Salmonella and Campylobacter on Broiler Farms

A kinetic research of Salmonella and Campylobacter contamination's on organic and conventional commercial broiler farms in relation to farm management in the Netherlands

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Foreword

This journal is written for the major thesis from the education Animal Science of the Wageningen Agricultural University in the Netherlands. It gives us 21 credit points. The thesis is a combination of the departments Adaptation Physiology and Animal Production Systems. We started at November 2002 and ended June 2003.

The purpose of this thesis was to investigate the kinetics of Salmonella and Campylobacter contamination's in conventional and organic broiler farms. We started in November whit our study. Due to the outbreak of Avian Influenza at the beginning of March we weren't able to finish our sampling period. We only had a few samples from the beginning of the rearing period from some farms. Not enough to say something about the kinetics, which was the major thing in our study.
We took a questionnaire on every farm, the purpose of that was to investigate if there was a relation between management and contamination's. The results of the questionnaire are in this report, but, off course, we could not make the comparison. We did a literature study about Salmonella and Campylobacter. And we had some results from (the beginning of) the sampling period. These tree things put together gives a detailed report for further investigation for students who can continue whit this research.

This report starts (after the abstract) with the introduction. In the introduction the original research questions, sub-questions, goal, problem definition and hypothesis of our research are given together with some background information.
In chapter 2 and 3 more information is given about Campylobacter and Salmonella, things like risk factors are mentioned.
In chapter 4 the material and method is given. After that, in chapter 5, the results are given. This report ends with a conclusion and discussion.

Our supervisors were:

- Dr. Ir. Rene Kwakkel, Animal Production Systems; Wageningen Agricultural University
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- Ing. Ria van der Hulst, ID-Lelystad: Division of Infectious Diseases and Chain Quality

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Abstract

This journal is written for the major thesis from the education Animal Science of the Wageningen Agricultural University in the Netherlands. The purpose of this thesis was to investigate the kinetics of Salmonella and Campylobacter contamination's in Dutch conventional and organic broiler farms in relation to farm management. Due to the outbreak of Avian Influenza at the beginning of March we weren't able to finish our sampling period, and through that, our study. Therefor we wrote a detailed report for further investigation for students who can continue whit this research.

Campylobacter and Salmonella infections are usually due to the consumption of contaminated eggs and egg-or meat-products. To lower the number of infected chicken flocks a better understanding of the epidemiology of both bacteria at the farm level is essential. In this study we tried this through measure the movement of the contamination during the rearing period and connect that with farm management. We also tried to investigate if there is a difference between organic farming systems and conventional farming systems.

To measure the kinetics of a contamination, we wanted to take samples on different days during the rearing period. Day 1, 17 and 35 of the conventional system and day 1, 17, 35, and 50 of the organic farming system. Samples also were taken in the empty house and of feed, water and floor cover of transport boxes.
In this study 10 conventional farms and 6 organic farms were enclosed. For Salmonella measurements we used overshoes and for Campylobacter transport swabs with agar, the samples were send to ID-Lelystad and analysed due to the general method used by PVE.
Because we want to know if contamination occurs at the slaughterhouse we also wanted to take samples (blood, "fresh" faeces, blood and breast skin) there.
To investigate if management is of influence on the contamination rate we took a questionnaire. According through earlier studies, there are a number of factors that are of influence on the prevalence of Campylobacter and Salmonella. In the questionnaire these factors were taken into account. The factors were about; surrounding, floor cover, rearing period, feed (-system), water system, climate, diseases, hygiene, outlet and the delivering of broilers. The questionnaire was analysed with SPSS. Thirteen organic and ten conventional farms were included in the questionnaire.
To measure the influence of the open-air run, at the organic farming system, we also wanted to take samples of the period the broilers get access to that. They get access to the outlet after the age of 40 days with a temperature above 15 degrees.

Because we couldn't finish our study there are no statistic-underlined results and we could not make a comparison between farm management and contamination rate.
We saw that Campylobacter occurred more in organic farms and Salmonella more in conventional farms. And that Campylobacter appeared after day 17 and Salmonella before day 17. No samples were analysed at the slaughterhouse and in the open-air run.
If this investigation is repeated we recommend to take the samples on the same days we did. For the questionnaire it is recommendable that the farmer fills it in during the growing period and not before. In that way you will get more detailed information about that period in particular and not about the rearing periods in general.

Table of contends

Chapter 1: Introduction

Food safety
Food safety is a concern of every individual. Consumers expect their food to be safe. Today the consumer can choose products of different production systems. But is there a difference in food safety between these systems?

Organic farming
'Organic' is a labelling term that denotes products that have been produced in accordance with organic standards. The organic labelling is not a health claim; it is a process claim.
The production of organic food of animal origin is done in many ways and uses many breeds. Therefore, a real comparison with conventional produced food is difficult. From the limited number of published data, it appears that the characteristics of quality of the products, the nutritionally and hygienic factors, are not very different in both systems of production. In some cases, organic food gets better marks, in others, conventional produced food scores higher.
Organic agriculture differs from conventional agriculture as it bans the use of synthetic agricultural inputs, such as synthetic pesticides, herbicides, veterinary drugs, fertilisers, fungicides, synthetic preservatives and additives. Thus potentially hazards of synthetics inputs residues are prevented (Kouba, jaartal). Is this the case for microbiological infections to? This is one of the questions of this study.
The 'organic' labelling provides assurance to consumers that no food ingredient has been subject to irradiation and that genetically modified organisms have been excluded. However, it seems that organic farming leads to a higher risk for the contamination of products by parasites of livestock and by microbes present in manure (Kouba, jaartal).

Numbers
In the Netherlands there was a production of 54,7 million broilers in the year 2002 (696.000 ton). There were a total of 1.027 broiler farms. 758.000 ton of the total poultry meat production was exported and 352.000 ton was imported. The consumption op broiler meat was in 2002 17,2 kg per person (www.pve.nl, 17-3-03).
In the Netherlands are 1568 organic farms, this is 1,74% (89.983) of the total farms. 49% of the organic farms is from the livestock-type (www.platformbiologica.nl, 17-3-03). 13 of them are organic broiler farms.

Occasion of research
The "Program Organic Farming" (intern rapport Praktijkonderzoek Veehouderij, 2002) wants to enlarge the organic farmers by solving bottlenecks that prevent the sector to enlarge and could harm the image or identity of this farming system.
The consumer has a growing interest in the product quality and development. Besides health, safety and taste of food and subjects like production methods and animal wellbeing becomes more important just like pollution to the environment and nature and landscape.
This research will concentrate on Salmonella and Campylobacter contamination in comparison to farm management in organic farming and conventional systems.
In the Dutch organic poultry production system there isn't, until now, much knowledge about health status and animal disease management.

Research goal

Kinetics of Salmonella and Campylobacter in relation to farm management in organic broiler farms in comparison to conventional broiler farms in the Netherlands.

Problem definition

Are there differences in appearance of Salmonella and Campylobacter in organic broiler farms in comparison to conventional broiler farms and plays management a role in this?

Sub-questions

1. If there is Salmonella or/and Campylobacter, what is the kinetics of it during the rearing period in organic farms in comparison to conventional broiler farms?
2. If there is a difference in appearance of Salmonella or/and Campylobacter contamination between organic and conventional broiler farms, is this due to management?
   And if so, which management aspects?
   - Surrounding + broiler data
   - Floor cover
   - Rearing period broilers
   - Water system
   - Climate
   - Diseases
   - Hygiene
   - Open-air run
   - Delivering broilers
3. If the broilers are negative tested for Salmonella or/and Campylobacter is this still the case at the slaughterhouse?
4. Broilers get access to the open-air run at the age of proximately 35 days, is this of influence on the contamination with Salmonella or/and Campylobacter? (only if broilers get access to the open-air run in this period!!)

Hypothesis

1. Salmonella appears more often in conventional broiler farms as in organic farms.
2. Campylobacter appears more often in organic broiler farms as in conventional farms.
3. Management influences the presence of Salmonella and Campylobacter.

In this paper a flock is defined as the chickens raised as a cohort in one compartment at the same time and a rearing period is the time when simultaneous flocks in the houses at the farm are raised.

Chapter 2: Literature

2.1 Campylobacter

2.1.1 Campylobacter in humans

Campylobacter jejuni is responsible for one of the most common types of food poisoning. Many cases of C. jejuni infection have been associated with the consumption of poultry meat (Oosterom e.a., 1984; Kapurud e.a., 1992 in Miwa e.a., 2002). Important sources of infections are inadequately heated poultry meat, poultry liver and cross-contamination of foodstuff through lack of kitchen hygiene (Skirrow, 1982; Skirrow and Blaser, 1992; Oosterom, 1994 in Atanassova, 1999).
From the food hygiene point of view, poultry thus plays an important role in the transmission of Campylobacter to man (Hood e.a., 1988; Jones e.a., 1991 a and b; Karib and Seeger, 1994; Lee e.a., 1994 in Atanassova, 1999).
For public health, it is important to lower the number of infected chicken flocks. To achieve this, a better understanding of Campylobacter epidemiology at the farm level is essential (Berndtson e.a., 1996 in Miwa e.a., 2002).

Results of studies on clinical Campylobacter cases in humans show a growing tendency (De Boer and Hahne, 1990; Jones e.a., 1991 a and b in Atanassova, 1999).
In several European countries, Campylobacter pathogenic to man have been isolated more frequently than Salmonella (Humphrey e.a., 1993; Bryan and Dole, 1995 in Atanassova, 1999 and Phillipps, 1995 in Refrégier-Petton e.a., 2001).

2.1.2 Transmission of Campylobacter

Campylobacter species have been isolated from a variety of animals (Luechtefeld and Wang, 1981 in Berndtson e.a., 1996) and are mostly considered as part of the normal intestinal flora (Berndtson e.a., 1996). Chickens can carry Campylobacter jejuni/coli in their intestinal sub-clinically (Berndtson e.a., 1996).
Many studies show that newly hatched and also young chickens do not excrete Campylobacter, and that vertical transmission is unlikely to occur (Shanker e.a., 1986 in Berndtson e.a., 1996). The Campylobacter colonisation appears at about 3 weeks of age (Shanker and Lee, 1983; Neill e.a., 1984; Annan-Prah and Janc, 1988 in Berndtson e.a., 1996) and, once settled, the bacteria are rapidly spread to all the birds within a flock (Smitherman e.a., 1984; Berndtson e.a., 1987 in Berndtson e.a., 1996). When colonised, chickens can excrete large numbers of Campylobacter (Berndtson e.a., 1992 in Berndtson e.a., 1996).
Infection and horizontal transmissions from one living animal to the other are of particular significance for the rate of detection of Campylobacter in poultry production. Very few infected animals can be the source of infection for the whole flock (Shanker e.a., 1990 in Atanassova, 1999).
Because of the widespread use of air-conditioning in pens, with optimal temperatures and humidity, seasonal influences on the spread of Campylobacter in production units hardly play a role. Where the inside climate cannot be standardised throughout the year,

a higher incidence of Campylobacter is observed in May and October (Doyle, 1984; Arwana, 1987; Wallance e.a., 1997 in Atanassova, 1999).
It is well known that higher isolation rates (71%) are found during warm seasons than during the winter (Zieger, 1993 in Atanassova, 1999).
Sources of contamination and routes of infection with Campylobacter are important for poultry fattening units (Doyle, 1984; Baker e.a., 1987 in Atanassova, 1999).
Campylobacter prevalence increased with age of chickens at slaughter. This was most obvious in flocks raised more than 6 weeks (Berndtson e.a., 1996).
Berndtson e.a. (1996) found that the general health status in flocks was not associated with Campylobacter infection rate, except in flocks starting with poor chicken material which showed higher Campylobacter prevalence.

2.1.3 Campylobacter problems in slaughterhouses

Intestinal contamination is the main source of broiler-carcass contamination at the slaughterhouse (Oosterom e.a., 1983 in Refrégier-Petton e.a., 2001).
During the slaughter process, damage to the intestinal tract can lead to direct contamination (Oosterom e.a., 1983; Jones e.a., 1991 a and b in Atanassova e.a., 1999). It is difficult to prevent carcass contamination with C. jejuni from their intestinal contents (Berndtson e.a., 1992 in Berndtson e.a., 1996).
Cross-contamination's are difficult to control during processing and lead to an increased risk of contamination of carcasses between different flocks at the and of the slaughtering process (Oosterom e.a., 1983 in Refrégier-Petton e.a., 2001, Rivoal e.a., 1999; Newell, e.a., 2001 in Miwa e.a., 2002). Contamination can also occur directly through air (Oosterom e.a., 1983; Jones e.a., 1991 a and b in Atanassova e.a., 1999).
Investigations at poultry slaughtering plants showed that there are many opportunities for contamination of carcasses (Oosterom e.a., 1983; Jones e.a., 1991 a and b in Miwa e.a., 2002).
The slaughtering plant is an important station for contamination with Campylobacter, carcasses contaminated are several times higher than animals entering plants (Hartog and De Boer, 1982; Izat e.a., 1988; Jones e.a., 1991 a and b in Atanassova , 1999).
At poultry slaughtering, the slaughter equipment and also the carcasses will be contaminated with intestinal contents including Campylobacters (Luechtefeld and Wang, 1981; Wempe e.a., 1983 in Berndtson e.a., 1996).

2.1.4 Results/numbers of Campylobacter

In a study in Germany, during a period of three years (1995-1997), 111 samples were analysed of broilers, 51 were positive tested (45,9%). Campylobacter jejuni biotype II was the most frequent isolate from broilers (46%) (Atanassova, 1999).
The detection rate in slaughtered broilers (45,9%) is similar to data from the UK and Northern Ireland, with 41 % Campylobacter positive poultry meat (Bryan and Doyle, 1995; Madden e.a., 1996; Bolton, 1996 in Atanassova, 1999).
In a study of Berndtson e.a. (1996) only 11% of 18 farms, followed during one year, were negative tested for Campylobacter during the whole sampling period. They found that even if one house is positive, it is possible to keep Campylobacters out of other houses on the same farm. During the entire sampling period, Campylobacters were detected in 27% of the investigated flocks. In most cases (89%), all ten pooled samples from the same flock were either negative of positive, which suggests that when Campylobacter are introduced into the flock, the bacteria are rapidly spread to most birds.

2.1.5 Factors of influence on the prevalence of Campylobacter contamination's

In table 1.1 and 1.2 there is a summary of factors, which are, according through earlier studies, of influence on the prevalence of Campylobacter contamination's.

Factor	**Influence**	**Source**
Flock size	1. Infection risk increases with flock size 2. No difference in Campylobacter prevalence due to flock size.	• (1) Berndtson e.a. (1992) in Berndtson e.a. (1996) • (1) Engvall e.a. (1986) in Berndtson e.a. (1996) • (2) Smitherman e.a. (1984) in Berndtson e.a. (1996)
Age of equipment	Not associated.	• Berndtson e.a. (1996)
Ceilings	Made of wood or concrete associated with less positive flocks than ceilings made of sheet metal.	• Berndtson e.a. (1996)
Floors	Made of wood were send with less positive flocks than concrete of asphalt floors.	• Berndtson e.a. (1996)
Food system	When placed in the anteroom instead of in the chicken-room the rate was higher.	• Berndtson e.a. (1996)
Empty period	Positive flocks increased with preceding shorter empty periods.	• Berndtson e.a. (1996)
Season	The risk of contamination is increased in summer/autumn.	• Refrégier-Petton e.a. (2001) • Kapperud e.a. (1993) in Refrégier-Petton e.a. (2001)
Ventilation	Houses with static air distribution have a higher contamination risk.	• Refrégier-Petton e.a. (2001)
Employee's	1. When two or more people are taking care of the flock the contamination risk is higher. 2. When one person takes care of several houses the contamination risk is also higher.	• (1) Refrégier-Petton e.a. (2001) • (2) Kapperud e.a. (1993); Berndtson e.a. (1996) in Refrégier-Petton e.a. (2001)

Table 1.1: Factors of influence on the contamination of Campylobacter

Factor	Influence	Source
Water	1. When the drinking water for the chickens was acidified the contamination risk is higher. 2. Non-disinfected surface water gives a higher contamination risk.	• (1) Refrégier-Petton e.a. (2001) • (2) Kapperud e.a. (1993) in Refrégier-Petton e.a. (2001)
Hygiene	1. Lack of hygienic practices gives a higher contamination risk. 2. No factor related to sanitary measures (such as clothing practices or change room) gives a lower contamination risk.	• (1) Van de Giessen e.a. (1996, 1998); Evans and Sayers (2000) in Refrégier-Petton e.a. (2001) • (2) Refrégier-Petton e.a. (2001)
Animals	1. Other animals on the farm give a higher contamination risk. 2. The presence of litter-beetles in the change room increased the risk.	• (1) Van de Giessen e.a. (1998) in Refrégier-Petton e.a. (2001) • (2) Refrégier-Petton e.a. (2001)
Treatments	The administration of an antibiotic treatment following a disease was a protective factor.	• Refrégier-Petton e.a. (2001)
Farm	1. In poultry farms with three or more houses the contamination risk is higher. 2. House surroundings give a higher risk.	• (1) Refrégier-Petton e.a. (2001) • (2) Kazwala e.a. (1990); Rivoal (2000) in Refrégier-Petton e.a. (2001)

Table 1.2: Factors of influence on the contamination of Campylobacter

2.2 Salmonella

2.2.1 Salmonella in humans

Salmonella contamination is of great concern to the poultry industry. Because more than 99% of the Salmonella strains so far identified cause no clinical disease in poultry, public health remains the most important concern for this pervasive organism. Human Salmonella infections are usually due to the consumption of contaminated eggs (Handzler e.a., 1994 a in Rose e.a., 2000) and egg-or meat-products (Descenclos e.a., 1996 in Rose e.a., 2000). Because of the many forms in which chicken-meat is consumed and the risks of cross-contamination to other foods, poultry meat-products become more and more implicated in food-borne Salmonellosis. (Lee, 1974; Hird e.a., 1993; Bryan and Doyle, 1995 in Rose e.a., 2000).

2.2.2 Transmission of Salmonella

Persistence of environmental contamination after sanitation occurred for at least 1 year in an empty poultry building (Davies and Wray, 1996 in Rose e.a., 2000). Salmonella persistence in broiler houses after cleansing and disinfecting is one of the main risk factors for the infection of the flock at the end of the rearing period (Lahellec e.a., 1986; Baggesand e.a., 1992; Rose e.a., 1999 in Rose e.a., 2000).
An important contamination period of flocks occurs during the first 2 weeks of the rearing period, during the rest of the period the number of flocks contaminated decreases (De Zutter e.a., 2001).

2.2.3 Salmonella problems in slaughterhouses

Slaughtering practices favour Salmonella dissemination and carcass contamination when a contaminated flock is processed on the slaughter line (Mead, 1993 in Rose e.a., 2000).

2.2.4 Results/numbers of Salmonella

In a nation-wide study conducted during 1990, reported by the National Food Administration, Salmonella was not isolated from any of 1809 samples of different food products, including meat at supermarkets and grocery stores. From 18.586 corresponding samples, mostly from restaurants, two (0,01%) were positive for Salmonella.
The very low prevalence of Salmonella in food at the consumer level is reflected by a corresponding low prevalence of domestically acquired Salmonella infections in humans. Only 15-20% of the reported Salmonella infections in humans are classified as domestic while 80-85% are contracted abroad. During the last 10-year period, the number of reported cases in humans has doubled from around 3000 to 6000. This increase is mostly due to an increased number of cases caused by S. enteritidis. During the last 10-year period, the number of reported S. enteritidis infections in humans has increased

from about 200 to 2000, of which 90% are acquired abroad, reflecting the international spread of S. enteritidis (Anderson e.a., 1992 in Wierup e.a., 1995). The majority of domestic cases of S. enteritidis are most likely the result of secondary infections from imported human cases and also from imported meat which, has a low level of Salmonella contamination not detected by import control.

2.2.5 Factors of influence on the prevalence of Salmonella contamination's

In table 2.1 and 2.2 there is a summary of factors which are, according through earlier studies, of influence on the prevalence of Salmonella contamination's.

Factor	Influence	Source
Disinfection	1. Absence of a terminal disinfection increases the risk. 2. Combined surface and pulse-fogging disinfection reduces prevalence.	• (1) Rose e.a. (2000) • (2) Gradel e.a. (2003)
Animals	1. Rodents increase the risks. 2. A systematic check of indoor rodent-bait depots reduces prevalence. 3. Other animals (including pets, insects, spiders, rodents and birds) on the farm are not a significant source for Salmonella.	• (1) Rose e.a. (2000) • (2) Gradel e.a. (2003) • (3) De Zutter e.a. (2001)
Farm	1. Having a large part of the access area to the house accessible to trucks increases the risk. 2. Gravel alongside the broiler house gives a reduced prevalence. 3. Ditch-water, puddles and other surfaces in the neighbourhood of the house are not a significant source of Salmonella. 4. Number of houses was not a significant factor for Salmonella contamination.	• (1) Rose e.a. (2000) • (2) Gradel e.a. (2003) • (3, 4) De Zutter e.a. (2001)
Treatment	Having had a disease leading to a treatment in the previous flock increases the risk.	• Rose e.a. (2000)
Litter	Not of influence.	• Rose e.a. (2000)

Table 2.1: Factors of influence on the contamination of Salmonella

Factor	**Influence**	**Source**
Equipment	Important factors for horizontal transmission are movable equipment after disinfection and cleaning.	• De Zutter e.a. (2001)
Cleaning	1. The cleansing procedure and the ability of the house to be cleaned and disfinceted is not of influence. 2. Antiseptic soap and water for washing hands in the anteroom reduces prevalence.	• (1) Rose e.a. (2000) • (2) Gradel e.a. (2003)
Hygiene	1. A hygiene barrier when removing death broilers reduces prevalence. 2. House hygiene is not a significant source.	• (1) Gradel e.a. (2003) • (2) De Zutter e.a. (2001)

Table 2.2: Factors of influence on the contamination of Salmonella

Chapter 3: Material and Method

3.1: Objects

This study was carried out from November 2002 to March 2003, samples were taken from January 2003 to March 2003. If all samples would be taken, the study carried out until May, but due to the Avian Influenza (see foreword) this was not possible.
The study involved 16 broiler farms spread through the Netherlands. To participate in the study, farms had to be commercial broiler farms. Six of the farms were organic, and ten of them were conventional.
There are a total of 13 organic broiler farms in the Netherlands (in 2002/2003), but only 6 of them gave there permission for this investigation.
All the conventional farms have a IKB-certification (Internal Chain Control). This certificate guarantees the quality and safety of Dutch poultry meat.
We spread a folder about our study under conventional farms, the farms included thus choose to co-operate themselves. In the conventional farming system only one flock was studied, it was selected on the basis of convenience (date has to be within the period available for the thesis). In the organic farming system more flocks were studied, also on the basis of convenience.

3.2: Samples

Salmonella:
For the Salmonella measurements the farmer walked with overshoes on given days (see for details table 3.2.1, 3.2.2 and 3.2.3) through the house, he repeated this one time.
According to De Zetter e.a. (2001) the overshoe method is the best one to measure a Salmonella contamination, you need at least 2 pair of overshoes and take samples on different times during the rearing period.
The shoes are made of plastic. Every time the farmer wears two pair of shoes across. The first pair was thrown away, it protected the top pair from contamination of the own shoes. The shoes were packed in a plastic bag with date, house number and farm number on it. It was sending within 48 hours to ID-Lelystad were it was analysed (see section 3.3).

Campylobacter:
For the Campylobacter measurements we used sterile transport swabs with agar (type: Copan Amies Agar Gel “Do All” Swabs). We used this type of swabs so the manure (and Campylobacter) taken doesn’t dehydrate. If it dehydrates the Campylobacter (if present) can’t be analysed.
According to De Zetter e.a. (2001) the most useful method for the measurement of Campylobacter is the analyse of cecal drops.
In this research the farmer took 10 samples of fresh manure on given days (see for details table 3.2.1, 3.2.2 and 3.2.3) spread through the house, during the house measurement he took samples of the surface area of given objects (see also table 3.2.2). Sometimes it gave problems to find fresh manure, for example during the first days of the rearing period, in that case the farmer took samples of any manure he could find. All 10 swabs were put together in a plastic bag with date, house number and farm number on it and was also send to ID-Lelystad within 48 hours.

3.2.1: Organic farms

Broilers:
As mentioned before, there are a total of 13 organic broiler farms in the Netherlands, only 6 of them gave there permission for the sampling and questionnaire, the other 7 only took the questionnaire. Broilers from the first 6 farms were of the Isa Hubert 957 type, the broilers of the last 7 farms were of the “Kemper-kip” type. Both are slow growing organic broilers. The rearing period takes about 81 days.

Overview measurements:
We took measurements on different days during the rearing period.
Table 3.2.1 gives an overview of measurements taken in the organic farms. The place, day and number of samples are noted.

	Swab; number per house (Schema monitoring vleessector, Internet)	**Overshoe; number of pair per house (Schema monitoring vleessector, Internet)**
Inside measurements		
House measurement (for details see table 3.2.2)	21	2
Day 1	10	2
Day 17	10	2
Day 35	10	2
Day 50	10	2
Final measurement	10	2
Outdoor measurements		
Floor measurement	10	2
Day 50	10	2
Final measurement	10	2

Table 3.2.1: Measurements of organic farms taken during the study

We choose for these specific days because they give an overview of the whole rearing period. The house measurement gives an overview of present Salmonella or Campylobacter bacteria before the broilers arrive. So you can explain, if broilers are tested positive on day 1, that this could be due to the hygiene of the house. Day 35 was chosen because on this day all the organic broilers are still inside the house (and because of the schedule of conventional farms, see 3.2.2), as said before, only after 40 days they get access to the outlet. Day 17 is chosen because this lays between day 1 and 35 and thus give information about the kinetics of an eventually present contamination. This is also the case for day 50, it lays just between the final measurement, samples taken just before slaughter, and day 35.

Outdoor measurements:
Organic broilers must have access to an outlet after the age of 40 days. In most cases this is only done if the weather is good, with temperatures above 15 degrees. Some farms give their broilers access to outlet throughout the whole year. Salmonella and Campylobacter measurements also take place in the outlet, but off course only if it is used. Before it will be used, a “floor measurement” is taken to measure the

contamination level of the “clean” ground. The same method is used as in the house (see for details table 3.2.1).

House measurement:
In table 3.2.1 we mentioned “house measurement”, this are samples taken in the empty house after cleaning or/and disinfection just before the broilers arrive. Table 3.2.2 shows exactly, on which places and how many swabs are taken.

Sample place in house	Number of swabs
Floor	4
Feed system	4
Drinking system	4
Wall	3
Sealing	2
Inlet	2
Hopper	1
Changing room	1

Table 3.2.2: House measurements

Other measurements on the farm:
Except the measurements mentioned before the farmer also took samples of:
- floor cover of the transport boxes (a total of 40 pieces (Schema monitoring vleessector, Internet)).
- feed (25 gram per delivery)
- drinking water (25 ml per house)

This was to investigate if contamination could be caused through external causes. And if these materials are contaminated if this gave a positive outcome for the broilers to.

Measurements during slaughtering:
After the rearing period samples were taken in the slaughterhouse:
- breast skin (1x15 per house)
- blood (1x30 per house)
- “fresh” faeces (2x15 per house)

This was done to investigate if a flock negative tested in the final measurement, still was in the slaughterhouse. Thus if they got infected during slaughtering. This measurement was done by all the flocks, also if a flock was negative tested.

3.2.2: Conventional farms

Broilers:
There were 10 conventional farms in this study. Seven of the farms used broilers of the Ross type. Two farms used broilers of the Cobb type. And one farm used Hubbard, this was a test. The rearing period is divided in two pieces. On day 35 the first broilers will be slaughtered, then after 40 days the reminding broilers are slaughtered.

Overview measurements:
We also took measurements on different days during the rearing period in conventional farms.
Table 3.2.3 gives an overview of the measurements taken. The place, day and number of samples are noted.

	Swab; number per house (Schema monitoring vleessector, Internet)	**Overshoe; number of pair per house (Schema monitoring vleessector, Internet)**
House measurement (for details see table 3.2.2)	21	2
Day 1	10	2
Day 17	10	2
Day 35	10	2

Table 3.2.3

Other measurements:
The same “other measurements” and “measurements during slaughter” are taken, as is the case in the organic farming system.

3.3: Sample analyse

Samples are send to ID-Lelystad by the farmer. In the laboratory they are analysed due to the general method used by PVE.

Campylobacter:
Each house is going to be monitored for Campylobacter infection by collection of swabs during the rearing period. These are going to be cultured according to conventional methods by ID-Lelystad. Faeces samples (swabs) are streaked onto CCDA (Charcoal Cefoperazone Deoxycholate Agar) plates. Floor cover samples are incubated on CCDB (Charcoal Cefoperazone Deoxycholate Broth) an after that on CCDA. Minimum of 5 specific colonies till one positive is found (if present; at < 5 specific colonies spread on plate they have to be confirmed).
Each house is classified as not infected with Campylobacter (negative) if all swabs and floor cover collected at each sampling time are Campylobacter culture-negative.

Salmonella:
Each house is going to be monitored for Salmonella infection by collection of overshoe's and floor cover. These are going to be cultured according to conventional methods by ID-Lelystad.

The sample is first pre-treated; dilute sample 1:10 in Buffered Peptone Water (BPW) After pre-treatment 0,1 ml BPW-culture is taken on MSRV (Modified Semi-solid Rappaport-Vassiliadis medium) plate. The plates are incubated respectively at 42°C for 24 h. Not suspicious or negative plates are incubated again, respectively at 42°C for 24 h. A suspicious MSRV plate demonstrate grow in agar and has a white/grey colour. Suspicious plates are incubated again on BGA (Briljant Groen Agar) plates. These plates are going to be incubated at respectively 37°C at 24 h. If the BGA plates forming pink colonies, these colonies are considerate to be positive.
Each house is classified as not infected with Salmonella (negative) if all shoes and floor cover collected at each sampling time are Salmonella culture-negative.

3.4: Questionnaire

To study the management factors of the farms we made a questionnaire, with questions about:

- Surrounding + broiler data
- Floor cover
- Rearing period broilers
- Feed
- Water system
- Climate
- Diseases
- Hygiene
- Outlet
- Delivering broilers

Al the 13 organic and 10 conventional farms give their permission for the questionnaire. If farms are positive tested we compared there management with negative tested farms, to look if something in the management could be the purpose of the contamination. For example; feed of farm A, B and C is positive tested for Salmonella, in the questionnaire the farmers said they never cleaned there feeding system. Farms which were negative tested, did clean their system. The questionnaire was analysed with SPSS.

Chapter 4: Results

4.1: Samples

4.1.1 Organic Farms

In the following tables the measurements of the organic farms are given, farm B is not included because we have no data of that one. The measurements are in chronological order.

Samples *Farm A1*	**Salmonella**	**Campylobacter**
Floor Cover	-	
House measurement	-	-
Feed 1	-	
Day 1	-	-
Water	-	
Feed 2	-	
Day 17	-	+
Feed 3	-	
Day 35	-	+

Table 4.1.1: Farm A1

Samples *Farm A2*	**Salmonella**	**Campylobacter**
Floor Cover	-	
House measurement	-	-
Feed 1	-	
Day 1	-	-
Water	-	
Feed 2	-	
Day 17	-	-

Table 4.1.2: Farm A2

Sample *Farm C*	**Salmonella**	**Campylobacter**
House measurement	-	-
Day 1	-	-
Floor Cover	-	
Feed 1	-	
Water	-	

Table 4.1.3: Farm C

Sample *Farm D*	**Salmonella**	**Campylobacter**
House measurement	-	
Floor cover	-	
Feed 1	-	
Day 1	-	
Water	-	-
Feed 2	+	
Day 17	-	-
Feed 2 (second measurement)	-	
Day 35	-	+

Table 4.1.4: Farm D

Sample *Farm F*	*Salmonella*	**Campylobacter**
House measurement	+	-
Water	-	
Floor Cover	-	
Feed 1	-	
Water	-	-
Day 1	-	-
Day 17	-	-

Table 4.1.5: Farm F

Sample *Farm E*	*Salmonella*	**Campylobacter**
Feed 1	-	
Water	-	
House measurement	-	
Floor Cover	-	
Day 1	-	-
Day 17	-	+
Feed house 1	-	
Feed house 2	-	
Feed house 3	-	
House measurement, house 3		-
House measurement, house 2		-

Table 4.1.6: Farm E

4.1.2: Conventional Farms

In the following tables the measurements of the conventional farms are given, farms R, Q and S are not included because we have no data from them. The measurements are in chronological order.

Sample *Farm M*	Salmonella	Campylobacter
House measurement	-	
Floor Cover	-	
Day 1	-	-
Feed, house 2	+	
Feed, old	-	
Feed, by products	-	
Feed 1	-	
Feed 2	+	
Day 17	+	-

Table 4.1.7: Farm M

Sample *Farm L*	Salmonella	Campylobacter
House measurement	-	-
Floor cover	-	
Feed 1	-	
Water	-	-
Day 1	-	-
Feed 2	-	
Day 17	+	-

Table 4.1.8: Farm L

Sample *Farm O*	*Salmonella*	Campylobacter
House measurement	-	
Floor cover	-	
Feed 1	-	
Water	-	
Day 1	-	-

Table 4.1.9: Farm O

Sample *Farm K*	Salmonella	Campylobacter
House measurement	-	

Floor Cover	-	
Feed 1	-	
Day 1	-	-
Water	-	
Feed 2	-	
Feed, by products	-	
Feed, by products	-	
Day 17	-	+

Table 4.1.10: Farm K

Sample *Farm N*	**Salmonella**	**Campylobacter**
HOUSE MEASUREMENT	-	-
Feed 1	-	
Water	-	-
Floor Cover	-	
Day 1	-	-
Feed, by products	-	
Feed 2	-	
Day 17	-	-

Table 4.1.11: Farm N

Sample *Farm P*	**Salmonella**	**Campylobacter**
HOUSE MEASUREMENT	-	
Floor Cover	-	
Feed 1	-	
Water	-	-
Day 1	-	-
Feed 2	-	
Day 17	-	-
Feed 3	-	

Table 4.1.12: Farm P

Sample *Farm T*	**Salmonella**	**Campylobacter**
Feed 1	-	
Water	-	
Day 1	+	-

Table 4.1.13: Farm T

4.2: Questionnaire

In this chapter some questions of the questionnaire are reported. The graphs and tables have the same numbers as the questions do.

Question 1: What is the flock complement?
In table 1 the number of animals per m^2 are given per system. The mean, minimum and maximum values are given. As you can see there is a big difference between the conventional and organic system. In the conventional system there are, on average, 16,5 animals per m^2 more. Only the flock complement of the indoor housing is given for the organic system.

System	Mean	Minimum	Maximum
Organic	8.7	5.5	10.0
Conventional	23.0	22.0	24.5

Table 1: animal per m^2

Question 2: Are there other animals on the farm?
In graph 2 the answer on the question "are there other animals" is given. As you can see in organic farming systems there are more other animals.

Graph 2: Other animals on farm

Question 3a: Are there other agricultural activities besides broilers?

In table 3a you can see that 6 organic and conventional farms have other agricultural activities besides broilers.

System	Yes	No
Organic	6	7
Conventional	6	4

Table 3a: Agricultural activities

Question 3b: If there are other agricultural activities, what kind?

The following answers were given.

Organic:
- Layers, turkeys
- Agriculture, meat
- Cattle
- Pigs (3x)

Conventional
- Agriculture and bulbgrower
- Agriculture (2x)
- Agriculture and plants
- Cattle
- Agriculture and pigs

Question 4: Is there a dunghill for the broilers?

System	Yes	No
Organic	8	5
Conventional	1	9

Table 4: Dunghill for broilers

Question 5: Is there a dunghill for other animals?

System	Yes	No
Organic	7	6
Conventional	3	7

Table 5: Dunghill for other animals

Question 6: Hygiene measurements taken

In table 6 the hygiene measurements taken on the different farms are given. In the first column the farming system is given, in the second one the number of the farm and in the third, and last one, the measurements taken.

System	Farm	Hygiene measurements
Organic	1	Clothes/shoes
	2	Clothes/shoes
	3	Combination off disinfection, equipment and clothes/shoes
	4	Clothes/shoes
	5	Clothes/shoes
	6	Changing room and clothes
	7	Nothing
	8	Combination off disinfection, equipment and clothes/shoes
	9	Combination off disinfection, equipment and clothes/shoes
	10	Combination off disinfection, equipment and clothes/shoes
	11	Combination off disinfection, equipment and clothes/shoes
	12	Combination off disinfection, equipment and clothes/shoes
	13	Combination off disinfection, equipment and clothes/shoes
Conventional	1	Combination off disinfection, equipment and clothes/shoes
	2	Combination off disinfection, equipment and clothes/shoes
	3	Combination off disinfection equipment and clothes/shoes
	4	Combination off disinfection equipment and clothes/shoes
	5	Clothes/shoes
	6	Combination off disinfection equipment and clothes/shoes
	7	Combination off disinfection equipment and clothes/shoes
	8	Clothes/shoes
	9	Clothes/shoes
	10	Combination off disinfection equipment and clothes/shoes

Table 6: Hygiene measurements

Question 7: Are there any other farms in the surrounding?

System	Yes	No
Organic	6	7
Conventional	8	2

Table 4.2.7: Surrounding farms

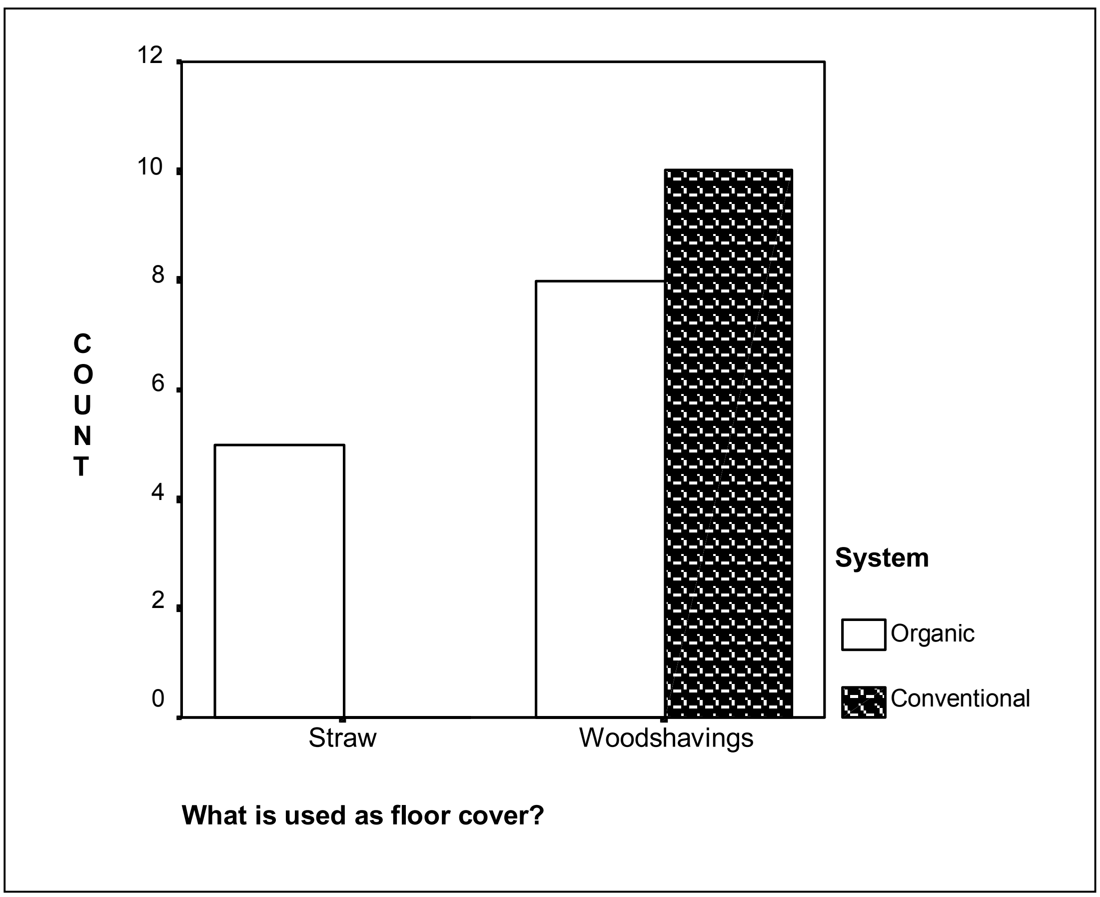
Question 8: Do other people get access to the broiler house?

System	Yes	No
Organic	1	12
Conventional	6	4

Table 4.2.8: Other people in house

Question 9: Which type of floor cover is used?

In graph 4.2.9 the type of floor cover is given. In the organic system both types, straw or woodshavings are used.



Graph 4.2.9: Floor cover

Question 10: Which lighting system is used?

In table 10 the lighting systems of the farms are given, in the second and third column the number of farms using the type mentioned in column 1 are given.

Lighting system	Organic	Conventional
Daylight	8	-
Bulb	-	2
TL	-	2
Daylight and TL	3	-
Daylight and bulb	2	-
Bulb and TL	-	3
TL and saving lamp	-	1
Bulb, TL and green lights	-	1
TL, sodium lighting and bulbs	-	1

Table 10: Lighting

Question 11:Which type of feed is used?

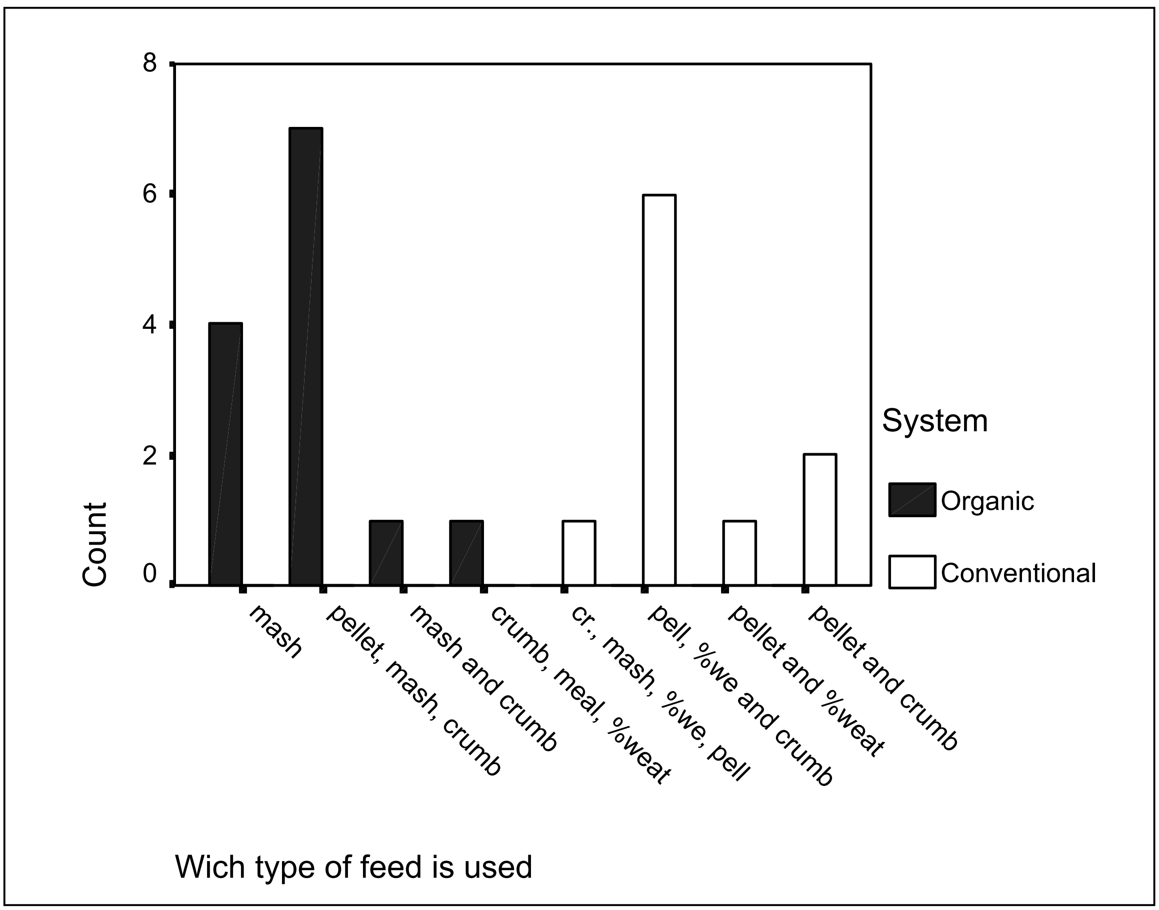


Table 11: Feed type used

Question 12: What is the way of feeding?

System	Limited	Unlimited	First unlimited, than limited	Other
Organic	0	13	0	0
Conventional	1	5	1	3

Table 12: Feeding

Question 13: What is the phase program of feed?

System	Start feed, grow 1 and grow 2	Start feed, grow 1, grow 2 and end feed	Start feed, grow 1 and end feed	Pre-starter, start feed, grow1, grow 2 and end feed	Pre-starter, start feed, grow 1 and end feed
Organic	10	1	2	-	-
Conventional	-	1	4	3	2

Table 13: Phase program

Question 14: Does feed contain anti-coccidiën device?

No organic farms uses anti-coccidiose, the conventional farms do.

Question 15: Are their substances added to feed for animal health care?

None of the farming systems add substances to their feed.

Question 16a: Are substances, for animal health, brought into the house or outlet?

In table 16a you can see the number of farmers who don't or does bring substances, for animal health, in the house. In table 16b the reason for doing this is given.

System	No	Yes
Organic	12	1
Conventional	9	1

Table 16a: Substances in house

Question 16b: Why farmers bring substances in house or outlet?

System	Don't bring substances in house/outlet	Precaution for coccidiose	Good intestinal development
Organic	12	1	-
Conventional	9	-	1

Table 16b: Reason for substances

Question 17: Which feeding system is used?

System	Feeding pan	Feeding chain	Tons	Feeding pans and tons	Feeding pans and feeding chain
ORGANIC	6	4	1	1	1
Conventional	9	-	-	-	1

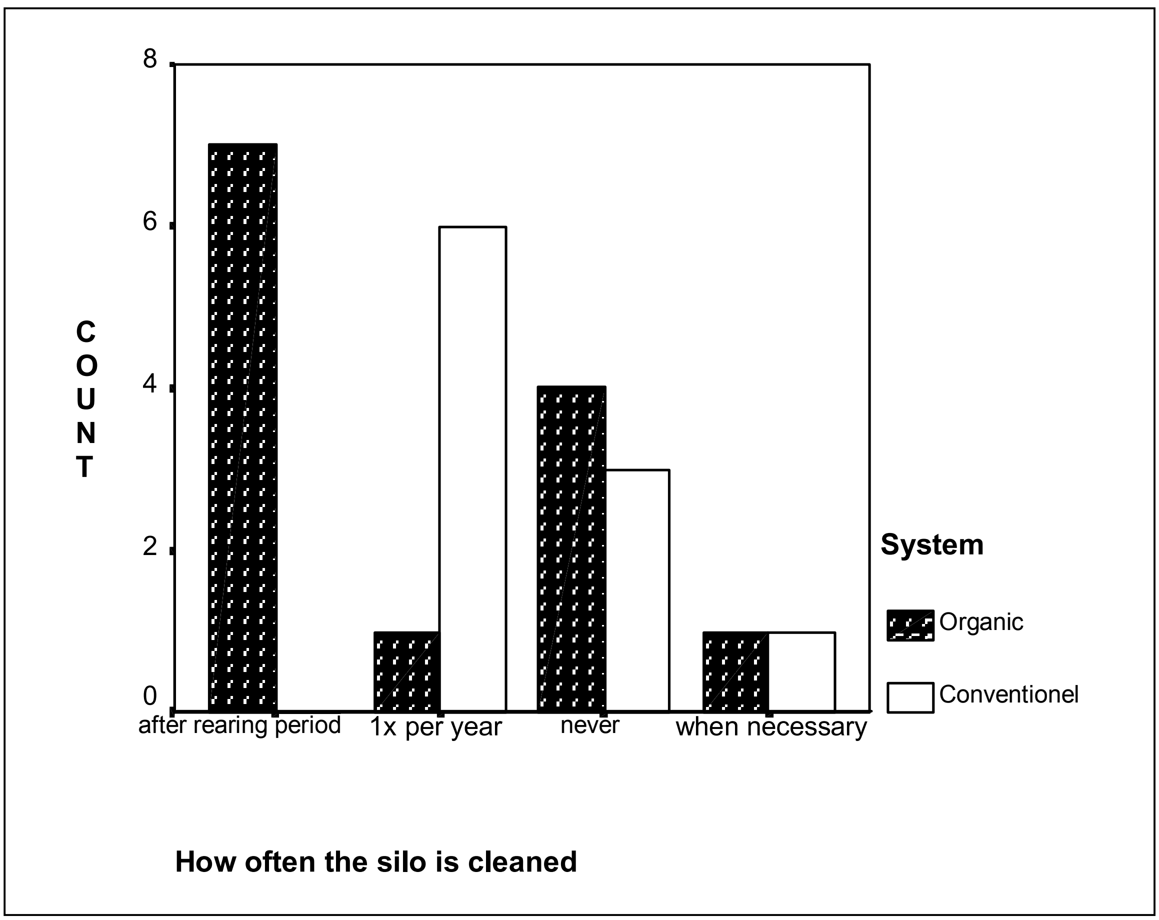
Table 17: Feeding systems

Question 18: Which feeding storage system is used?

System	Silo with outlet funnel (metal)	Silo with outlet funnel (polyester)
Organic	3	10
Conventional	1	9

Table 18: Feeding storage system

Question 19: How often the silo is cleaned?



Graph 19: Silo cleaning

Question 20: Which drinking water system has been used?

System	Open	Nipples	Nipples and cups	Open and cups
Organic	8	3	1	1
Conventional		10	-	-

Table 20: Drinking system

Question 21: Which water source is used?

System	Tap-water	Water from own source	Tap-water en water from own source
Organic	11	2	-
Conventional	4	5	1

Table 21: Water source

Question 22: Is drinking water checked on quality en composition?

System	No	Yes
Organic	10	3
Conventional	3	7

Table 22: Checking drinking water

Question 23: Are the waterworks during the rearing period cleaned and / or rinsed?

System	No	Yes
Organic	6	7
Conventional	4	6

Table 23: Cleaning water system

Question 24: Is there a water storage?

System	No	Store barrel	Floating tank	Store barrel and floating tank
Organic	2	2	9	-
Conventional	7	2	-	1

Table 24: Water storage

Question 25a: Are substances provided through the water for animal health care?

System	No	Yes
Organic	8	5
Conventional	-	10

Table 25a: Substances in water

Question 25b: What is the main reason for providing substances through the water?

System	Not filled in	Curative (when broilers are ill)	Preventive (for precaution)
Organic	9	2	2
Conventional	2	7	1

Table 25b: Reason for providing substances in water

Question 26: Which heating system is used?

System	Local heating	Space heating
Organic	8	5
Conventional	-	10

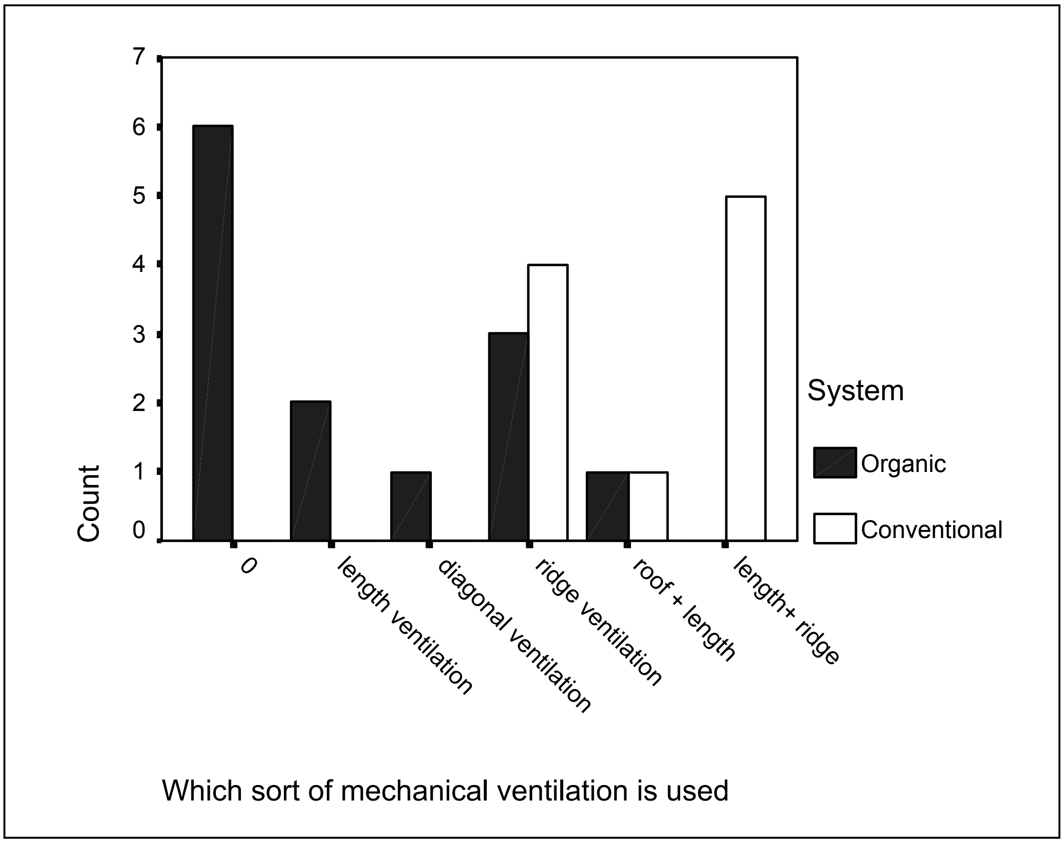
Table 26: Heating system

Question 27: What ventilation system is used?

System	Natural	Mechanical	Natural and Mechanical
Organic	9	1	3
Conventional		10	-

Table 27: Ventilation system

Question 28: Which type of mechanical ventilation is used?



Graph 28: Mechanical ventilation types

Question 29:Do they use a vaccine for IB?

	Now answer	No	Yes
Organic	1	4	8
Conventional	-	5	5

Table 29: Vaccination for IB

Question 30: Do the use a vaccine for NCD?

In table 30 the number of farms are mentioned who use a vaccine for NCD.

Organic	13
Conventional	10

Table 30: Vaccination for NCD

<u>Question 31: Do they use a vaccine for Gumboro?</u>

System	No answer	No	Yes
Organic	1	4	8
Conventional	-	-	10

Table 31: Vaccination for Gumboro

<u>Question 32: Do they use a vaccine for Marek?</u>

System	No answer	No	Yes
Organic	1	7	5
Conventional	-	10	-

Table 32: Vaccination for Marek

<u>Question 33: Do they use a vaccine for Salmonella?</u>

System	No answer	No	Yes
Organic	1	11	1
Conventional	-	10	-

Table 33: Vaccination for Salmonella

<u>Question 34a: Were there any problems with diseases last year?</u>

System	No	Yes
Organic	12	1
Conventional	5	5

Table 34a: Problems with diseases

<u>Question 34b: If so, what kind of diseases?</u>

System	No answer	Coccidiose	Coli	Other
Organic	12	1	-	-
Conventional	5	2	1	2

Table 34b: Kind of diseases

Chapter 5: Conclusion and discussion

In this chapter the conclusion and discussion are mentioned. As we explained earlier it was not possible to finish this study, so we wrote a detailed proposal for further investigation. Therefor we divided the sub questions over paragraphs. For each sub question we first give our results (and literature study), secondly mentioned the things, which still needs to be investigated, and finally give modifications and recommendations about the question and research methods. We don't say anything about the hypothesis, because this is not possible with the results we have.

5.1: Kinetics

Question:
If there is Salmonella or/and Campylobacter, what is the kinetics of it during the rearing period in organic farms in comparison to conventional broiler farms?

See for detailed results chapter 4.

Campylobacter:
Four farms were positive tested for Campylobacter.
A total of three farms (2 organic and 1 conventional) were positive on day 17. One of them (organic) was also positive tested on day 35, the other two were not tested on that day.
One organic farm was positive tested on day 35.

Salmonella:
One organic farm tested positive on Salmonella during the house measurement (before arrival of the broilers in an empty house), after that it was negative.
By one conventional farm the feed was tested positive two times, the same farm tested positive on day 17.
One conventional farm was tested positive on day 1 and another one was tested positive on day 17.

We can't say much about kinetics. For Campylobacter only one farm was tested again after a positive test. It was positive again. For Salmonella also only one farm was tested again after a positive test, this farm was the second time negative. There was also one farm tested positive after use of Salmonella contaminated feed.
So we have to little evidence to say something about kinetics.
One thing you can see is that there are more cases of Campylobacter in organic farms (3 times) as is the case in conventional farms (1 time). And there are more cases of Salmonella in conventional farms (3 times) as is the case in organic farms (1 time). But also in this case we have to little evidence to give a statistical correct answer.

In literature we didn't found much about kinetics or about a comparison between the two farming systems. A number of authors (Shanker and Lee, 1983; Neill e.a., 1984; Annan-Prah and Janc, 1988 in Berndtson e.a., 1996) said that colonisation of Campylobacter appears at about 3 weeks of age. Our results show the same (day 17).
Berndtson e.a. (1996) concluded that Campylobacter prevalence raises in flocks raised more than six weeks. We didn't come that far with this study to conclude that.

About Salmonella De Zutter e.a. (2001) concluded in their study that the first two weeks of the rearing period is an important contamination period, during the rest of the period the number of flocks contaminated decreases. We found contamination's during the first 17 days, but didn't test after that anymore.

We think the method we used and the days on which we (would) test are good, no modifications needed. Important is to keep in contact with the farmers so you know for sure that they take and send the samples, and do so on the right day. We had farms who didn't.

5.2: Management

Question:
If there is a difference in appearance of Salmonella or/and Campylobacter contamination between organic and conventional broiler farms, is this due to management?
And if so, which management aspects?
- *Surrounding + broiler data*
- *Floor cover*
- *Rearing period broilers*
- *Water system*
- *Climate*
- *Diseases*
- *Hygiene*
- *Open-air run*
- *Delivering broilers*

In the first paragraph of this chapter the number of contamination's is given. If we make the assumption that these results are significant for all the farms, we can say that Salmonella appears more in conventional farms and Campylobacter appears more in organic farms. There are to little farms in this research to give statistical underlined answers if this difference is due to management.
The questions of the questionnaire were, according to us, good. Maybe it is recommendable to make a questionnaire that the farmer fills in during the growing period and not before. In that way you will get more detailed information about that period in particular and not about the rearing periods in general.

5.3: Slaughterhouse

Question:
If the broilers are negative tested for Salmonella or/and Campylobacter is this still the case at the slaughterhouse?

There were no measurements taken in the slaughterhouse, due to avian influenza. We tried to get old data of Salmonella and Campylobacter contamination's but we didn't get permission to use them. For the kinetics it is important to get the slaughterhouse information. Many studies showed that the slaughtering plant is an important station for contamination with, for example, Campylobacter, there are more carcasses contaminated than animals entering the plants (Hartog and De Boer, 1982; Izat e.a., 1988; Jones e.a., 1991a and b in Atanassova, 1999). We recommend making contact with the slaughterhouses before the start of the study.

5.4: Influence of open-air run

Question:
Broilers get access to the open-air run at the age of, proximately, 35 days, is this of influence on the contamination with Salmonella or/and Campylobacter? (only if broilers get access to the open-air run in this period!!)

In the period we took the samples, the broilers didn't get access to the open-air run. To investigate this factor you have to sample in periods with temperatures above 15 degrees.

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