling.
Differences were observed between campylobacter prevalences in broilers from the current study and as measured by the industry (PVE). Whereas the PVE measured a continuing decrease in campylobacter contamination both at farm level and at slaughtering (66), in our programme an increase in campylobacter occurrence at farm level was observed in 2000. Equally, an increase in contamination of retail products was observed for *Campylobacter* spp. (61). The difference observed at farm level may be caused by differences in sampling strategy. In the poultry industry's programme either two pairs of overshoes per house or twice 15 faeces swabs are collected (farmer's choice). Thus differences between the programmes exist in both material and numbers of the samples. Also, age at sampling is different; whereas in this surveillance programme broiler flocks are preferably sampled at an age of 4-6 weeks, the industry measures the campylobacter status of birds that are due to be delivered to the slaughterhouse. This, however, would be expected to result in a higher prevalence estimate, since increasing age is a risk factor for campylobacter occurrence (8; 18).
The presented seasonal pattern for *Campylobacter* spp. showed a peak of campylobacter contamination of broiler flocks in the period May – July. Although the presented smoothing

technique is in essence not suitable to accurately estimate the onset and length of the seasonal peak, it is valuable in defining the period with the highest relative risk attributable to seasonal fluctuations for campylobacter occurrence in a flock. Due to the Action Plan (39) several hygiene related precautions have already been implemented on farms, but until now, they do not have the desired reducing effect on campylobacter prevalence. Possibly, the motivation of the farm holders to uphold high hygienic standards subsides with time. Therefore, maintaining more strict hygiene standards in the period of increased relative risk due to seasonal fluctuations might be considered as an alternative strategy for reduction of the prevalence. For this, however, it will be necessary to analyse the seasonal fluctuations in more detail in order to fully identify the period of peak occurrence.

Identification of campylobacter species gave similar results for all three years. The predominant campylobacter species in broilers was *C. jejuni* (approx. 80% of the isolates), which is in accordance with previous studies (18; 23). The main species in finishing pigs was *C. coli* (over 90% of the isolates), which agrees with findings described in other reports (4; 55). Campylobacteriosis in humans is often caused by *C. jejuni*, which may be seen as an indication of the relative importance of broilers as source of infection. Also in dairy cattle *C. jejuni* was the predominating species, whereas in veal calves the majority of the campylobacter positive herds showed the presence of *C. coli*. However, *C. jejuni* was also prevalent in veal calves, either in a simultaneous presence of *C. coli* or alone.

Campylobacter contamination of veal and beef products in retail stores is normally very low (0.4% for beef/veal meat; 1.5% for mixed beef/veal and pork meat) (60), suggesting that risk of human campylobacteriosis related to the consumption of veal calf and beef products is relatively small.

E. coli O157

For *E. coli* O157 in dairy cattle herds, isolation percentages were 5%, 9% and 6% in 1998, 1999 and 2000, respectively. The examination of veal calf samples resulted in percentages of 5%, 13% and 11% of positive herds in consecutive years. In neither animal species a trend in annual prevalence estimates was observed, although data for veal calves showed a tendency toward an increasing trend (P=0.0712). However, this tendency was not observed in an increase in the annual peak prevalences in consecutive years when examining the seasonal variation graphs, but shows an increased period of contamination of herds throughout a year for 1999 and 2000 when compared to 1998. Analysing data from later years will give more insight in the possibility of a gradual increase in *E. coli* O157 occurrence in time.

In 2000, the method of determining the *E. coli* O157 status of a herd was changed from examining one mixed sample of the (maximally 5) pooled samples per herd into examination of all five pooled samples individually. Unpublished data show that approximately 40% more herds were defined positive when analysing the individual pooled samples. In theory, this additional pooling should not alter the performance of the test, since the detection probability for *E. coli* O157 in animal faeces reaches 50% when 0.49 colony forming units (CFU) per gram faeces are present (11). The presence of at least one CFU per gram will then be detected with a probability of 77% (interpolation of the results from Dam-Deisz and Evers (11)).

Bacterial counts using the most probable number method on dairy cattle samples collected in this surveillance study indicated a 'worst' estimate of 24 CFU per gram of faeces from a positive farm (95% CI: 4 – 99; data not shown). However, at low contamination levels thorough mixing of the collected faecal material before taking the 10g portion of faeces to be examined is the crucial factor.
In dairy cattle and veal calves, 32 of 33 and all 35 isolates, respectively, possessed either one or both of the genes that enables the production of shiga-like toxin and thus are potentially pathogenic to humans.
In a Dutch study on contamination of cows at slaughter in 1995 and 1996, approximately 11% of 540 cows and less than 1% of 397 veal calves were found contaminated with *E. coli* O157 (27). Most of the isolates obtained in this study also contained the virulence genes SLT and/or eae. Another study, conducted in retail stores, revealed a contamination percentage of less than 1% for pork, beef and veal meat together (60). Nevertheless, human STEC-infections have frequently been attributed to cattle products (21; 36). Besides, *E. coli* O157 reaches the human population through other pathways, such as direct transmission (29), raw milk (21) or fruits and vegetables (1; 6).

Conclusions
Salmonella spp. are frequently present in faecal samples from layers, broilers and pigs. The observed decreasing trend in annual prevalence estimates for broilers since 1998 may be related to the implementation of the Action Plan by the PVE in 1997 (39). Also the prevalence of *S.* Enteritidis in broilers has substantially decreased in time. No obvious decreasing prevalence is observed for layers, however. At serotype level, *S.* Enteritidis was present at a constant level in layers, indicating the further need for control or intervention measures.
An increase of *S.* Typhimurium DT104 was observed in finishing pigs during the study period. *S.* Paratyphi B var. Java emerged in broilers during the study years, whereas *S.* Infantis contamination decreased. The level of salmonella contamination of pig herds was shown to be substantial, advocating the need of an integrated control programme, both at farm level and at slaughter, following the example of the poultry sector.
Campylobacter spp. was isolated from all four examined animal species and each of them can be considered as a animal reservoir. For broilers, a non-significant increase was observed in 2000 and a tendency toward an overall decreasing trend has been observed ($P<0.09$). Data indicate that the aim in reduction of campylobacter contamination has not been reached in 2000, but a positive effect of the Action Plan might be present. Monitoring and analysing data from later years will conclude on this.
E. coli O157 was present in dairy cattle at a similar level each year. However, in the upcoming years an increasing pattern in *E. coli* O157 occurrence in veal calves might develop, considering the indications for an increasing trend. Examination of the individual pooled samples proved to be a better examination technique than aggregating the pooled samples further in the laboratory.

As for the risk factors for *E. coli* O157, management factors such as purchase of animals and the supply of beet pulp were found to be risk related. Also, the seasonal fluctuations presented in Appendix 4C were observed in this analysis. Further studies on these factors should be performed to develop an effective intervention strategy. However, since cross-contamination in slaughterhouses can not yet be prevented, intervention strategies should be based on an integrated chain approach.

Analysis of risk factors for campylobacter occurrence in broiler flocks revealed that most potential risk factors were directly or indirectly related to hygiene or environmental transmission. However, several hygienic measures have already been implemented in the poultry industry, without resulting in a reduction of campylobacter prevalence (39). More emphasis on maintaining the hygienic measures especially in the period of peak occurrence of *Campylobacter* spp. might help reduce the prevalence. Furthermore, additional experimental studies should be conducted to identify the most effective control measures.

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Appendix 1a Primary sample sizes for layers

1998

Number of layer farms:	2,488[#]
Average number of layer houses on farms:	1.5
Number of flocks raised per house per year:	1
Total number of flocks raised per year:	3,732
Expected prevalence *Salmonella*:	20%
Expected prevalence *E. coli* O157	<5%
Fixed confidence level:	90%
Fixed accuracy level:	±5%
Calculated sample size:	**174**

[#] obtained from the report 'Zoonosen en zoönoseverwekkers Nederland, Juli 1996' (5)

1999

Number of layer farms:	2,822[*]
Average number of layer houses on farms:	1.5
Number of flocks raised per house per year:	1
Total number of flocks raised per year:	4,233
Expected prevalence *Salmonella*:	30%
Expected prevalence *E. coli* O157:	<5%
Fixed confidence level:	90%
Fixed accuracy level:	±5%
Calculated sample size:	**216**

* Source: Animal Health Service, 1998

2000

Number of layer farms:	2,085[*]
Average number of layer houses on farms:	1.5
Number of flocks raised per house per year:	1
Total number of flocks raised per year:	3,127
Expected prevalence *Salmonella*:	30%
Fixed confidence level:	90%
Fixed accuracy level:	±5%
Calculated sample size:	**216**

* Source: Animal Health Service, 1999

Appendix 1b Primary sample sizes for broilers

1998

Number of broiler farms:	1,301[#]
Average number of broiler houses on farms:	1.8
Number of flocks raised per house per year:	6
Total number of flocks raised per year:	14,051
Expected prevalence *Salmonella*:	25%
Expected prevalence *Campylobacter*:	40%
Expected prevalence *E. coli*:	<5%
Fixed confidence level:	90
Fixed accuracy level:	±5%
Calculated sample size:	**260**

[#] obtained from the report 'Zoonosen en zoönoseverwekkers Nederland, Juli 1996' (5)

1999

Number of broiler farms:	1,245[*]
Average number of broiler houses on farms:	1.8
Number of flocks raised per house per year:	6
Total number of flocks raised per year:	13,466
Expected prevalence *Salmonella*:	30%
Expected prevalence *Campylobacter*:	40%
Expected prevalence *E. coli* O157:	<5%
Fixed confidence level:	90%
Fixed accuracy level:	±5%
Calculated sample size:	**260**

* Source: Animal Health Service, 1998

2000

Number of broiler farms:	2,540[*]
Average number of broiler houses on farms:	1.8
Number of flocks raised per house per year:	6
Total number of flocks raised per year:	27,432
Expected prevalence *Salmonella*:	25%
Expected prevalence *Campylobacter*:	30%
Fixed confidence level:	90%
Fixed accuracy level:	±5%
Calculated sample size:	**216**

* Source: Animal Health Service, 1999

Appendix 1c Primary sample sizes for finishing pigs

1998

Number of finishing pig farms:	19,627[#]
Average number of pig houses on farms:	2
Number of production rounds per year:	3
Total number of herds raised per year:	117,762
Expected prevalence *Salmonella*:	50%
Expected prevalence *Campylobacter*:	<5%
Fixed confidence level:	90%
Fixed accuracy level:	±5%
Calculated sample size:	**271**

[#] obtained from the report 'Zoonosen en zoönoseverwekkers Nederland, Juli 1996' (5)

1999

Number of finishing pig farms:	18,642[*]
Average number of pig houses on farms:	2
Number of production rounds per year:	3
Total number of herds raised per year:	111,852
Expected prevalence *Salmonella*:	50%
Expected prevalence *Campylobacter*:	50%
Expected prevalence *E. coli* O157:	<5%
Fixed confidence level:	90%
Fixed accuracy level:	±5%
Calculated sample size:	**271**

* Source: Animal Health Service, 1998

2000

Number of finishing pig farms:	15,275[*]
Average number of pig houses on farms:	2
Number of production rounds per year:	3
Total number of herds raised per year:	91,542
Expected prevalence *Salmonella*:	30%
Fixed confidence level:	90%
Fixed accuracy level:	±5%
Calculated sample size:	**216**

* Source: Animal Health Service, 1999

Appendix 1d Primary sample sizes for dairy cattle

1998

Number of broiler farms:	37,465[#]
Total number of herds per year:	37,465
Expected prevalence *Salmonella*:	<5%
Expected prevalence *Campylobacter*:	10%
Fixed confidence level:	90%
Fixed accuracy level:	±3%
Calculated sample size:	**271**

[#] obtained from the report 'Zoonosen en zoönoseverwekkers Nederland, Juli 1996' (5)

1999

Number of dairy farms:	34,596[*]
Total number of herds per year:	34,596
Expected prevalence *Salmonella*:	<5%
Expected prevalence *Campylobacter*:	15%
Expected prevalence *E. coli* O157:	5%
Fixed confidence level:	90%
Fixed accuracy level:	±3%
Calculated sample size:	**143**

* Source: Animal Health Service, 1998

2000

Number of dairy farms:	50,533[*]
Total number of herds per year:	50,533
Expected prevalence *Salmonella*:	<5%
Expected prevalence *E. coli* O157:	5%
Fixed confidence level:	90%
Fixed accuracy level:	±3%
Calculated sample size:	**143**

* Source: Animal Health Service, 1999

Appendix 1e Primary sample sizes for veal calves

1998

Number of broiler farms:	2,334*
Average number of broiler houses on farms:	3
Number of herds raised per house per year:	2
Total number of herds raised per year:	14,004
Expected prevalence *Salmonella*:	5%
Expected prevalence *Campylobacter*:	<5%
Fixed confidence level:	90%
Fixed accuracy level:	±3%
Calculated sample size:	**135**

obtained from the report 'Zoonosen en zoönoseverwekkers Nederland, Juli 1996' (5)

1999

Number of broiler farms:	2,444*
Average number of broiler houses on farms:	3
Number of herds raised per house per year:	2
Total number of herds raised per year:	14,664
Expected prevalence *Salmonella*:	<5%
Expected prevalence *Campylobacter*:	>50%
Expected prevalence *E. coli* O157:	<5%
Fixed confidence level:	90%
Fixed accuracy level:	±3%
Calculated sample size:	**135**

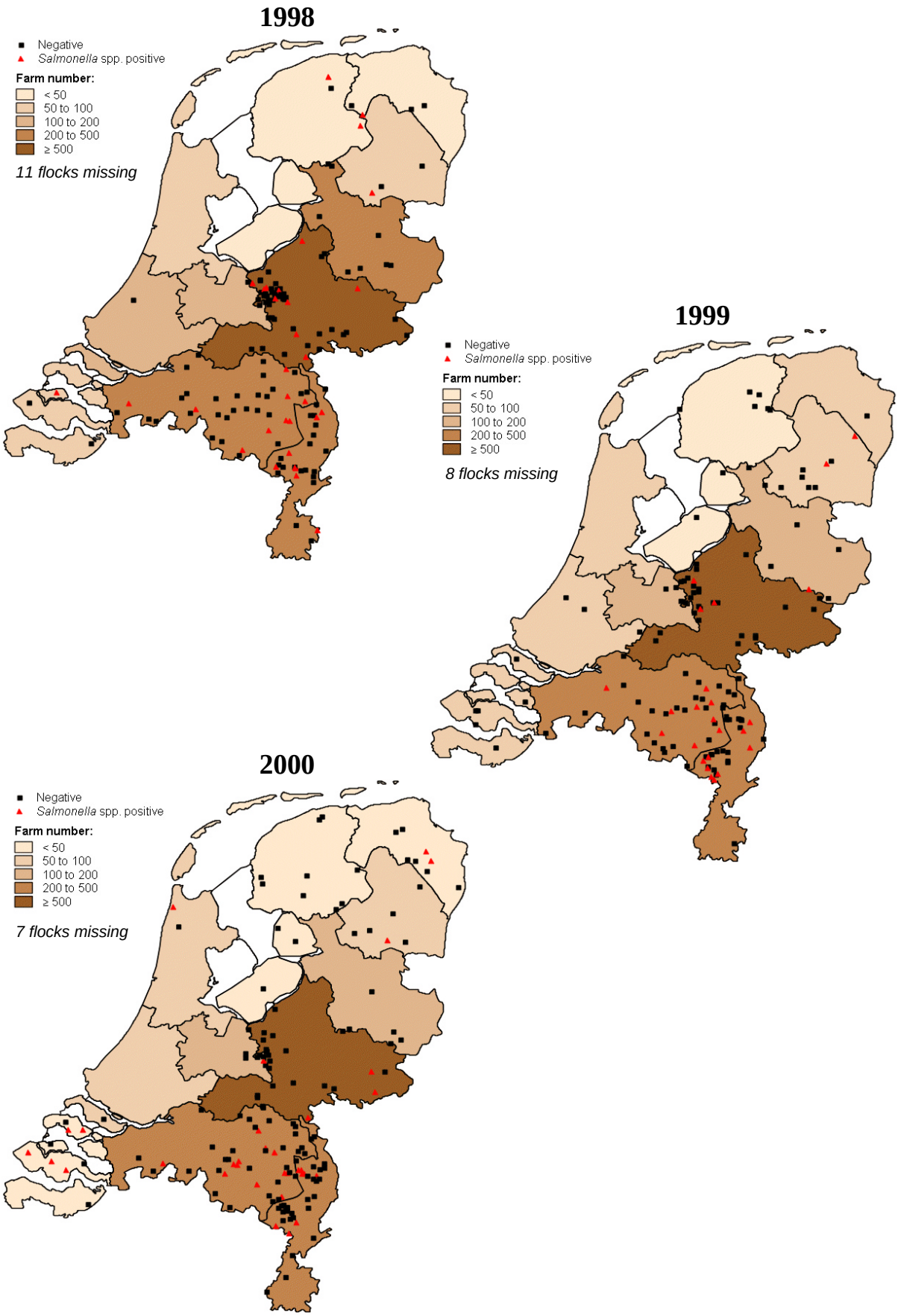
* Source: SKV, 1998

2000

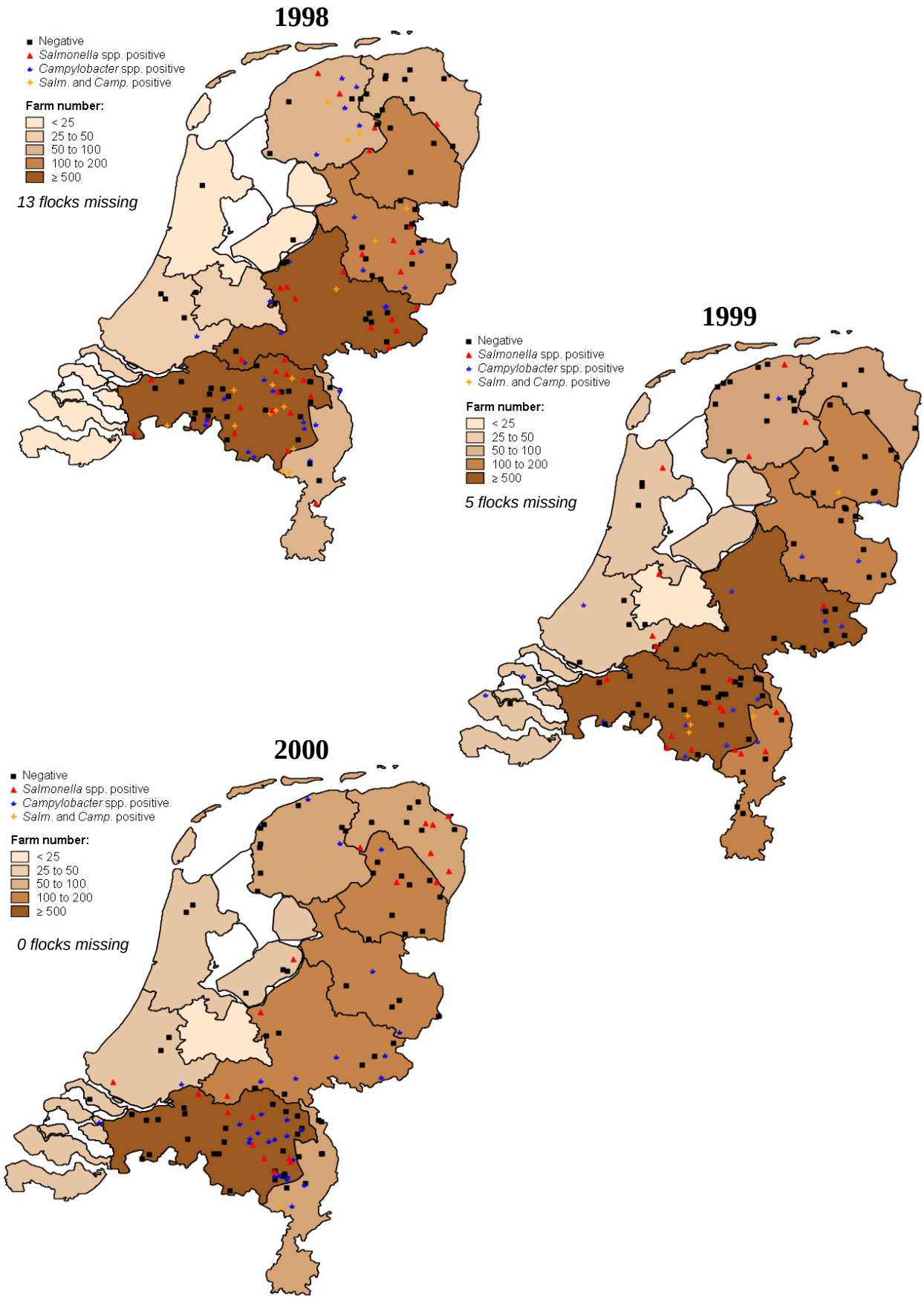
Number of broiler farms:	±2,000*
Average number of broiler houses on farms:	3
Number of herds raised per house per year:	2
Total number of herds raised per year:	12,000
Expected prevalence *Salmonella*:	5%
Expected prevalence *E. coli* O157:	5%
Fixed confidence level:	90%
Fixed accuracy level:	±3%
Calculated sample size:	**135**

* Source: Animal Health Service, 1999

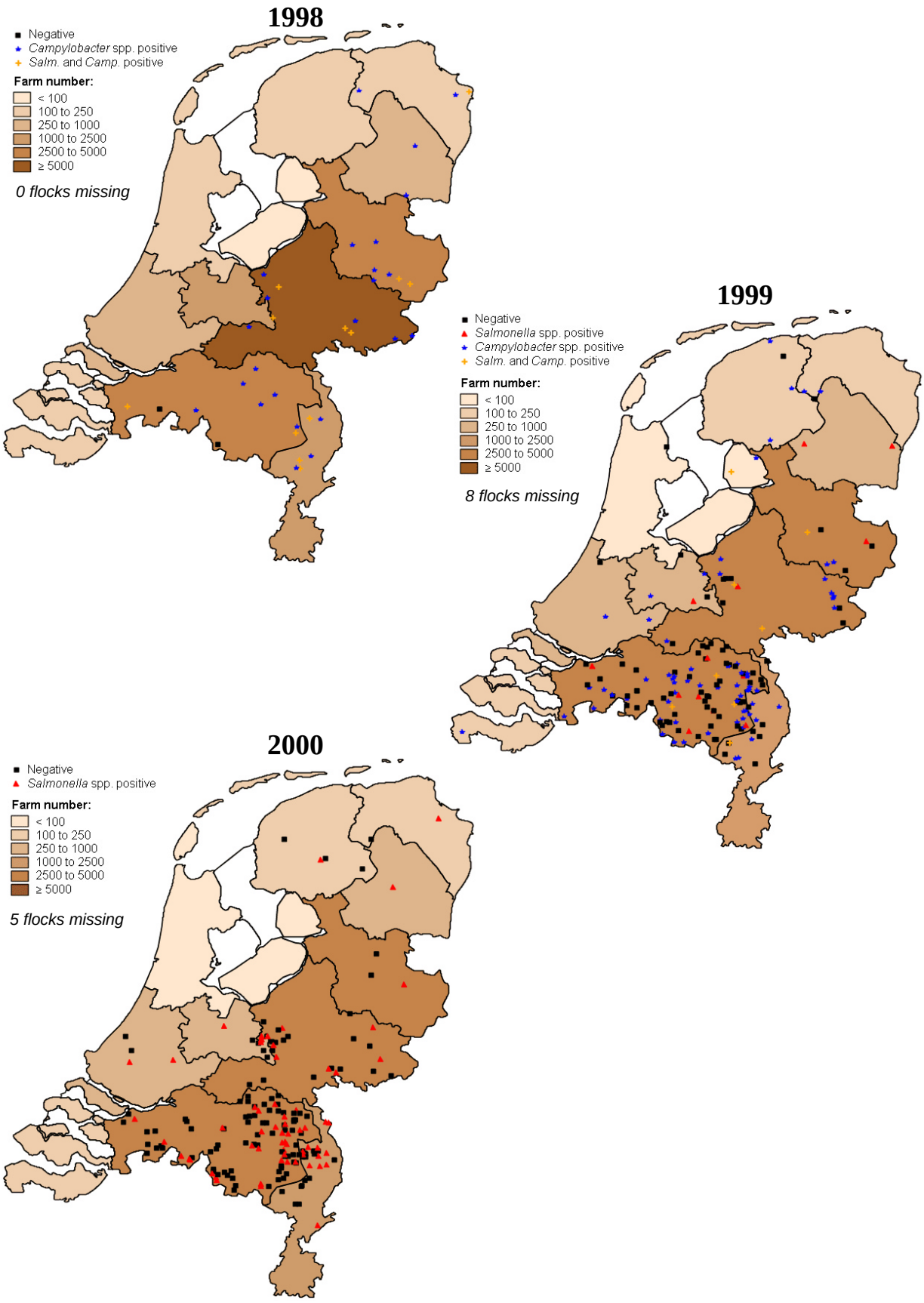
Appendix 2a Geographical distribution of layer flocks



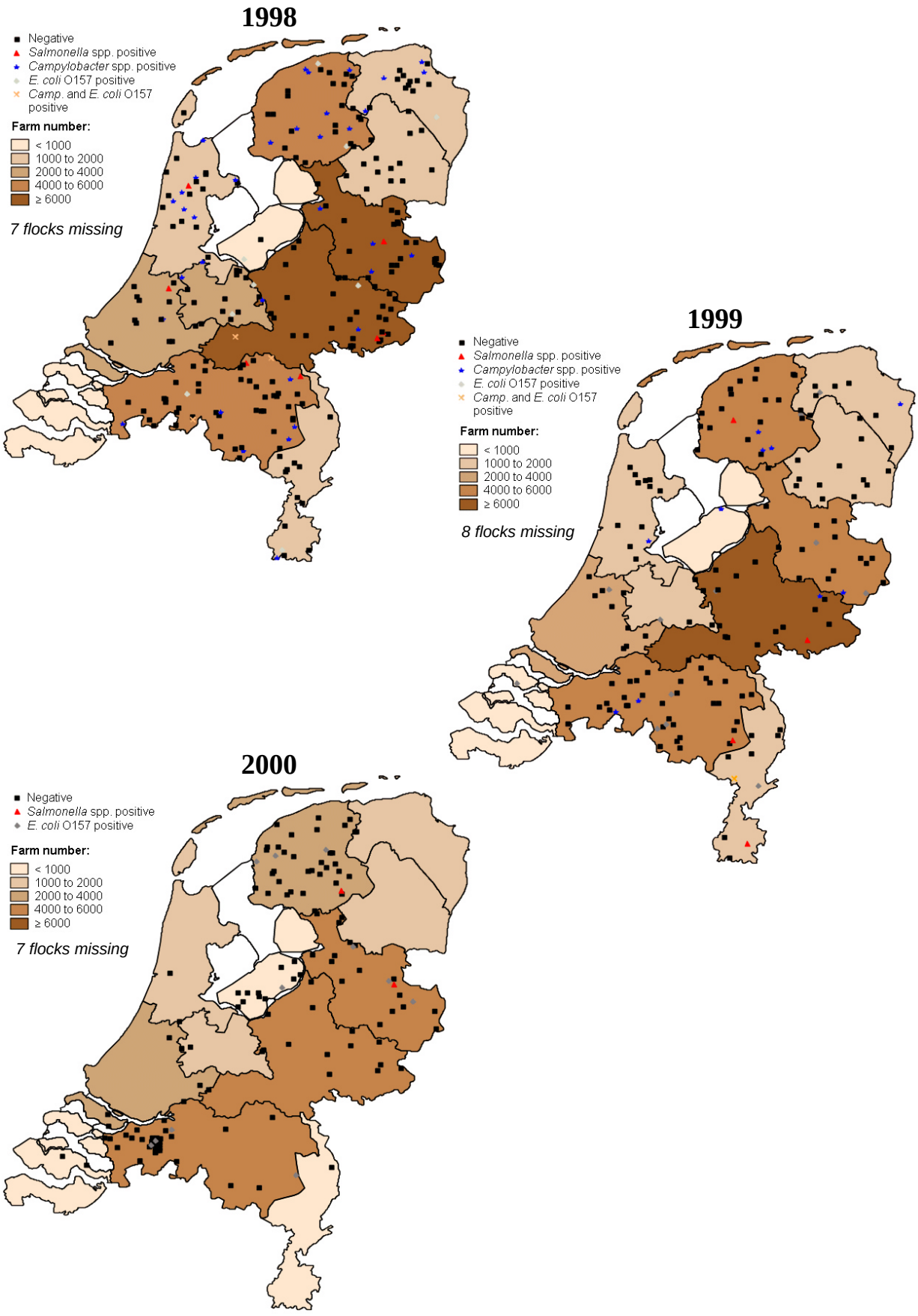
Appendix 2b Geographical distribution of broiler flocks



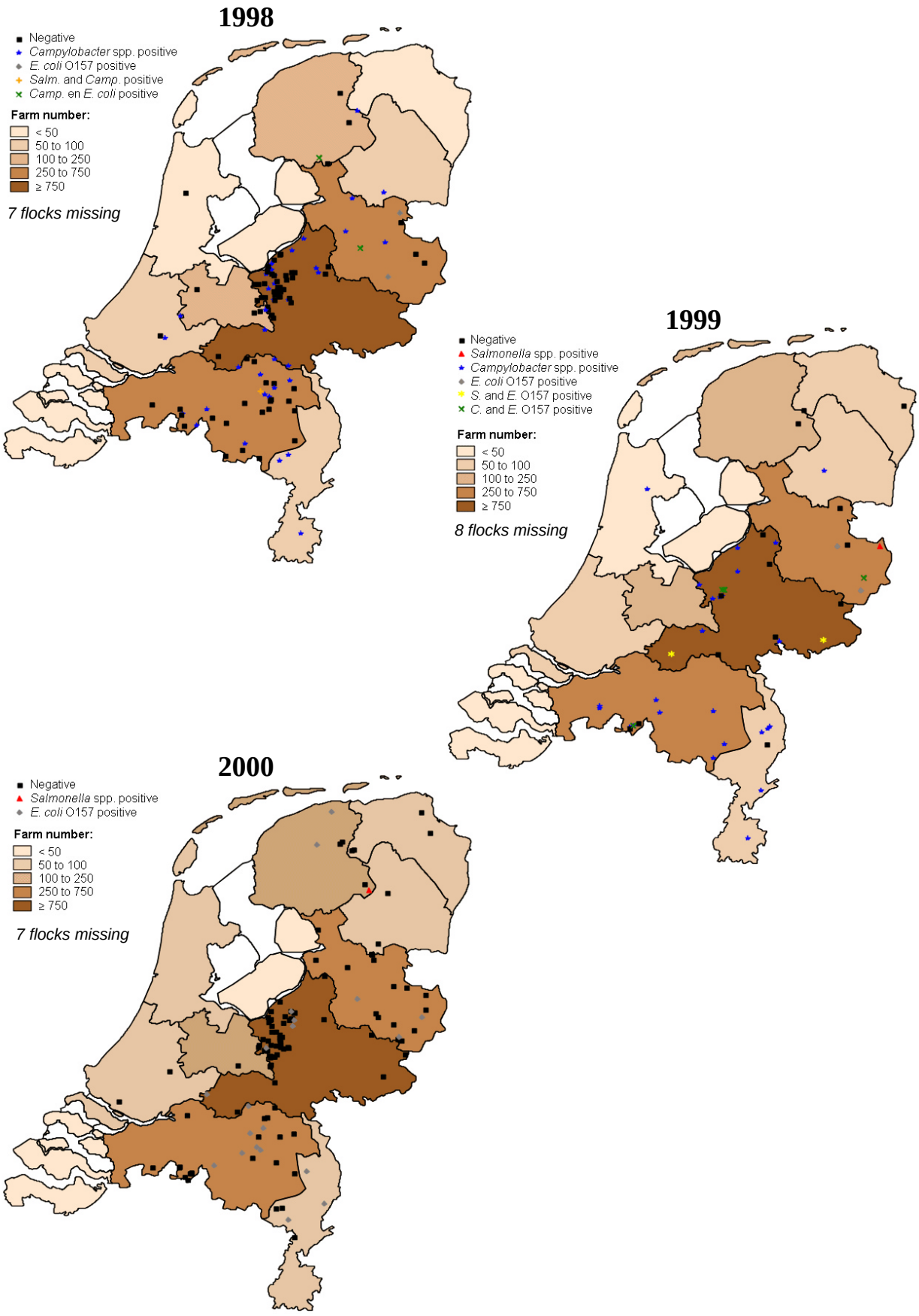
Appendix 2c Geographical distribution of finishing pig herds



Appendix 2d Geographical distribution of dairy herds



Appendix 2e Geographical distribution of veal calf herds



Appendix 3 The effect of discordance in regional spread of farms between the database and The Netherlands on the annual prevalence estimates

For adjusting the annual prevalences, weighting based on relative spread of farms and the estimated prevalence was used, according to eqn. (6).

$$\hat{P} = \frac{1}{A} \times \sum_{i=1}^{5} a_i p_i \qquad (6)$$

$\hat{P}$ = the adjusted prevalence
A = the actual number of farm in The Netherlands
a_i = the actual number of farms in region i
p_i = estimated prevalence for region i (p_i)

The adjusted prevalence estimates are summarised in the following table per bacterium.

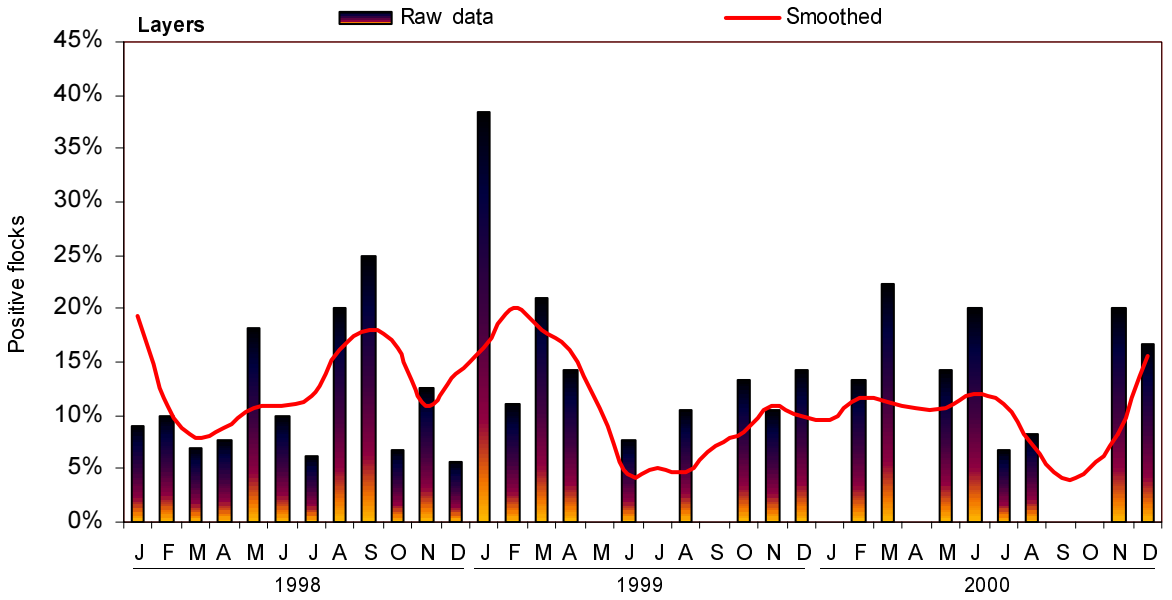
Salmonella	1998		1999		2000	
	measured	adjusted	measured	adjusted	measured	adjusted
Layers	23.7	20.0	22.6	19.7	20.5	17.2
Broilers	31.8	29.5	20.7	18.5	16.5	15.7
Finishing pigs	34.2	31.8	12.7	16.1	15.5	16.1
Dairy cattle	2.7	3.0	2.4	2.1	1.3	1.7
Veal calves	0.7	0.6	5.0	5.3	0.8	0.9

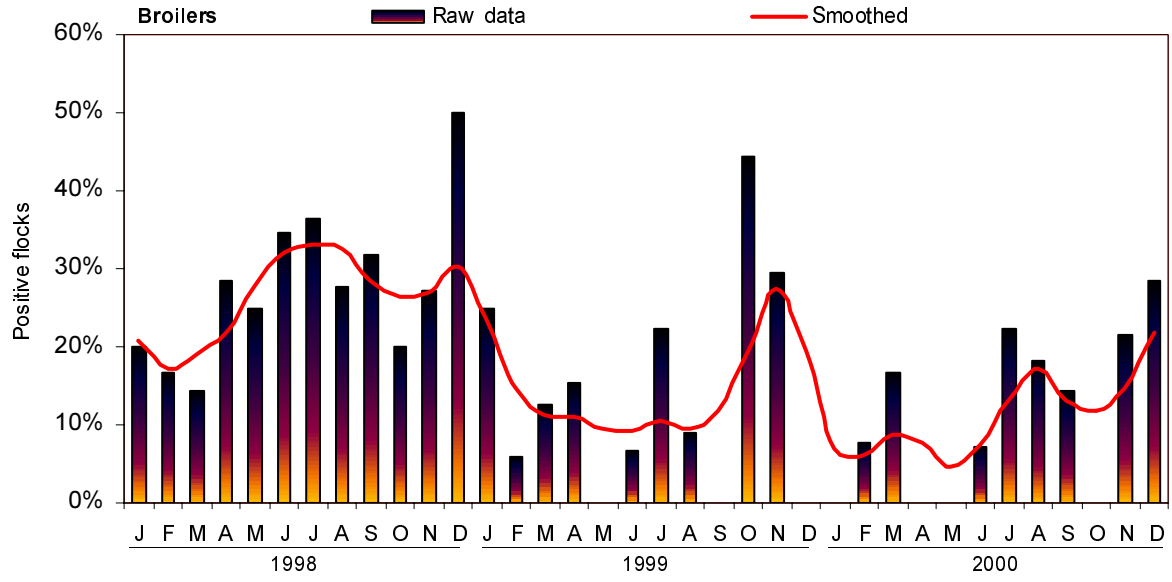
Campylobacter	1998		1999		2000	
	measured	adjusted	measured	adjusted	measured	adjusted
Broilers	30.7	29.3	17.5	17.4	23.6	23.6
Finishing pigs	97.4	85.6	45.5	44.6	n.d.*	n.d.
Dairy cattle	31.5	30.5	6.5	6.3	n.d.	n.d.
Veal calves	83.9	85.6	58.3	57.1	n.d.	n.d.

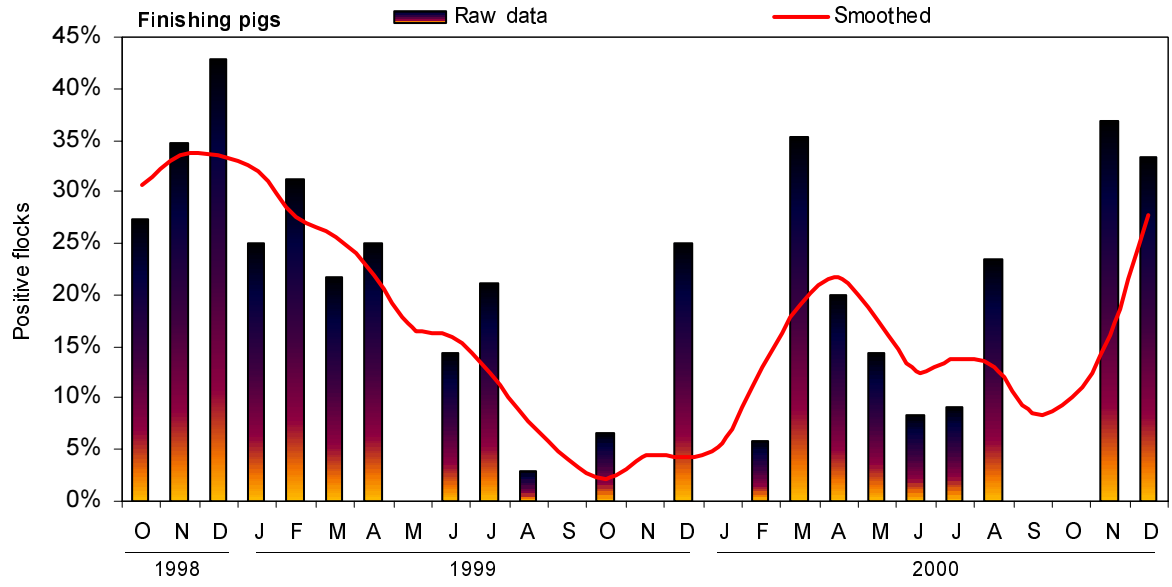
* not determined

E. coli O157	1998		1999		2000	
	measured	adjusted	measured	adjusted	measured	adjusted
Dairy cattle	5.2	4.9	8.6	8.6	8.9	7.6
Veal calves	5.3	5.5	13.3	13.6	17.3	15.5

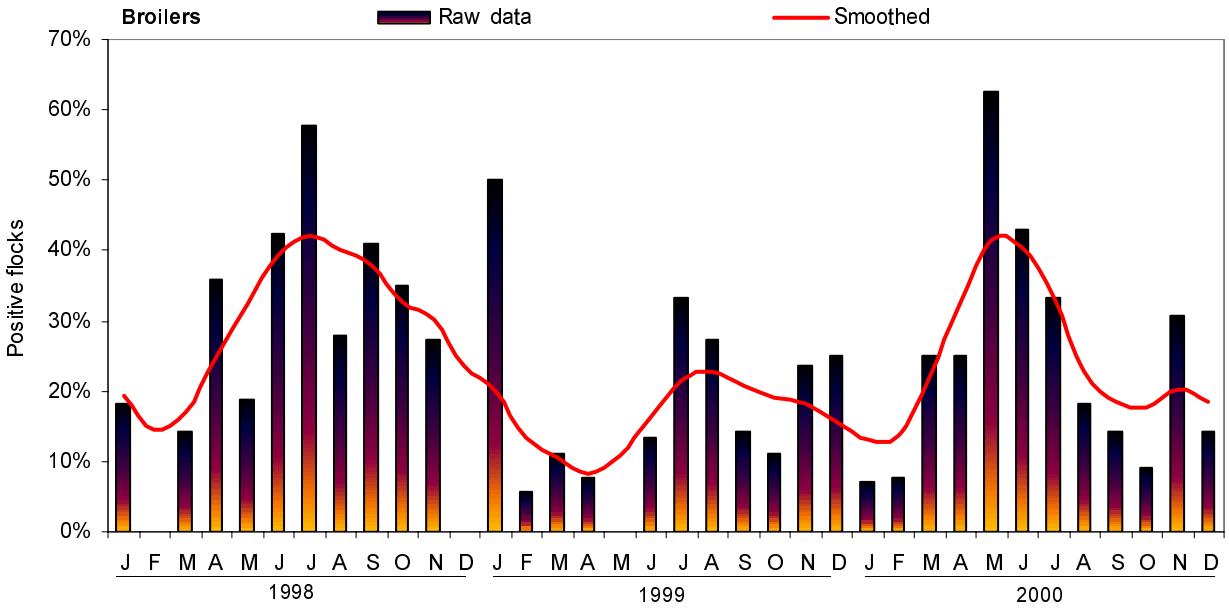
Appendix 4a Smoothed graphs for *Salmonella* spp. in layers, broilers and finishers (RV-data)



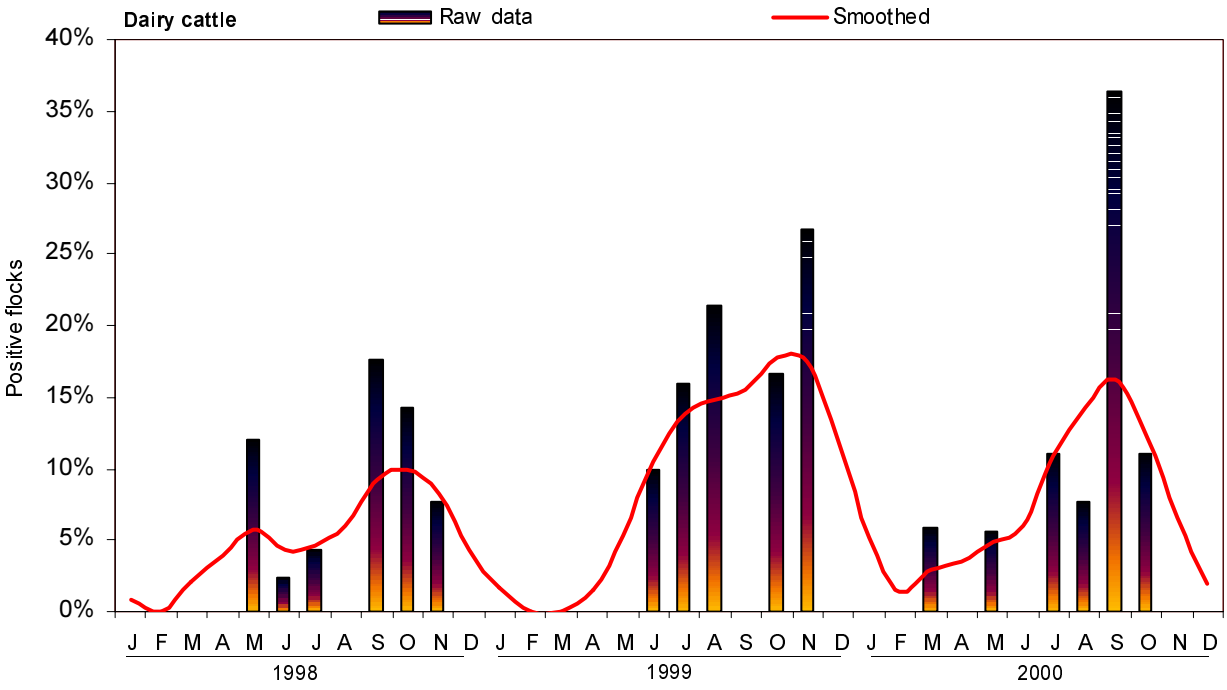


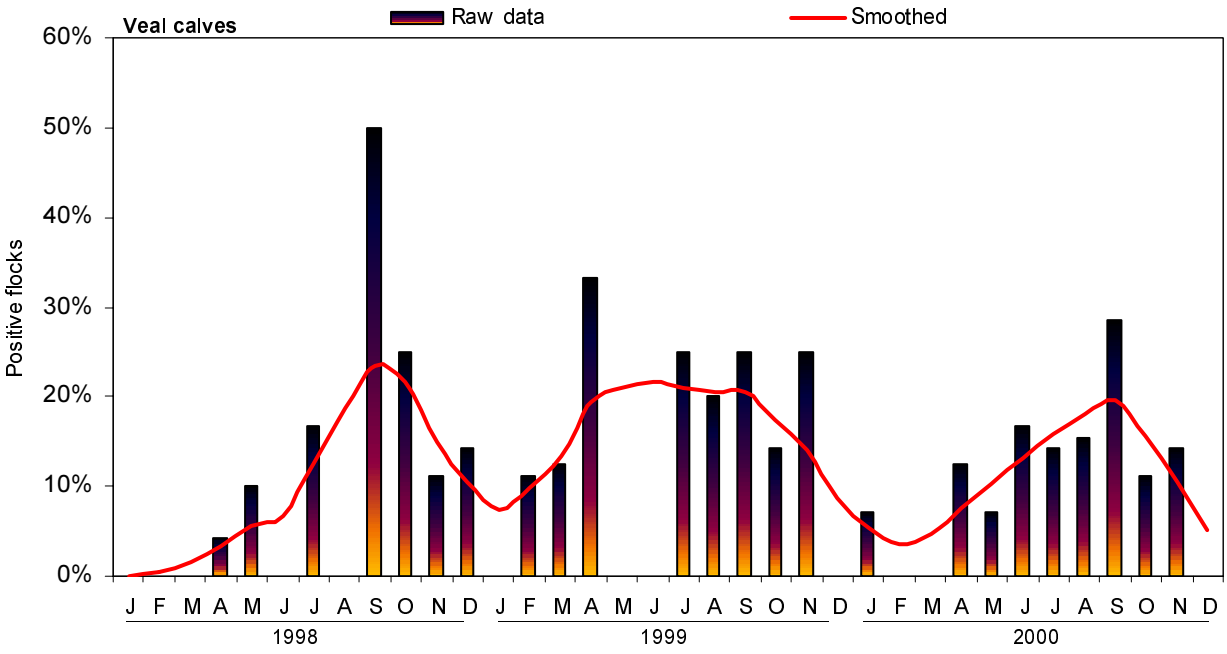


Appendix 4b Smoothed graph for *Campylobacter* spp. in broilers



Appendix 4c Smoothed graphs for *E. coli* O157 in dairy cattle and veal calves





Appendix 5a Isolated salmonella serotypes in 1998

	Number of flocks/herds positive				
Serotype	**Layers**	**Broilers**	**Finishing pigs**	**Dairy cattle**	**Veal calves**
S. Bareilly	1				
S. Braenderup	8				
S. Brandenburg			1		
S. Cerro	1				
S. Derby			2		
S. Enteritidis pt 1		1			
S. Enteritidis pt 3	1				
S. Enteritidis pt 4	19	8			
S. Enteritidis pt 6	7	1			
S. Enteritidis pt 6a		2			
S. Enteritidis pt 7	4	1			
S. Enteritidis pt 8	2				
S. Enteritidis pt 14	1				
S. Enteritidis pt 18	1				
S. Enteritidis pt 21	1				
S. Enteritidis pt 23	2				
S. Enteritidis pt 28	1	1			
S. Enteritidis pt 32	1				
S. Enteritidis pt 35	3	1			
S. Enteritidis pt 38	1				
S. Enteritidis app	1	1			
S. Enteritidis np		1			
S. Enteritidis os		1			
S. Enteritidis ors		1			
S. Enteritidis nt		1			
S. Enteritidis nct	1				
S. Give			1		
S. Hadar		3			
S. Heidelberg		6			
S. Indiana		5			
S. Infantis	4	16	2		
S. Linvingstone		1		1	
S. London			1		
S. Mbandaka	2	5			
S. Montevideo	2	1			
S. Oranienburg		1			
S. Paratyphi B. var Java		4		1	
S. Rissen	1				
S. Senftenberg		1			
S. Typhimurium app				1	
S. Typhimurium ft 11	1				
S. Typhimurium ft 62			1		
S. Typhimurium ft 80			1		
S. Typhimurium ft 171			1		
S. Typhimurium ft 295			1		
S. Typhimurium ft 296		1			

Continued

Serotype	Number of flocks/herds positive				
	Layers	**Broilers**	**Finishing pigs**	**Dairy cattle**	**Veal calves**
S. Typhimurium ft 301			1		
S. Typhimurium ft 353			1		
S. Typhimurium ft 401			1		
S. Typhimurium ft 506				1	
S. Typhimurium ft 510				1	
S. Typhimurium ft 530			1		
S. Typhimurium ft 690		1			
S. Typhimurium app		2			
S. Uganda		1			
S. Virchow	4	1			
S. nt	1	2			1
S. nct	2	4		2	

pt phagetype
ft faagtype (Dutch nomenclature)
app atypical phage pattern
np no phage reaction
nt not typable
nct not completely typable

N.B.: *Salmonella* Typhimurium DT104 includes the Dutch phage types (ft) 401 and 506.

Appendix 5b Isolated salmonella serotypes in 1999

	Number of flocks/herds positive				
Serotype	**Layers**	**Broilers**	**Finishing pigs**	**Dairy cattle**	**Veal calves**
S. Agona	1			1	
S. Bareilly	1				
S. Berta		1			
S. Bovismorbificans	1				
S. Braenderup	5				
S. Brandenburg		1			
S. Cerro	1				
S. Derby			5		
S. Dublin					1
S. Enteritidis pt 4	13	8	1		
S. Enteritidis pt 6	1				
S. Enteritidis pt 23	1				
S. Enteritidis pt 25	1				
S. Enteritidis pt 28	3				
S. Enteritidis pt 35	1	1			
S. Enteritidis app	1				
S. Enteritidis np		2			
S. Give			2		
S. Heidelberg	1	1			
S. Idikan		1			
S. Infantis	5	5			
S. Kentucky		1			
S. Linvingstone	5		3		
S. London			2		
S. Mbandaka	1	3			
S. Montevideo	1				
S. Panama	1	2	1		
S. Paratyphi B. var Java	1	4			
S. Rissen	2				
S. Saintpaul		1			
S. Typhimurium app	1		1		
S. Typhimurium ft 2		1			
S. Typhimurium ft 61			1		
S. Typhimurium ft 251			1		
S. Typhimurium ft 350			2		
S. Typhimurium ft 506			2		
S. Typhimurium ft 507		1			
S. Typhimurium ft 510			3	1	
S. Typhimurium ft 654				1	
S. Typhimurium ft 656			1		
S. Typhimurium np			2		
S. Typhimurium nt			1		
S. Uganda		3			
S. nt	1	2	1		2
S. nct		1		1	

pt: phagetype app: atypical phage pattern nt : not typeable
ft: faagtype (Dutch nomenclature) np: no phage reaction nct: not completely typeable

Appendix 5c Isolated Salmonella serotypes in 2000

	Number of flocks/herds positive				
Serotype	**Layers**	**Broilers**	**Finishing pigs**	**Dairy cattle**	**Veal calves**
S. Agona	1				
S. Anatum	1		1		
S. Bareilly	2				
S. Braenderup	4				
S. Brandenburg			6		
S. Derby			8		
S. Enteritidis pt 1		1			
S. Enteritidis pt 4	15	1			
S. Enteritidis pt 7	2				
S. Enteritidis pt 7a	1				
S. Enteritidis pt 11	1				
S. Enteritidis pt 14b	1				
S. Enteritidis pt 21		1			
S. Enteritidis pt 23	1				
S. Enteritidis pt 28	2				
S. Enteritidis np	1				
S. Goldcoast			1		
S. Hadar		1			
S. Heidelberg		1			
S. Infantis	6	1	4		
S. Kedougou			1		
S. Linvingstone	2		8		
S. London			3		
S. Manhattan		2	1		
S. Mbandaka	2	3			
S. Montevideo	1				
S. Ohio			1		
S. Orion	1				
S. Panama			3	1	
S. Paratyphi B. var Java		8	1		
S. Tennessee	1				
S. Thompson	1	1			
S. Typhimurium app			5		
S. Typhimurium ft 20			1		
S. Typhimurium ft 60			2		
S. Typhimurium ft 61			1		
S. Typhimurium ft 296			2		
S. Typhimurium ft 340			1		
S. Typhimurium ft 344			1		
S. Typhimurium ft 351			1		
S. Typhimurium ft 353			3		
S. Typhimurium ft 401			1		
S. Typhimurium ft 506			6		
S. Typhimurium ft 507			2		

Continued

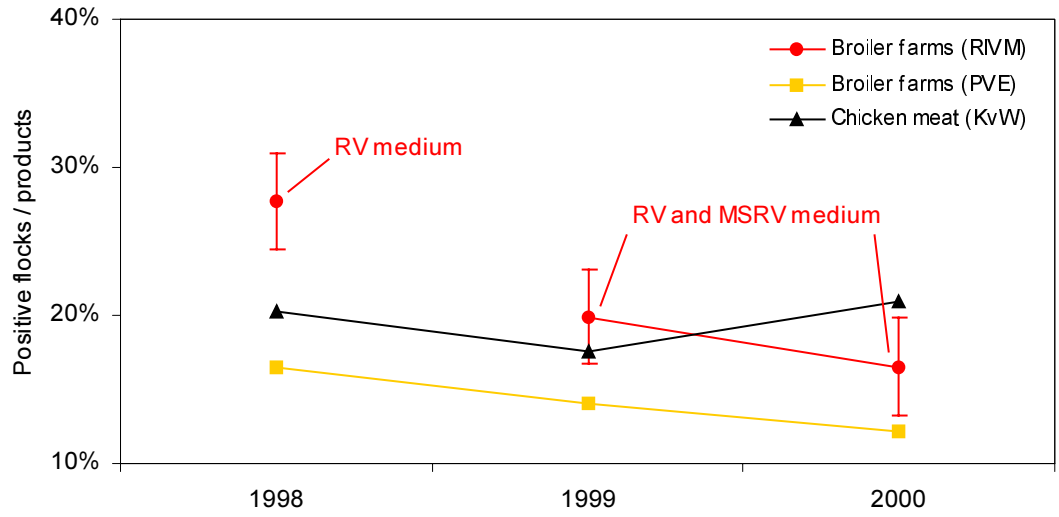
Serotype	Number of flocks/herds positive				
	Layers	**Broilers**	**Finishing pigs**	**Dairy cattle**	**Veal calves**
S. Typhimurium ft 510			7		
S. Typhimurium ft 655			2		
S. Typhimurium np			8		
S. Virchow		4			
S. Worthington		1			
S. nt			1		
S. nct			1	1	1

pt phagetype
ft faagtype (Dutch nomenclature)
app atypical phage pattern
np no phage reaction
nt not typable
nct not completely typable

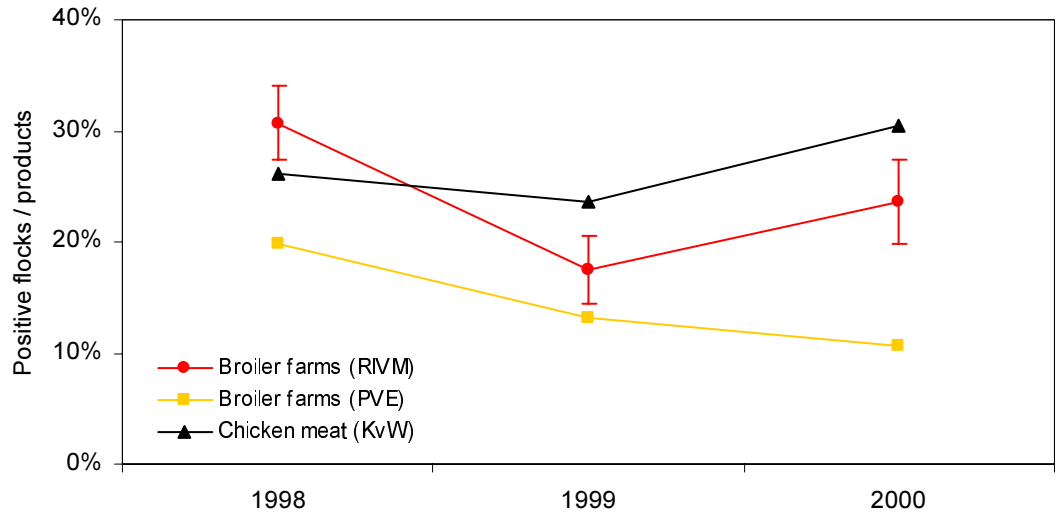
Appendix 6 Comparison between data from three different monitoring programmes on *Salmonella* spp. and *Campylobacter* spp. in broilers

Comparison of salmonella and campylobacter prevalences found in this monitoring programme in broiler flocks, the industry's monitoring in broiler flocks and the KvW monitoring on chicken end-products in retail stores. Protocol for the PVE monitoring can be found in the Action Plan (39), the sampling procedure for the KvW monitoring in Van der Zee *et al.* (59).

***Salmonella* spp.**



***Campylobacter* spp.**



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