The occurrence of Campylobacter spp. in broiler flocks in Patio and conventional housing systems in the Netherlands

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Abstract

Background: Campylobacteriosis is the most frequently reported foodborne disease in the EU since 2005. Monitoring at Dutch slaughterhouses revealed that 44.5% of broiler flocks tested positive for *Campylobacter* spp. Because approximately 80% of human campylobacteriosis cases are related to strains from the chicken reservoir, reducing the number of *Campylobacter* colonized flocks will have a significant contribution to public health.

Aim of study: The aim of this study was to compare two broiler housing systems, the Patio system (Vencomatic Group, Eersel, the Netherlands) and conventional poultry floor housing systems, for the presence of *Campylobacter* spp. The hypothesis was that introduction of *Campylobacter* is less likely to occur in Patio systems than in conventional broiler housing systems, because of specific differences in biosecurity regarding the number of insects and intensity of human traffic, especially around thinning of the flock.

Materials and methods: Four Patio systems were matched to four control houses and progeny of the same broiler breeder flock was placed in both housing systems at approximately the same time. A questionnaire with specific *Campylobacter* related biosecurity questions (CAMPAS checklist) filled out by the farmer was used to assess the level of biosecurity on all farms. Two rounds of sampling of cecal droppings were performed, around day 28, before partial thinning and day 38, after partial thinning. Sticky flytraps were placed in all broiler houses for 14 days to evaluate the number of insects.

Results and discussion: The CAMPAS checklist revealed that biosecurity risk levels were scored lower by farmers of Patio systems (on average 4.0) than by farmers of conventional broiler houses (10.7). Broiler flocks on all farms tested negative for the presence of *Campylobacter* during the first round of sampling. At the second round of sampling, all four Patio systems tested negative, whereas one out of four control houses was *Campylobacter jejuni* positive. Although the difference in the proportion of positive houses of 0.25 (25%) between both housing systems was statistically significant, the actual difference in *Campylobacter* prevalence for Patio systems versus conventional broiler houses may range between 0.6% and 80.6% at 95% confidence level. Consequently, more flocks need to be sampled to accurately compare prevalence in both housing systems. Insects were present in both types of housing systems. Category 3 insects (with sizes between 5-10 mm), that may comprise flies that are known to be able to transfer *Campylobacter* to the broiler flock, were present in both housing systems. The mean differences in insects caught between the housing systems in both weeks and the number of category 3 insects were not statistically significant. Therefore it cannot be concluded that there is an actual difference between the housing systems in terms of the number of insects. In the past years the relationship between biosecurity and the introduction of *Campylobacter* in broiler flocks has been well established. However, it is also recognized that biosecurity alone cannot ensure *Campylobacter* negative flocks. Therefore studies into complementary measures to reduce colonization of broilers with *Campylobacter* should be conducted. Further research with regard to the occurrence of *Campylobacter* in Patio systems and conventional broiler housing systems is recommended.

Introduction

Poultry meat is an important protein source worldwide. The average consumption in the European Union (EU) in 2015 was 22.5 kg/capita and 22.1 kg/capita for the Netherlands. Unlike red meat, poultry meat consumption is expected to increase reaching 22.8 kg/capita by 2025, due to its affordability and healthy image (AVEC, 2016). Poultry meat is also considered to be a potential source of various biological hazards that threaten human public health. In a qualitative risk assessment *Campylobacter* spp., *Salmonella* spp. and Extended-Spectrum Beta-Lactamase/AmpC gene-carrying bacteria have been identified as the most relevant hazards (EFSA, 2012). Campylobacteriosis is the most frequently reported foodborne disease in the EU since 2005 (EFSA, 2015).

Human Campylobacteriosis

Each year over 190,000 laboratory confirmed cases of human campylobacteriosis are reported in the EU. Because not all cases of *Campylobacter* foodborne infections are thought to be reported, the actual number of cases is estimated around nine million in the EU only (EFSA, 2017; EFSA, 2015). In the Netherlands, the number of human campylobacteriosis was estimated to be 83,300 cases in 2015 (Uiterwijk *et al.*, 2015). Annual costs of *Campylobacter* infections in the Netherlands are estimated at €21-36 million, due to direct health care costs and indirect non-health care costs, defined as the value of production lost to society (Mangen *et al.*, 2007).

Campylobacter causes a self-limiting gastroenteritis with watery to bloody diarrhoea, abdominal pain, nausea and fever in humans (Wagenaar *et al.*, 2015). Several sequelae can complicate the disease. In 0.1% of the cases complicating demyelinating disorders, named Guillain-Barré Syndrome and Miller-Fisher Syndrome, can occur, leading to progressive paralysis and even death. Other chronic sequelae linked to gastrointestinal infection with *Campylobacter* are reactive arthritis, post-infectious irritable bowel syndrome and inflammatory bowel disease (Havelaar *et al.,* 2012; Haagsma *et al.*, 2010; Wakerley *et al.*, 2016).

The majority of human infections originate from chicken products, through eating undercooked chicken meat or other cross-contaminated food products (Doorduyn *et al.*, 2010). *Campylobacter jejuni* is the predominant species causing human campylobacteriosis, followed by *Campylobacter coli* (Mughini-Gras *et al.*, 2012). International travel, environmental sources, such as recreational waters, and direct contact with farm animals are also significant risk factors for human infection with *Campylobacter* spp. (Domingues *et al.*, 2012).

Campylobacter spp. and broilers

Campylobacter, the name literally means 'curved rod', is a gram-negative, microaerophilic bacterium that lives in the digestive tract of birds and mammals (Bolton, 2015). Experimental inoculation has demonstrated that chickens are highly susceptible to colonization with *Campylobacter* (Cawthraw *et al.*, 1996). The thermophilic character of *C. jejuni* and *C. coli* in combination with the avian body temperature of 41-42 °C makes birds preferred hosts for these organisms (Wagenaar *et al.*, 2015). The mucus layer of cecal crypts is the predominant colonization site. Colonization does not lead to any clinical signs and therefore the organism is considered to be part of the normal enteric flora. Colonized broilers generally carry 10^6-10^8 cfu/g *C. jejuni* in their ceca (Hermans *et al.*, 2012; Sahin *et al.*, 2002). Once *Campylobacter* is introduced in a broiler flock, transmission is rapid: in a flock of 20,000 broilers, the within-flock prevalence of *C. jejuni* increases to 95% within 4.4 to 7.2 days (van Gerwe *et al.*, 2009).

During a field-study in the Netherlands a typical pattern was discovered: colonization is detectable in broiler flocks from 3-4 weeks of age and *Campylobacter* stays present up to slaughter in all colonized flocks (Jacobs-Reitsma *et al.*, 1995). A similar pattern of rising percentages of *Campylobacter* positive flocks with increasing age has been described in several other articles (van de Giessen *et al.*, 2006; Hermans *et al.*, 2012; Bull *et al.*, 2006). There is conflicting evidence about the role of vertical transmission in the epidemiology of *Campylobacter* spp.

Although the prevailing view is that vertical transmission does not play a major role in the introduction of *Campylobacter* to broilers, its role is not absolutely excluded (Sahin *et al.,* 2015, Agunos *et al.*, 2014). Multiple hypotheses about the inability to culture *Campylobacter* in chickens under two weeks of age have been posed. The best accepted hypothesis is that this so-called lag phase is caused by maternal antibodies which prevent *Campylobacter* from colonizing the intestinal tract (Sahin *et al.*, 2003; Newell *et al.*, 2003).

On-farm risk factors for the introduction of *Campylobacter* spp. are divers: insects, human traffic, other livestock adjacent to the farm, poor biosecurity, free-range or organic housing systems, partial depopulation and various other factors are thought to play a role. Table 1 in annex 1 provides an overview of these risk factors, according to numerous studies. Food and water are assumed to play a role in the horizontal spread of *Campylobacter* after introduction in the broiler flock (Tangkham *et al.*, 2016).

Campylobacter spp. and broiler meat

Risk assessments have revealed that about 50-80% of campylobacteriosis cases in humans are related to strains from the chicken reservoir but that and handling, preparation and consumption of broiler meat accounts for 20-30% of the human cases (EFSA, 2011). The level of contamination on the exterior of the chicken carcass and in the intestine directly influences the level of bacteria on the final product for the consumers (Northcutt *et al.*, 2003; Berrang *et al.*, 2004; Pacholewicz *et al.*, 2015). Large numbers of *Campylobacter* may contaminate poultry carcasses when intestines leak or rupture during processing in the slaughter house (Berrang *et al.*, 2001; Seliwiorstow *et al.*, 2015). A 2 log reduction of *Campylobacter* on chicken carcasses could lead to 30 times reduced incidences of campylobacteriosis associated with the consumption of chicken meat (Rosenquist *et al.*, 2003). This reduction could be achieved with physical or chemical processing of poultry meat after slaughter, because *Campylobacter* is sensitive to many environmental stresses. However, irradiation procedures and the treatment of carcasses with chemical substances is poorly accepted by consumers (Wagenaar *et al.*, 2015). Moreover, many of these strategies are unattractive to meat processors from both a logistic and economical point of view (Havelaar *et al.*, 2007). On 1 January 2018 EU regulations on *Campylobacter* monitoring in slaughterhouses will be implemented. Already since 1 March 2014 all Dutch slaughterhouses voluntarily monitor their meat processing with Process Hygiene Conventional (NEPLUVI, 2017). In order to manage *Campylobacter* levels on poultry meat, a comprehensive approach is required, involving broiler farms and slaughterhouses. Clearly, it is important to reduce the number of contaminated incoming flocks at the slaughterhouse. Because *Campylobacter* spreads rapidly through a flock after introduction, conventional measures should be targeted at the risk of introduction on the broiler farm. In addition to conventional measures for the reduction of contaminated meat, kitchen hygiene also plays an important role in the prevention of human campylobacteriosis.

Hypotheses

The aim of this study is to compare two broiler housing systems: the Patio system (Vencomatic Group, Eersel, the Netherlands) and conventional poultry floor housing systems, for the presence of *Campylobacter* spp. The hypothesis is that the introduction of *Campylobacter* is less likely in Patio systems than in conventional broiler housing systems, because of specific differences in biosecurity. It is known that biosecurity plays a crucial role in the introduction of *Campylobacter* on broilers farms (Agunos *et al.*, 2014; Newell *et al.*, 2011). Flies and other insects are known to introduce *Campylobacter* in broiler flocks (Hald *et al.*, 2004; Hald *et al.*, 2008; Bahrndorff *et al.*, 2013; Royden *et al.,* 2016). It is hypothesized that flies and other insects are less likely to enter Patio systems than conventional broiler houses. This would be due to the relatively closed ventilation system in a Patio system in comparison with conventional housing systems (van de Ven *et al.*, 2009). In addition to this, insects are also less likely to enter the Patio system during thinning activities, because during this process there is almost no air contact between the outdoor environment and the inside of the broiler house. In conventional poultry houses a large opening is necessary during thinning activities, often the size of an overhead door, and therefore flies are more likely to enter these poultry houses.

Human traffic on broiler farms is another possible route for the introduction of *Campylobacter,* because *Campylobacter* can be brought into the poultry house from the external environment, for instance through footwear (Evans and Sayers, 2000; Newell *et al.*, 2011). Thinning a flock is a significant risk factor for the introduction of *Campylobacter,* because potentially contaminated materials, clothes and transport crates from catching crews are brought into the poultry houses (Allen *et al.*, 2008; Hald *et al.*, 2001; Hue *et al.*, 2010; Smith *et al.*, 2016). In Patio systems farm workers, catching crews and transport crates cannot enter the chicken habitat and litter physically. It is therefore hypothesized that introduction of *Campylobacter* in Patio systems is less likely to occur than in conventional broiler housing systems.

Motivation

The Vencomatic group (Eersel, the Netherlands) is specialized in poultry housing, egg handling and climate for any type of poultry house. Their most important goals are to improve efficiency, profitability of poultry production and sustainability. One of their initiatives to help achieve those goals is to contribute in finding a solution for the public health issue of *Campylobacter* infections on broiler farm level. The Patio system is designed to house broilers in a system that ensures high animal welfare and biosecurity levels. The system is constructed into multiple compartments and each compartment consists of two rows of six identical levels (Patio units) on top of each other. The Patio system combines a brooding phase with on-farm hatching, and therefore eggs are placed in the system at day 18 of the brooding phase. The broiler habitat comprises one Patio unit, with dimensions of 90 m (length) x 2.34 m (width) x 0.75 m (height) on average. Because of these dimensions a Patio unit cannot be entered by humans, only manually from the sides. Chicks are housed on wood shavings or pelletised straw covering the floor of the Patio units, which are synthetic Patio belts. The conveyer belts are used to depopulate the Patio units, automatically separating the manure from the broilers (van de Ven *et al.*, 2009).

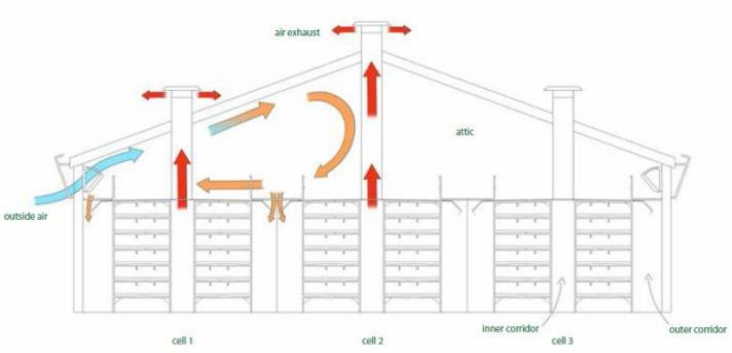


Figure 1: The Patio system consists of multiple compartments (cells)

Figure 2: One compartment consists of two rows of six Patio units (levels) on top of each other

Figure 3: The broiler living habitat

Materials and methods

Study design

A longitudinal cohort study on presence of *Campylobacter* spp. in two different broiler housing systems was conducted from May to June 2017 in the Netherlands. In the Netherlands 629 broiler farms were operational in 2016 (Agrimatie, vleeskuikens, 2016). The majority of these farms were conventional poultry houses and only six Patio systems were operational. For this study, 4 Patio systems and 4 conventional houses were selected. The selection of Patio systems was based on their cycle and suitability for visiting and taking samples in the months May and June. The selection criteria for the conventional houses were that they were not free-range or organic, not used on-farm hatching and that they performed partial thinning of the flock at least once during the cycle. The farms were situated in 4 different provinces of the Netherlands: 1 Patio system and 1 conventional broiler house in Zeeland, the other 3 Patio systems in Noord-Holland, 2 conventional broiler houses in Noord-Brabant and 1 conventional broiler house in Drenthe. The average number of broilers in the conventional houses was 33,365, which is 11,635 broilers less than the average number of broilers housed per poultry house in the Netherlands, namely 45,000 (KWIN, 2016). In the Patio system, the average number of broilers was 157,750 per system and 52,583 per compartment.

The Patio systems and conventional houses were matched to form 4 pairs in total: Patio 1-conventional 1, Patio 2-conventional 2, Patio 3-conventional 3 and Patio 4-conventional 4. In both housing systems of one pair, progeny from the same broiler breeder flock and from the same hatchery was placed at approximately the same time. Although it is assumed that vertical transmission does not play a major role in the epidemiology of *Campylobacter*, using chickens from the same parent stock rules out two confounders, namely the differences between both housing systems in terms of genotypic variation between chickens and possible vertical transmission of *Campylobacter*. For the Patio system day 0 refers to day of hatch and for conventional broiler houses day 0 was the day one-day-old chicks arrived at the farm. The difference between the start of two matching cycles of one pair, was 6 days on average (range 3-13 days).

Farms were visited 3 times during one production round: on about 21 days, 28 and 38 days. An overview of the exact days and dates farms were visited is provided in table 1. There is a variation in days because farms were not visited during the weekends or national holidays and because day of slaughter varied between farms.

Table 1: An overview of days and dates farms were visited

	Visit 1		**Visit 2**		**Visit 3**	
Activities	Place flytraps in house Fill out CAMPAS checklist		Replace flytraps in house Sample collection round 1		Collect removed flytraps Sample collection round 2	
Housing system						
Patio 1	Day 21	26-05-2017	Day 28	02-06-2017	Day 38	12-06-2017
Conventional 1	Day 21	23-05-2017	Day 28	30-05-2017	Day 37	09-06-2017
Patio 2	Day 21	26-05-2017	Day 28	02-06-2017	Day 38	12-06-2017
Conventional 2	Day 21	01-06-2017	Day 28	08-06-2017	Day 36	16-06-2017
Patio 3	Day 21	01-05-2017	Day 28	08-05-2017	Day 39	19-05-2017
Conventional 3	Day 21	04-05-2017	Day 28	11-05-2017	Day 35	18-05-2017
Patio 4	Day 19	03-05-2017	Day 26	10-05-2017	Day 38	22-05-2017
Conventional 4	Day 20	17-05-2017	Day 27	24-05-2017	Day 40	06-06-2017

Literature research

Literature research was done to determine important on-farm risk factors for the introduction of *Campylobacter* in broiler flocks. CAB Abstracts and Google scholar were used for this research and searching terms were 'on-farm risk factors, *Campylobacter,* broilers' and other specific searching terms referring to risk factors directly, such as 'partial depopulation, broilers, *Campylobacter*'. Hypotheses were formulated and the study design was determined using both the knowledge about risk factors and about Patio systems.

CAMPAS

The CAMPAS checklist was developed by Wageningen University and Research to inventory relevant risk factors regarding the introduction of *Campylobacter* on poultry farms in a structured and reproducible way (CAMPAS, 2017). In this study the CAMPAS checklist was used both to define the level of biosecurity and to gain insight in the exact risk factors present on the farms. Farmers filled out the CAMPAS checklists themselves. Risk factors were arranged in five different categories, which were *farm site*, *farm hygiene*, *stable hygiene*, *materials and vehicles* and *pest conventional*. For each category, a certain score was assigned to the farm, based on the number of risk factors. The range of scores per category is 0.0 to 6.0 and in total 30 points can be acquired. A lower score is indicative for better biosecurity on the farm. This CAMPAS checklist can be found in annex 2.

Sample collection

1. Swabs of cecal droppings

Swabs of cecal droppings were collected at day 28 (range 26-28) before partial thinning and at day 38 (range 35-40) after partial thinning on all participating farms. The second round of sampling was performed about 7 days (range 5-8) after the partial thinning. As stated before, thinning may affect the presence of *Campylobacter* because of the involved risk of introduction. Samples were taken before transport to the slaughterhouse and not at the slaughterhouse, because of the risk of cross-contamination and potentially false positive results (Herman *et al.*, 2003).

The average number of broilers in a conventional broiler system in the Netherlands is 45,000 and in the Patio system approximately 50,000 broilers are housed per compartment (KWIN, 2016). Sample size calculation, performed with the website Ausvet, Epitools, indicated that one set of seven pools was needed to be able to detect at least 10% *Campylobacter* positive animals at a 95% confidence level in a population of 45,000-50,000 animals. One COPAN Transystem® dry swab (COPAN, Btescia, Italy) was used for every pool and the swab itself was inserted successively and directly into five fresh cecal droppings. The Amies Agar Gel Medium transport tubes were used for storage of each individual pool. The pools were stored at room temperature during transport to the laboratory. In the conventional broiler houses, the samples were taken randomly through the houses in such a way that the front, middle and back of the house were covered and no cecal dropping was sampled two times. In the Patio systems, one pool was taken from five cecal droppings distributed over the length of one Patio unit (level). In one Patio compartment, the first 3 pools were taken from level 1, 3 and 5 from one row and the next 4 pools from levels 1, 2, 4 and 6 of the other row. The number of pools taken per sample collection round per housing system are displayed in annex 3.

The diagnostic test for the presence of *Campylobacter* is derived from the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (OIE, 2017). Bacterial analysis was performed in the laboratory for Clinical Infectiology at the Faculty of Veterinary Medicine, University of Utrecht. Test specificity is 100% and the sensitivity is known to be high, but the exact percentage is unknown. It is estimated that sensitivity of the test is 90% (personal communication prof. dr. J.A. Wagenaar).

In the laboratory each swab representing one pool of five cecal samplings was streaked directly onto Charcoal Cefoperazone Deoxycholate (CCD) blood-free selective agar (Oxoid, Basingstoke, UK). These CCDA plates were incubated at 42 °C during 48 hours in a microaerobic atmosphere (6% O_2, 6% CO_2, 4% H_2 in N_2) and assessed afterwards by experienced laboratory personnel. Characteristic *Campylobacter* colonies were gram-stained and examined by microscope for typical spiral-shaped, gram-negative bacteria. When bacteria matched the previous description, the original colony was transferred from the CCDA plate onto a Columbia agar with sheep blood plus plate (Oxoid, Basingstoke, UK) and incubated for 48 hours. MALDI-TOF was then used to confirm the presence of *Campylobacter* on the plate and to identify the subtype.

2. Flytraps

In order to catch flies and insects in both housing systems, sticky sheets from the Silvalure system® were applied and replaced weekly, as described in Hald *et al.*, 2008. The size of all sheets was 20 x 30 cm (see photo 1). Flytraps were placed in all housing systems for exactly 14 days during one round: they were placed in the houses at about 21 days, replaced at 28 days and removed at 35 days.

Photo 1: Example of a flytrap

The number of sheets was estimated based on the surface of the broiler living area in both housing systems. This included all area chickens themselves have entrance to and excluded other spaces, such as the ante room. Exact data about these surfaces per farm was not yet available before the study design was determined and therefore an average was calculated. The average broiler living area in one Patio compartment was 2530 m^2, based on the dimensions of the 4 Patio systems participating in this research and 2250 m^2 in an average conventional housing system in the Netherlands (KWIN, 2016). Nine sheets were placed in conventional broiler houses and ten sheets per Patio compartment, since the living area of broilers in Patio systems was on average 1.12 times larger. The exact dimensions of the broiler living areas were known after all farms had been visited. A correction factor was used to calculate the number of caught insects, as shown in table 2, based on the assumption that the number of insects was equally distributed throughout the houses.

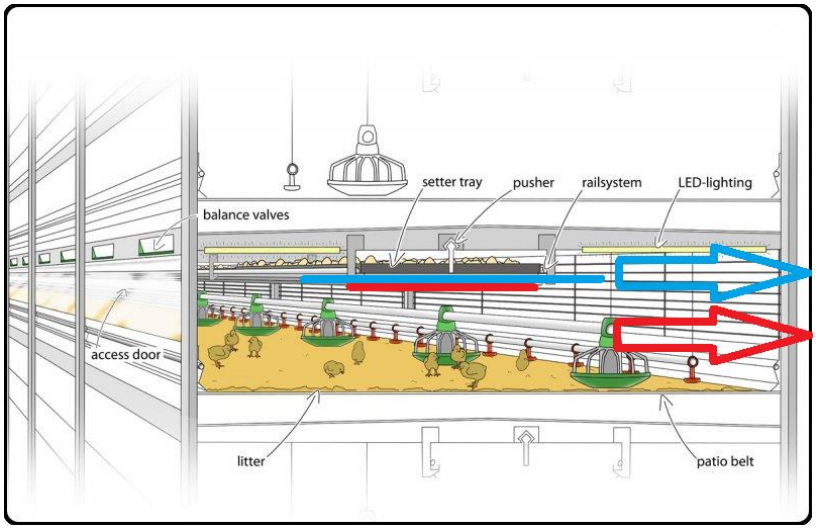
Table 2: Details used for the calculation of the flytrap correction factor per farm

Farm	Surface living area (per compartment) in m^2	Number of flytraps placed	m^2 sampled per flytrap	Correction factor
Patio 1	2571	10	257.1	1.18
Patio 2	2571	10	257.1	1.18
Patio 3	2185	10	218.5	1.00
Patio 4	3000	10	300.0	1.37
Conventional 1	1443	9	160.3	0.73
Conventional 2	1836	9	204.0	0.93
Conventional 3	1474	9	163.8	0.75
Conventional 4	1000	9	111.1	0.51
Average	**2073.5**		**218.3**	

The flytraps were positioned in the houses avoiding places with (high) air velocity, since true flies (order Diptera) are not likely to be in such places (personal communication H.H. Ellen and J.W.M. van Schip). In conventional housing systems nine traps were equally distributed within the houses: 3 sheets were placed in the forward half of the house, evenly distributed over the width of it. 3 sheets were placed in the middle and 3 sheets in the back end of the house, in the exact same way, at about 50 cm above the floor. Photo 2 shows how the flytraps were attached to the feed-or drinking lines inside these houses.

In the Patio housing systems two different methods were used for the positioning of the flytraps inside the houses. In the Patio systems 3 and 4 flytraps attached to metal meshes were placed onto the rails of the setter trays inside the Patio units, which was also the chicken habitat. Figure 1 and photo 3 visualise this method. With this method, flytraps were hanging approximately 50 cm above the floor.

Photo 2: Example of a flytraps placed in a conventional broiler house



Metal mesh

Attached flytrap

Figure 4: Schematic view of a metal mesh with flytrap placed inside a Patio unit

Photo 3: Example of a metal mesh with flytrap placed inside a Patio unit

The system of placing metal meshes with flytraps on the setter trays, appeared to be not robust enough: many traps were separated from the metal meshes and could not be retrieved. It was therefore decided to use a different method in Patio system 1 and 2. In these systems the flytraps were tacked onto the system in the inner corridor, between the two rows of an compartment and at different levels, as shown in photo 4. An overview of the locations and number of flytraps placed in both housing systems is given in annex 4.

Photo 4: Example of a flytrap attached to the Patio system in the compartment inner corridor

In addition to the sheets in the living area of the broilers, in the poultry house, additional sheets were placed in variable locations expected to be possible entrance locations for insects on the farm. These locations included the hygiene barrier, close to the overhead door and close to the ventilation fans. Data from these flytraps were not included in the comparison of the influx of insects between the housing systems, because they could not be standardised.

The sticky side of all individual collected traps was covered with cling film, in order to protect the traps from clinging together. The collected traps were taken to the lab on the Faculty of Veterinary Medicine, University Utrecht and stored in a dry place at room temperature. Farmers removed the flytraps themselves exactly seven days after the second visit, i.e. around day 35. Again, traps were individually covered with cling film and stored by the farmer on a dry place at room temperature. The removed traps were taken to the same laboratory in Utrecht during the last visit.

After collection of all flytraps from all housing systems, the number of caught insects was quantified. Determination of caught insects was not possible because that requires a detailed study of the morphology of the insects, which was impossible due to the inability to detach the insects from the sticky sheets. The insects were quantified and subdivided into one of five categories, based on its size from its head (antennas excluded) to the terminal abdominal segment. The categories are catalogued in table 3. The categories were used to obtain a general impression of the -potential- types of insects and flies present on the traps. For instance: houseflies (*Musca domestica*), which are known for their ability to introduce *Campylobacter* in broiler flocks, are about 7 mm long and would therefore belong to category 3 in this study (Smallegange and Den Otter, 2007).

Table 3: Method used to assess caught insects on flytraps

Category	Length of head to terminal abdominal segment in mm
1	<2,5
2	2,5-5
3	5-10
4	10-15
5	>15

Statistical analysis

1. Swabs of cecal droppings

The bacteriological analysis of the swabs (pools) resulted in either a positive or negative result per housing system per sampling round. For statistical analysis for each sampling round two-way contingency tables of observed frequencies was made. McNemar's test was used to test the null hypothesis that the true proportions of *Campylobacter* positive housing systems were equal. The McNemar's test and 95% confidence interval were calculated. The result was considered statistical significant if the *p*-value is <0.05.

2. Flytraps

Several assumptions were made with regard to the flytraps. First of all, the number of insects on the unusable or lost flytraps were assumed to be equal to the average of the flytraps analysed for that housings system in the same week. The number of insects was corrected for the true surface of the broiler living areas per housing system, as shown in table 2, assuming that insects were equally distributed over these surfaces in all houses. Finally, the assumption was made that both methods (1 and 2) for catching insects in Patio systems were comparable. For the statistical analysis of the flytrap data an independent *t*-test with bootstrapping was performed in IBM SPSS 24 on the number of insects per housing system per week, the numbers of insects per farm per week, the number of category 3 insects/flies per housing system per week and the difference between the number of insects on flytraps and category 3 insects for both types of housing system between the two weeks. Bootstrapping was performed because the data was not expected to be normally distributed.

Results

Literature research

Horizontal transmission plays the most important role in the epidemiology of *Campylobacter* spp. in broiler flocks. Various risk factors for the introduction of these bacteria are known. Annex 1 provides an overview of risk factors.

CAMPAS

Table 4 and diagram 1 display the scores of the CAMPAS checklist per farm. Patio 4 had the lowest CAMPAS score: 1.6 and conventional house 1 had the highest CAMPAS score: 13.5. According to these results, biosecurity levels were scored higher by farmers of Patio systems than by farmers of conventional broiler houses: the average Patio system score was 4.0 and conventional house score was 10.7. *Farm hygiene*, *materials and vehicles* and *pest control* were the categories that particularly determined the differences between both housing systems.

Table 4: Schematic view of the CAMPAS scores per farm

Farm	1 Farm site	2 Farm hygiene	3 Stable hygiene	4 Materials and vehicles	5 Pest conventional	Total score
Patio 1	0.0	2.2	0.5	0.4	1.3	**4.4**
Patio 2	0.0	2.2	0.5	0.4	1.3	**4.4**
Patio 3	2.6	1.7	0.5	0.0	0.9	**5.7**
Patio 4	1.0	0.6	0	0.0	0.0	**1.6**
Conventional 1	2.6	3.3	3.0	2.0	2.6	**13.5**
Conventional 2	2.2	3.3	1.5	1.5	1.7	**10.2**
Conventional 3	1.9	2.8	2.3	1.1	2.6	**10.7**
Conventional 4	1.9	2.8	0.7	1.1	1.7	**8.2**

Diagram 1 provides a schematic view of the CAMPAS scores per individual farm. The most important finding was that the level of biosecurity in Patios systems was generally considered better than in conventional housing systems.

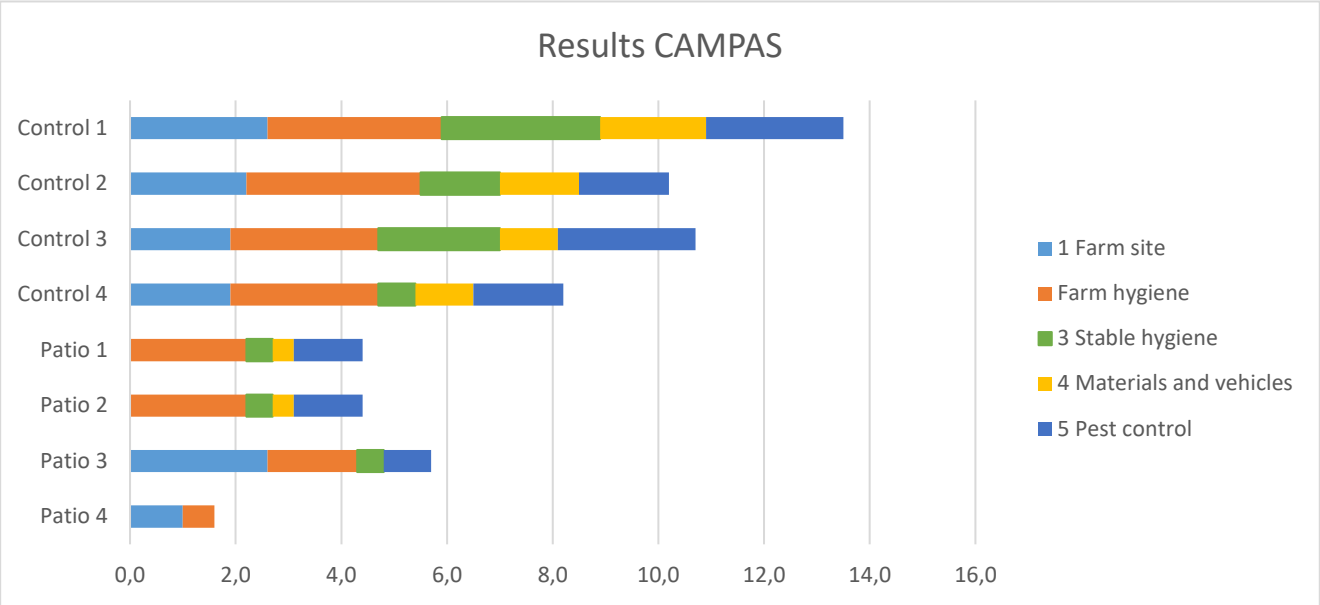


Diagram 1: CAMPAS scores per farm per category

Sample collection

1. Swabs of cecal droppings

Table 5 displays the results of the first round of sampling. Broiler flocks in all housing systems tested negative for the presence of *Campylobacter*. No statistical analysis was performed on data from the first round of sampling, because all samples were negative for *Campylobacter*.

Table 5: Presence of Campylobacter per matched pair of Patio and control stables in the first round of sampling

Housing system		**Conventional house**		**Total no. pairs**
		Positive	Negative	
Patio system	Positive	0	0	0
	Negative	0	4	4
Total no pairs.				4

Table 6 displays the results of the second round of sampling. Broiler flocks in all Patio systems tested negative for the presence of *Campylobacter.* Three conventional houses tested negative and conventional house 1 tested positive: *Campylobacter jejuni* colonies were present in all seven tested pools. Between the two housing systems the proportion of positive outcomes was compared. For Patio zero out of four systems was positive, whereas one out of four control stables was positive, which results in a difference in the proportion of positive stables of 0.25 (25%) between both housing systems. This difference in farm prevalence was statistically significant with a 95% confidence interval for this proportion difference of 0.006-0.806. This indicates that we can be 95% confident that the actual difference in *Campylobacte*r prevalence for Patio systems versus conventional broiler houses ranged between 0.6% and 80.6%, which implies that the reliability of the data is very low.

Table 6: Presence of Campylobacter per matched pair of Patio and control stables in the second round of sampling

Housing system		Conventional house		Total no. pairs
		Positive	Negative	
Patio system	Positive	0	0	0
	Negative	1	3	4
Total no. pairs				4

2. Flytraps

The average number of insects is displayed in diagram 2 for the first week (t=1) and second week of measurement (t=2) for both housing systems. Apparently, insects emerged in both housing systems, but not to the same extent. In control stables in total more insects were caught than in Patio systems. In the first week the average number of insects caught in control stables was 69.4 and 33.2 in Patio systems. In the second week the average number of insects caught in control stables was 107.0 and 42.7 in Patio systems. In the second week the number of caught insects had increased in both housing systems and this increase was larger in the control stables.

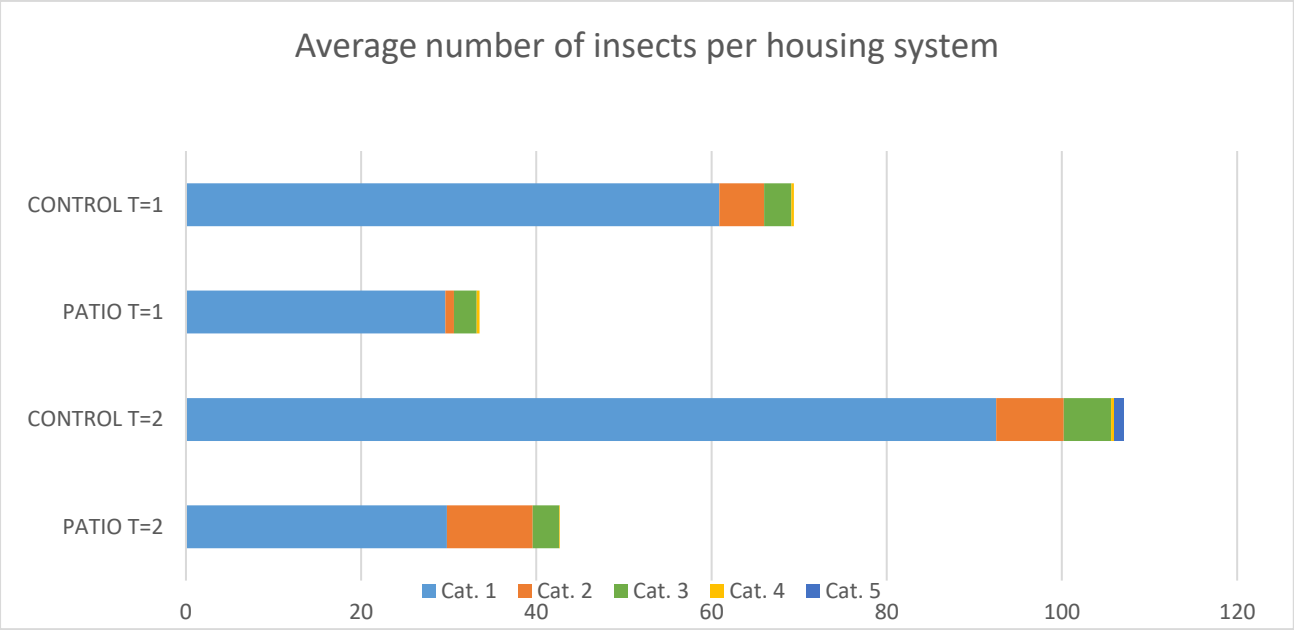


Diagram 2: Average number of insects per control stable or Patio compartment in the first week (t=1) and the second week (t=2)

An independent *t*-test with bootstrapping was performed in SPSS 24 on the data from the flytraps. The total number of insects on the traps in both weeks was corrected for the surface covered by one flytrap on all individual farms, as described in the materials and methods section. Table 7 displays the results of the statistical analysis in SPSS. The mean difference between both housing systems was 35.5 flies in the first week (t=1) and 63.3 in the second week (t=2). The difference between the two weeks was an increase of 28.9 flies from the first to the second week for both housing systems together. This increase was visible in both housing systems from the first to the second week. These mean differences and the difference in number of insects between both weeks were not statistically significant. Therefore it cannot be concluded that there is an actual difference between the number of insects between both housing systems and between the two weeks of measurement in both housing systems together.

Table 7: Results independent t-test with bootstrapping in SPSS 24 total number of insects

Moment	Mean difference in number of insects between housing systems	*p*-value	95% confidence interval
t=1	35.5 insects	0.120	3.7 to 66.4 flies
t=2	63.3 insects	0.158	5.1 to 134.3 flies
Difference between t=1 and t=2	28.9 insects	0.416	0.5 to 72.4 flies

The number of insects caught per individual farm showed quite some variation. Diagram 3 shows the number of insects caught per control stable in both weeks and diagram 4 shows the same for all Patio systems. Note that the number of insects per Patio system represents the average number of insects per compartment.

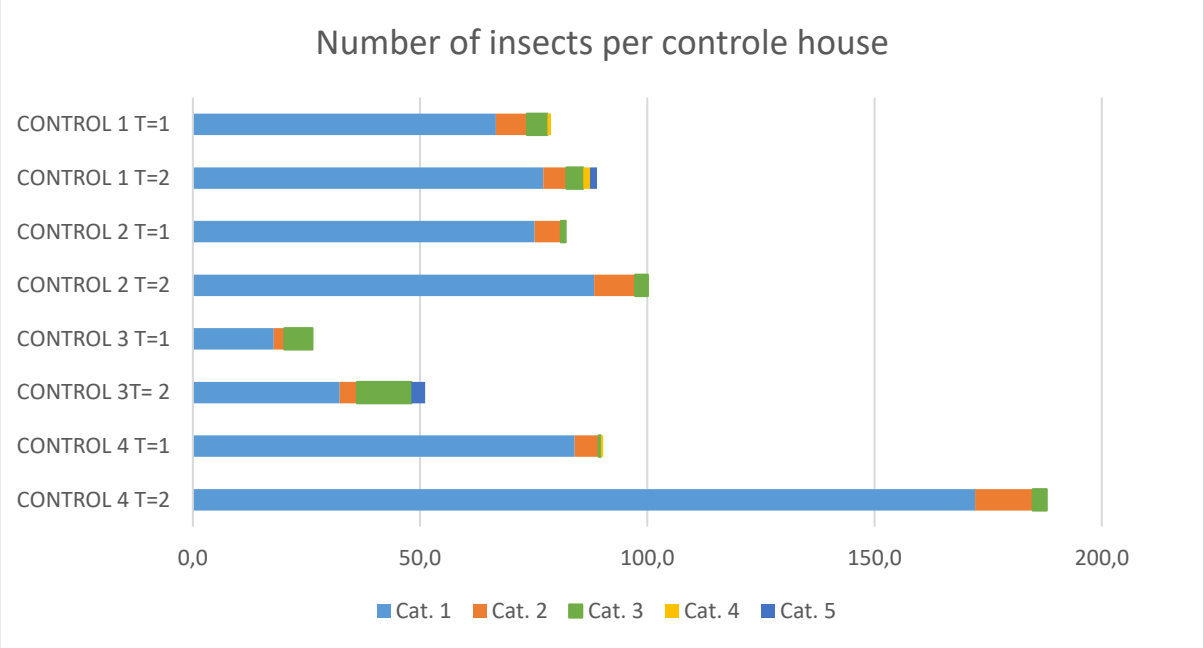


Diagram 3: Average number of insects per control stable in the first week (t=1) and the second week (t=2)

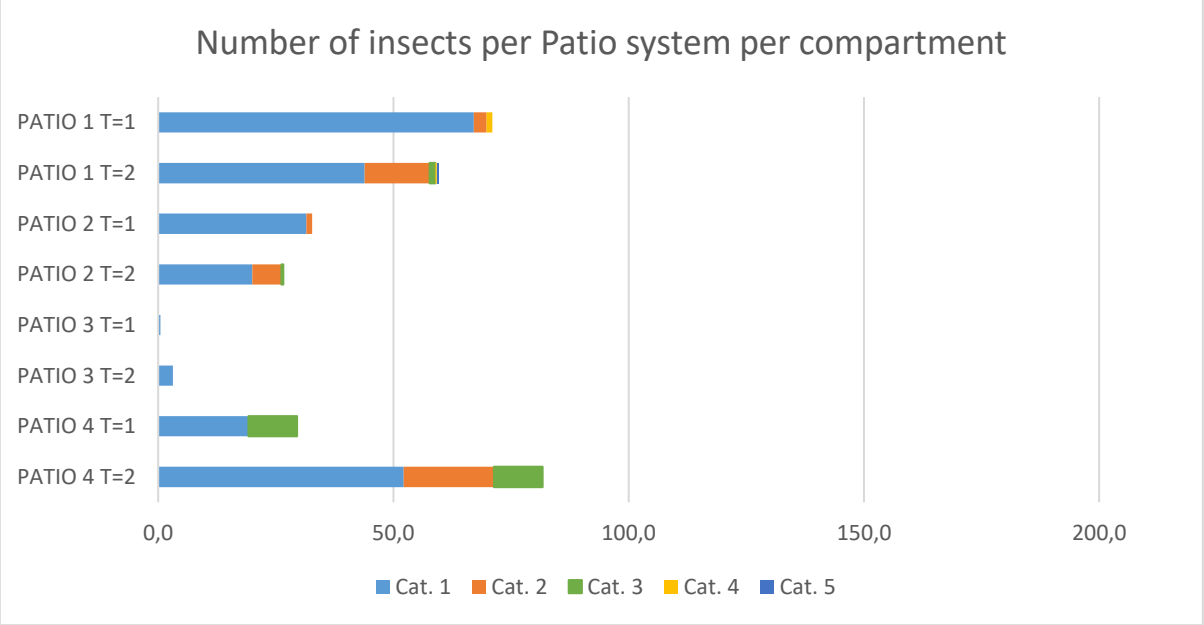


Diagram 4: Average number of insects per Patio system per compartment in the first week (t=1) and the second week (t=2)

Table 8 displays the average percentages of insects per category in both housing systems in both weeks of measurement. Category 1 insects represented the majority of insects caught. Category 3 insects were more present in Patio systems in both weeks than in control stables: 7.8% vs. 4.4% and 7.0% vs. 5.0%. Category 5 insects were only present in control stables during the second week of measurement. Note that no statistical analysis was performed on the data from table 8.

Table 8: Average percentage of insects per category in both housing systems in both weeks

Housing system	Week (t=x)	Cat. 1 0-2.5 mm	Cat. 2 2.5-5 mm	Cat. 3 5-10 mm	Cat. 4 10-15 mm	Cat. 5 >15 mm	Sum
Patio system	1	89.0	2.9	7.8	0.3	0.0	100.0
Control stable	1	87.9	7.3	4.4	0.5	0.0	100.0
Patio system	2	69.4	23.0	7.0	0.2	0.0	100.0
Control system	2	86.4	7.1	5.0	0.4	1.1	100.0

Category 3 insects were present in both housing systems and more often in Patio systems than control stables. Table 9 shows the results of the statistical analysis of category 3 insects in both housing systems. The mean difference in category 3 insects between both housing systems is 0.5 in the first week and 2.4 in the second week. The difference between both weeks is an increase of 2.0 insects in both housing systems together. This increase was visible in both housing systems from the first to the second week. These differences were not statistically significant. Therefore it cannot be concluded that there is an actual difference in category 3 insects between both types of housing systems.

Table 9: Results independent t-test with bootstrapping in SPSS 24 for category 3 insects

Moment	Mean difference category 3 insects between housing systems	*p*-value	95% confidence interval
t=1	0.5 insects	0.876	-4.8 to 6.0 flies
t=2	2.4 insects	0.559	-8.5 to 3.8 flies
Difference in cat. 3 insects between t=1 and t=2	2.0 insects	0.210	0.87 to 4.6 flies

Discussion

In this longitudinal cohort study, two housing systems were compared for the presence of *Campylobacter* spp. The hypothesis was that the introduction of *Campylobacter* spp. was less likely to occur in Patio systems than in conventional broiler housing systems, because of specific differences in biosecurity. This hypothesis was based on two underlying hypotheses. The first hypothesis was that the level of influx of insects in the Patio system was less than in conventional housing systems, due to a relatively closed ventilation system. Moreover, insects were also less likely to enter the Patio system during thinning activities. Secondly, it was thought that the introduction of *Campylobacter* in Patio systems was less likely to occur because farm workers, catching crews and transport crates cannot enter the chicken habitat and litter physically.

All pools in all 4 Patio stables and 4 conventional control stables, collected prior to partial thinning, around day 28, tested negative for the presence of *Campylobacter*. Around day 38, 5-8 days after partial thinning, all Patio systems were negative and one of the conventional broiler houses (control stable 1) tested positive. This result was statistically significant, but the actual difference in *Campylobacter* farm prevalence for conventional houses vs. Patio systems ranged between 0.6% and 80.6%, which implies that the reliability of the data is very low. A larger sample size or testing more consecutive broiler flocks on the farms is preferred to obtain more a reliable estimation of farm prevalence. Partial thinning was performed in both housing systems between the two rounds of sampling. It is known that partial thinning is a significant risk factor for the introduction of *Campylobacter* in the remaining broiler flock (Smith *et al.*, 2016, Hald *et al.*, 2000, Hue *et al.*, 2010). In the study of Allen *et al.* flocks became entirely positive within 2-6 days of the start of thinning and in this study 5-8 days passed between thinning activities and the second round of sampling, which could have enabled potentially introduced *Campylobacter* to spread within the broiler flock (Allen *et al.,* 2008). Future studies could focus on this moment of thinning by sampling the broiler flock more intensively, short before and short after thinning and taking samples of materials used, such as vehicles, clothing, footwear and transport crates of the catching crew. This would provide more insight as to in which extent partial thinning may be the explanation when a broiler flock tests positive for *Campylobacter.* Another known risk factor for introduction is human traffic. The fact that we visited the farms 3 times may also have posed a risk for introduction. However, strict biosecurity protocols were followed which makes this route of introduction less likely.

Vertical transmission as source of *Campylobacter* in conventional broiler house 1 is unlikely. Broilers in Patio system 1 originated from the same broiler breeder flock and hatchery, and remained *Campylobacter* negative. Furthermore, during the first sampling moment at day 28 conventional broiler house 1 tested negative. If vertical transmission would have played a role, it was expected that both broiler flocks would test positive at the same time, after maternal antibody titres would have decreased (Sahin *et al.,* 2003). Theoretically, the *Campylobacter* strains should correspond and be of the same genotype as the parent breeder flock. However, many studies reported that *Campylobacter* strains colonizing broiler flocks have different genotypes than their parent breeder flocks, which makes vertical transmission less likely than horizontal transmission from the environment (Sahin *et al.*, 2015). Patio system 1 could also have stayed negative because of antibiotic treatment. Antibiotics were administered to two broiler flocks of one pair. In Patio system 1 the antibiotic trimethoprim-sulfamethoxazole was administered from day 18-22 (Methoxasol-T©) and day 24-26 (Methoxasol 20/100©) and tylosin (Tylan© WO) from day 26-29 (CBG-MEB, 2017). The antibiotic trimethoprim-sulfamethoxazole is effective against both gram positive and gram negative bacteria and is also eliminated through the feces. Tylosin is mostly effective against gram positive bacteria and some gram negative bacteria, including *Campylobacter* spp. *Campylobacter* is most likely resistant to trimethoprim-sulfamethoxazole (both to the trimethoprim and sulpha component). However, *C. jejuni*, and to a lesser extend *C. coli*, are susceptible for macrolides including tylosin. This treatment could have influenced the results (Fliegelman *et al*., 1985, Osaili and Alaboudi, 2017).

In control stable 1 phenoxy-methylpenicillin (Phenoxypen© WSP) was administered from day 14-19. This small spectrum beta lactam antibiotic is used against gram positive bacteria and no effect from the administration of this product is expected on possible present *Campylobacter* spp. in the broiler flock.

On the positive control farm, *Campylobacter jejuni* was isolated from all pools. *Campylobacter jejuni* is known to be the most prevalent *Campylobacter* spp. in poultry (EFSA, 2010). It is also the subspecies responsible for 80% of human *Campylobacter*iosis cases in the European Union (EFSA, 2015). It should be noted that the test sensitivity of 90% was assumed to detect at least 10% *Campylobacter* positive broilers in a flock, but this sensitivity was not certain. Given a transmission rate of 2.37 ± 0.295 infections per colonized bird per day, 10% prevalence is accomplished very quickly. This transmission rate implies that in a flock of 20,000 broilers within-flock prevalence of *C. jejuni* would increases to 95% within 4.4 to 7.2 days after colonization of the first broiler (van Gerwe *et al.*, 2009). Rapid spread of *Campylobacter* in the broiler flock ensures the flock to be either entirely positive or negative at slaughter (Jacobs-Reitsma *et al.*, 1995, Wagenaar *et al.*, 2013). It is unknown if test sensitivity truly was 90%. If test sensitivity had been 80% one set of 7 pools sampling 5 cecal droppings per pool would have revealed a prevalence of 20%. The chance would still have been very high that we could have measured *Campylobacter* colonization of a broiler flock, even if the true test sensitivity had been lower.

It is noteworthy that control farm 1, which tested positive for *Campylobacter* at about 38 days, had the highest score in the CAMPAS checklist. However, although certain risk factors for the introduction of *Campylobacter* were applicable to control stable 1, such as drinking water from a well, other livestock adjacent to the broiler farm, multiple broiler houses on the premises, the presence of pets on the farm, etc., these factors were also present on several other farms that stayed negative. Regarding the CAMPAS score control stable 1 appeared to have the highest 'risk' of *Campylobacter* being introduced compared to the other farms, based on biosecurity. However, it is important to note that the CAMPAS checklist was filled in by the farmers themselves and that their individual interpretation of questions may have influenced the outcome. Also, it is known that not one biosecurity related factor predominates, but that improved biosecurity can decrease the risk of a flock becoming *Campylobacte*r-positive (Newell *et al.,* 2011). The CAMPAS results showed that Patio systems in general have better biosecurity levels than conventional ground stables, which strengthens the hypothesis that *Campylobacter* is less likely to occur in Patio systems.

The prevalence of *Campylobacter* positive flocks in this study, based on the results of one round of sampling and eight sampled flocks, is estimated to be 12.5%. This deviates from the average prevalence of *Campylobacter* positive flocks in the Netherlands. A weekly monitoring carried out from January to December 2016 in all 16 poultry slaughter houses in the Netherlands showed that 55.5% of the incoming flocks were not or very low shedding and 44.5% of the flocks was high shedding *Campylobacter* (>10.000 CFU/g cecal feces). The number of incoming colonized batches differed between slaughter houses with a range of 17 to 100% (NEPLUVI, 2017). The estimated prevalence in this study is not accurate, because only eight flocks were sampled and this number is insufficient to calculate a reliable average. In addition, the prevalence of *Campylobacter* shows strong seasonality in North European countries, with a gradual rise in spring and peak in July and August (Jore *et al.*, 2010, Ellis-Iversen *et al.*, 2009, Lawes *et al.*, 2012). In this study, all sampling rounds were performed in May and June. It is possible that *Campylobacter* prevalence will be higher in broiler flocks and the potential difference between the two housing systems more accentuated, when samples are taken during these peak months.

The population broilers used in this experiment was moderately representative for the population broilers in the Netherlands. To a certain extent, the selection of the farms with the two different housing types was random. CAMPAS revealed that the level of biosecurity differed between farms and in general between the two types of housing systems. The question is whether in this study the difference of *Campylobacter* between housing systems has been studied properly, given the fact that biosecurity levels of both housing systems and farms were not equal. In further research, it would be best to match biosecurity levels between farms to ensure that the housing system is the only explanatory variable of the difference in occurrence of *Campylobacter*.

It was hypothesized that flies and other insects were less likely to enter Patio systems than conventional broiler houses. This would be due to the relatively closed ventilation system. Insects were less likely to enter Patio systems during thinning activities, because there is almost no air contact between the outdoor environment and inside of the broiler house. This study showed that insects emerge in both housing systems, but not to the same extent. In control stables in total more insects were caught during both weeks of measurement than in Patio systems. In the second week the number of caught insects had increased in both housing systems. The earlier posed hypothesis could explain the difference in number of insects between both housing systems. It is possible that the amount of air contact between the outdoor environment and inside of the broiler house during thinning activities has had an effect on the increasing number of insects in the second week in all control stables. However, it is not known whether insects caught on the flytraps were insects that actually entered the broiler houses with the influx of ventilation air or that their habitat was already inside of the broiler house. Poultry houses are suitable living and breeding areas for houseflies (*Musca domestica;* Insecta: Diptera: Musciadae) because of the temperature, humidity and abundance of food (Mul *et al*., 2015). There are many other factors that may influence the number of insects in broiler stables, such as season and weather conditions, the number of flies in the outdoor environment of the stables and the flow of ventilation air (m^3/h) (Hansson *et al.*, 2007, Hald *et al.,* 2008). In this study, no correction for the difference of these factors between farms was performed. In all 4 control stables no insect/fly control measures were taken. In the ante rooms Patio system 1 and 2 electric flytraps with blue light were used and in Patio system 4 chemical insect repellent was used in the ante room and office. In Patio system 3 no insect control measures were taken. The use of anti-insect control measures could have influenced the number of insects caught in both housing systems, although in Patio system 3 the lowest number of insects was caught in comparison with the other Patio systems. The occurrence of certain risk factors on farms, such as insects or pests could of course directly influence the motivation of farmers to take such control measures. This may explain the absence of both control measures against insects/flies and the low number of insects caught in Patio system 3.

It has not been studied whether the insects caught in this study were *Campylobacter* positive or negative. To study this, insects had to be detached from the traps and analysed with PCR. This analysis is costly and the information it would provide in this study was considered doubtful, because no differentiation was possible from the glue traps between *Campylobacter* positive insects that entered the poultry house with the influx of ventilation air or insects that picked up *Campylobacter* from feces within the poultry house in case of a *Campylobacter* positive broiler flock. In other studies polyester nets were used to trap insects in ventilation vents and wall inlets (Hald *et al*., 2004, Hald *et al.*, 2008). This method is better suitable for determination whether caught insects carried *Campylobacter* spp. or not.

On average 83% of the caught insects in both housing systems in this study together were very small and belonged to category 1. In another study in which glue traps were used to catch insects in broiler houses, 79.7% of the caught insects were 1-4 mm (Hald *et al.*, 2008). The importance of these small flies in the transfer of *Campylobacter* to broiler flocks has not been studied, but technically all insects may be mechanical vectors for bacteria like *Campylobacter* (Smallegange and Den Otter, 2007).

Primarily the housefly (*Musca domestica*) is considered to be an important temperature related factor in the epidemiology of *Campylobacter* (Hald *et al.*, 2004; Royden *et al.*, 2016). In Denmark, multiple studies have been performed with the application of flyscreens, showing significant reductions in the number of *Campylobacter* positive flocks (Hald *et al.*, 2007; Bahrndorff *et al.*, 2013). Although insects could not be determined from the flytraps, the insects belonging to category 3 could fulfil a signalling function for the risk *Campylobacter* is introduced to a broiler flock by houseflies. In this study category 3 insects emerged in both types of housing systems in both weeks of measurement and it can therefore not be excluded that insects could play a role in the introduction of *Campylobacter* in broiler flocks in these housing systems.

The mean differences in insects caught between the housing systems in both weeks and the difference in number of insects from both housing systems between both weeks were not statistically significant. The difference in category 3 insects was also not statistically significant. Therefore it cannot be concluded that there is an actual difference between the housing systems in terms of the number of insects. In addition, many assumptions have been made regarding this study of insects and no corrections have been implemented for other factors that may influence the number of insects in the broiler houses. In further studies, these factors should be taken into account when determining the study design.

In the past years the relationship between biosecurity and the introduction of *Campylobacter* in broiler flocks has been well established. However, it is also recognized that biosecurity alone cannot ensure *Campylobacter* negative flocks (EFSA, 2011). Therefore studies into complementary measures to increase resistance to, or reduce colonization of broilers with *Campylobacter* should be conducted. Further research about the occurrence of *Campylobacter* in Patio systems and conventional broiler housing systems is recommended. Further studies into housing systems should correct for the different levels of biosecurity on farms. It is recommended to use a larger sample size to obtain a more reliable estimation of farm prevalence.

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Annexes

Annex 1

Risk factor	Article(s)	Additional information
Increasing age	Jacobs-Reitsma *et al.*, 1995 Newell *et al.,* 2003 Barrios *et al.*, 2006 Van de Giessen *et al.*, 2006 McDowell *et al.*, 2008 Wagenaar *et al.*, 2015	
	Bouwknegt *et al.*, 2004	Age 29–35 days (OR = 2.34) and 36–42 days (OR = 3.96) compared to 22–28 days.
	Lawes *et al.*, 2012	Increasing bird age (40–41 days, OR 3.18; 42–45 days, OR 3.56; o46 days, OR 13.43).
Increasing flock size	Barrios *et al.*, 2006 Guerin *et al.*, 2007 Näther *et al.*, 2009 Hermans *et al.*, 2012	
Multiple broiler houses on the premise	McDowell *et al.*, 2008 Lyngstad *et al.*, 2008 Wagenaar *et al.*, 2015 Høg *et al.*, 2016	
	Bouwknegt *et al.*, 2004	Five or more broiler houses on the premises (OR = 3.02).
Presence of other livestock adjacent to the broiler house	Katsma *et al.*, 2007 Hansson *et al.*, 2010 Patriarchi *et al.*, 2011 Newell *et al.*, 2011 Hermans *et al.*, 2012 Agunos *et al.*, 2014 Wagenaar *et al.*, 2015	
	Hald *et al.*, 2000	Presence of animals in the vicinity of the broiler house on farms with a missing hygiene barrier (OR = 7.0, 1.6 < OR < 33.9). Livestock other than chickens on farms with a missing hygiene barrier (OR = 7.6, 1.4 < OR < 44.9).
	Bouwknegt *et al.*, 2004	The presence of other farm animals on the farm (OR = 1.88); the presence of animals on farms within 1 kilometre (OR = 9.56).

	Ellis-Iversen *et al.,* 2009	Cattle on or adjacent to the farm increased the risk (OR 1.7, CI95% 1.1;2.7)
The execution of partial thinning	Hermans *et al.*, 2012 Patriarchi *et al.*, 2011 Newell *et al.*, 2011 Ellis-Iversen *et al.*, 2009 Wagenaar *et al.*, 2015 Smith *et al.*, 2016	
	Hald *et al.*, 2000	Dividing the flock into batches for staggered slaughter (OR = 6.8, 1.2 < OR < 49.3).
	Lawes *et al.*, 2012	Previous partial depopulation of the flock [odds ratio (OR) 5.21].
Presence of pets on the farm	Hermans *et al.*, 2012 Agunos *et al.*, 2014 Wagenaar *et al.,* 2015	
Increased prevalence in the summer months	Nylen *et al.*, 2002 Van de Giessen *et al.*, 2006 McDowell *et al.*, 2008 Jore *et al.*, 2010 Newell *et al.*, 2011 Chowdhury *et al.*, 2012 Hermans *et al.*, 2012 Wagenaar *et al.*, 2015	
	Bouwknegt *et al.*, 2004	Summer (OR = 3.48) and fall (OR = 2.59) compared to winter.
	Ellis-Iversen *et al.*, 2009	Risk of *Campylobacter* colonization was highest in July (OR 3.4), August (OR 3.4) and September (OR 3.7).
	Lawes *et al.*, 2012	Slaughter in the summer months (categorized as June, July and August; OR 14.27) or autumn months (categorized as September, October and November; OR 1.70).
Presence of manure adjacent to the broiler house	Newell *et al.*, 2011	
	Arsenault *et al.*, 2007	Odds 5.2 times higher for flocks with manure heap >200 m from the poultry house
Vertical ventilation systems in the broiler house	Barrios *et al.,* 2006	Vertical ventilation systems were strongly associated with positive flocks (OR = 5.3).
	Guerin *et al.*, 2007	Vertical (OR 2.7) or vertical and horizontal (OR 3.2)ventilation shafts.

Organic and free-range broiler flocks	Näther *et al.*, 2009 Vandeplas *et al.*, 2010 Newell *et al.*, 2011 Tangkham *et al.*, 2015	
Presence of wild rodents on the farm	McDowell *et al.*, 2008 Newell *et al.*, 2011	
Increasing age of the broiler house	Newell *et al.*, 2011 Høg *et al.*, 2016	
Increasing human traffic on the farm	Newell *et al.*, 2011	
Presence of stagnant water or puddles on the farm	Newell *et al.*, 2011	
Drinking water for broilers from surface water or a well	Lyngstad *et al.*, 2008 Newell *et al.*, 2011 Hermans *et al.*, 2012 Høg *et al.*, 2016	
Contaminated transport crates	Newell *et al.*, 2011 Agunos *et al.*, 2014 Patriarchi *et al.*, 2011	
Poor biosecurity on the farm	Lyngstad *et al.*, 2008 McDowell *et al.*, 2008 Hansson *et al.*, 2010 Newell *et al.*, 2011 Høg *et al.*, 2016	
	Hald *et al.*, 2000	Lack of a hygiene barrier (odds ratio (OR) = 3.1, 1.1 < OR < 9.3).
Presence of insects and flies in the broiler house	Hald *et al.*, 2007 Hald *et al.*, 2008 Newell *et al.*, 2011 Bahrndorff *et al.*, 2013 Agunos *et al.*, 2014 Royden *et al.*, 2016	
Shorter length of downtime	Lyngstad *et al.,* 2008 Newell *et al.*, 2011	
	Hald *et al.*, 2000	A down period of less than 14 days (OR = 5.0, 1.2 < OR < 22.6).
Drinking nipples with cups	Høg *et al.*, 2016 Näther *et al.*, 2009 Rushton *et al.*, 2009	
Unchlorinated drinking water	Newell *et al.*, 2011	
	Ellis-Iversen *et al.*, 2009	Chlorinated drinking water reduced the risk (OR 0.5; CI95% 0.2-0.9).
Presence of biofilm in the drinking lines	Newell *et al.*, 2011	

Bijlage I – Checklist Campylobacter op vleeskuikenbedrijf

Algemeen

Bedrijfsnaam	
Naam pluimveehouder	
Locatie	
Datum bedrijfsbezoek	
IKB Certificering	Ja/nee
Status Campylobacter	Onbekend/negatief/positief
Staltype	
Aantal vleeskuikens	
Neventak	

Bedrijfstype en kenmerken

Stal	Aantal dieren	Leeftijd	Soort pluimvee	Houderijsysteem
1				
2				
3				
4				

1. Bedrijfsterrein

1.1	Is het bedrijfsterrein afgesloten met een hek of ketting?	Ja/nee	Indien nee, 1 punt	
1.2	Is het terrein rondom de stallen vrij van materialen?	Ja/nee	Indien nee, 1 punt	
1.3	Is het terrein schoon en opgeruimd?	Ja/nee	Indien nee, 1 punt	
1.4	Is het terrein rondom de stallen vrij van begroeiing?	Ja/nee	Indien nee, 1 punt	
1.5	Zijn de loop- en rijpaden en het terrein rondom de stallen verhard?	Ja/nee	Indien nee, 1 punt	
1.6	Zijn vleeskuikens de enige landbouwhuisdieren op de locatie?	Ja/nee	Indien nee 1 punt	
1.7	Komen andere landbouwhuisdieren voor in de directe omgeving van de locatie?	Ja/nee	Indien ja, 1 punt	
1.8	Wordt mest uitgereden van landbouwhuisdieren in de directe omgeving van de stallen?(weide/land)	Ja/nee	Indien ja, 1 punt	

1.9	Zijn open mesthopen/opslag aanwezig op het bedrijfsterrein?	Ja/nee	Indien ja, 1 punt	
1.10	Komen huisdieren (honden/katten/..) voor op het bedrijfsterrein?	Ja/nee	Indien ja, 1 punt	
1.11	Komen huisdieren (honden/katten/..) voor in de pluimveestallen?	Ja/nee	Indien ja, 1 punt	
1.12	Gaat de aan- en afvoer volgens het schone en vuile weg principe?	Ja/nee	Indien nee, 1 punt	
1.13	Wordt alle voeder (tevens ruwvoeders van bijvoorbeeld eigen teelt) opgeslagen in gesloten silo's waar vogels of ongedierte niet bij kunnen?	Ja/nee	Indien nee, 1 punt	
1.14	Wordt afval bewaard in afgesloten containers?	Ja/nee	Indien nee, 1 punt	
1.15	Wordt stro/strooisel en afleidingsmateriaal zo opgeslagen dat er geen vogels of ongedierte bij kunnen?	Ja/nee	Indien nee, 1 punt	
1.16	Staan voersilo's op een verharde ondergrond?	Ja/nee	Indien nee, 1 punt	
1.17	Worden eventuele voerresten direct verwijderd?	Ja/nee	Indien nee, 1 punt	
1.18	Loopt afvoer van water van daken van bedrijfsgebouwen via dakgoten/regenbuizen?	Ja/nee	Indien nee, 1 punt	
1.19	Is het bedrijfsterrein goed ontwaterd en vrij van vijvers en wateropvang?	Ja/nee	Indien nee, 1 punt	

2. Bedrijfshygiëne

2.1	Wordt all-in- all-out op bedrijfsniveau toegepast?	Ja/nee	Indien nee, 1 punt	
2.2	Is een hygiënesluis/omkleedruimte aanwezig op de scheiding van het schone en vuile bedrijfsgedeelte?	Ja/nee	Indien nee, 1 punt	
2.3	Is een goed zichtbaar hygiëne-instructieprotocol aanwezig voor bezoekers?	Ja/nee	Indien nee, 1 punt	

2.4	Is een voorziening voor het wassen van de handen aanwezig?	Ja/nee	Indien nee, 1 punt	
2.5	Is een operationele douche aanwezig?	Ja/nee	Indien nee, 1 punt	
2.6	Betreden medewerkers en bezoekers het bedrijfsterrein altijd via de hygiënesluis?	Ja/nee	Indien nee, 1 punt	
2.7	Maakt iedereen op het bedrijf gebruik van bedrijfseigen kleding/wegwerpkleding?	Ja/nee	Indien nee, 1 punt	
2.8	Maakt iedereen op het bedrijf gebruik van bedrijfseigen schoeisel/overschoentjes?	Ja/nee	Indien nee, 1 punt	
2.9	Wordt bedrijfs- en stalkleding na ieder gebruik gewassen?	Ja/nee	Indien nee, 1 punt	
2.10	Wordt de erfverharding ontsmet na ontvangst van eendagskuikens?	Ja/nee	Indien nee, 1 punt	
2.11	Wordt de erfverharding ontsmet voor en na het uitladen?	Ja/nee	Indien nee, 1 punt	

3. Stalhygiëne

3.1	Zijn gaten of kieren in de buitenmuren of deuren(inclusief luchtinlaten) aanwezig waar ongedierte- of insecten door heen kunnen komen?	Ja/nee	Indien ja, 1 punt	
3.2	Zijn openingen van ramen of luchtinlaten aanwezig waar vogels door heen kunnen komen?	Ja/nee	Indien ja, 1 punt	
3.3	Zijn drangers aanwezig op alle loopdeuren?	Ja/nee	Indien nee, 1 punt	
3.4	Zijn de oppervlakten in de stal glad, zonder beschadigingen, gaten, kieren (en daardoor goed te reinigen?)	Ja/nee	Indien nee, 1 punt	
3.5	Is een betonnen of asfalt verharding voor de toegangsdeuren aanwezig?	Ja/nee	Indien nee, 1 punt	

3.6	Is een voorlokaal aanwezig, afgescheiden van de dierverblijven?	Ja/nee	Indien nee, 1 punt	
3.7	Is er slechts één toegang aanwezig tot de vleeskuikenstal tijdens de productieperiode?	Ja/nee	Indien nee, 1 punt	
3.8	Is een schoeiselontsmettingsbak of -mat aanwezig bij de entree?	Ja/nee	Indien nee, 1 punt	
3.9	Is er een strikte, fysieke scheiding tussen het schone en vuile gedeelte?	Ja/nee	Indien nee, 1 punt	
3.10	Zijn mondkapjes, hoofdbedekkingen en handschoenen aanwezig?	Ja/nee	Indien nee, 1 punt	
3.11	Is er een voorziening aanwezig om handen te wassen met zeep?	Ja/nee	Indien nee, 1 punt	
3.12	Wordt schoeisel altijd gewisseld voor het betreden van de dierruimten?	Ja/nee	Indien nee, 1 punt	
3.13	Wordt altijd omgekleed in staleigen kleding en schoeisel voor de dierruimten worden betreed?	Ja/nee	Indien nee, 1 punt	
3.14	Wordt staleigen kleding en schoeisel enkel gedragen in de dierruimten en het schone deel? (nooit mee naar buiten)	Ja/nee	Indien nee, 1 punt	
3.15	Worden de handen voor het betreden van de dierruimten altijd gewassen en gedesinfecteerd?	Ja/nee	Indien nee, 1 punt	
3.16	Zijn de afvalbakken afgesloten?	Ja/nee	Indien nee, 1 punt	
3.17	Wordt het voorlokaal frequent bezemschoon gemaakt?	Ja/nee	Indien nee, 1 punt	
3.18	Vindt vliegenbestrijding plaats in het voorlokaal?	Ja/nee	Indien nee, 1 punt	
3.19	Is er een voetbad/laarzenwasser aanwezig op het bedrijf?	Ja/nee	Indien nee, 1 punt	
3.20	Worden alle voetbaden ververst wanneer deze visueel gecontamineerd zijn?	Ja/nee	Indien nee, 1 punt	

3.21	Worden alle staloppervlakten gereinigd tussen de rondes?	Ja/nee	Indien nee, 1 punt	
3.22	Wordt alle apparatuur (drinkers, voerpannen) grondig gereinigd en ontsmet tussen de rondes?	Ja/nee	Indien nee, 1 punt	
	(Indien sprake van tussentijds uitladen)			
3.23	Zijn de materialen van de vangploeg gereinigd en ontsmet voordat de ploeg het bedrijf betreedt?*	Ja/nee	Indien nee, 1 punt	
3.24	Neemt de vangploeg specifieke IKB hygiënemaatregelen in acht bij het uitladen?	Ja/nee	Indien nee, 1 punt	
3.25	Wordt het binnenkomen van insecten via open deuren tegengegaan (door bijvoorbeeld een luchtgordijn)	Ja/nee	Indien nee, 1 punt	

4. Materialen en voertuigen

4.1	Worden de wielen en wielkasten van alle wagens voor het betreden van het bedrijfsterrein gereinigd/ontsmet?	Ja/nee	Indien nee, 1 punt	
4.2	Worden de wielen en wielkasten van alle wagens bij het verlaten van het bedrijfsterrein gereinigd/ontsmet?	Ja/nee	Indien nee, 1 punt	
4.3	Wordt uitsluitend gebruik gemaakt van bedrijfseigen materialen (vaccinatieapparatuur, gereedschap etc.)	Ja/nee	Indien nee, 1 punt	
4.4	Zijn alle in de stal benodigde materialen en -hulpmiddelen zoals bezems, emmers, kruiwagens, etc. staleigen?	Ja/nee	Indien nee, 1 punt	
4.5	Worden alle staleigen materialen en hulpmiddelen gereinigd en ontsmet tussen opeenvolgende rondes?	Ja/nee	Indien nee, 1 punt	
4.6	Worden dode dieren dagelijks uit de stal verwijderd?	Ja/nee	Indien nee, 1 punt	

4.7	Is de kadaveropslag gekoeld, afsluitbaar en visueel schoon?	Ja/nee	Indien nee, 1 punt	
4.8	Worden hulpmiddelen voor het verplaatsen van dode dieren gereinigd en ontsmet na gebruik?	Ja/nee	Indien nee, 1 punt	
4.9	Bevindt de aanbiedingsplaats van kadavers zich buiten of aan de buitenrand van het bedrijfsterrein?	Ja/nee	Indien nee, 1 punt	
4.10	Worden kadaverbakken/tonnen altijd gereinigd en ontsmet na het legen?	Ja/nee	Indien nee, 1 punt	
4.11	Wordt mest direct na het leegkomen van de stal verwijderd?	Ja/nee	Indien nee, 1 punt	
4.12	Wordt mest afgevoerd in een afgedekte/gesloten mestcontainer/mesttrailer?	Ja/nee	Indien nee, 1 punt	
4.13	Zijn mestcontainers/mesttrailers schoon voordat deze op het bedrijfsterrein worden toegelaten?	Ja/nee	Indien nee, 1 punt	
4.14	Is mest van elke productieronde volledig afgevoerd van het bedrijfsterrein?	Ja/nee	Indien nee, 1 punt	
4.15	Wordt een eventuele mestplaats na afvoer gereinigd en ontsmet?	Ja/nee	Indien nee, 1 punt	
4.16	Wordt erfverharding na afvoer van mest gereinigd en ontsmet?	Ja/nee	Indien nee, 1 punt	

5. Ongediertewering- en bestrijding

5.1	Wordt ongediertebestrijding uitgevoerd door een professioneel bedrijf dat hiervoor erkenning heeft (zoals IKB-PBS erkenning)	Ja/nee	Indien nee, 1 punt	
5.2	Is er een ongediertebestrijdingsplan voor het weren en bestrijden van ratten en muizen rond de pluimveestallen?	Ja/nee	Indien nee, 1 punt	
5.3	Vind wisseling van werkzame stof in	Ja/nee	Indien nee, 1 punt	

	ongediertebestrijdingsmiddelen frequent plaats?			
5.4	Wordt het aanwezige grasland rondom de stallen kort gehouden?	Ja/nee	Indien nee, 1 punt	
5.5	Zijn de stallen vrij van ratten en muizen (of uitwerpselen en vraat)?	Ja/nee	Indien nee, 1 punt	
5.6	Zijn de stallen vrij van wilde vogels?	Ja/nee	Indien nee, 1 punt	
5.7	Zijn de stallen vrij van insecten?	Ja/nee	Indien nee, 1 punt	
5.8	Zijn gaten of kieren aanwezig in de buitenmuren of deuren (inclusief luchtinlaten) waar ongedierte en insecten doorheen kunnen komen?	Ja/nee	Indien ja, 1 punt	
5.9	Kunnen vogels binnen komen via openingen van ramen of luchtinlaten?	Ja/nee	Indien ja, 1 punt	
5.10	Kan vogelpoep binnen komen via luchtin- of uitlaten (met name via het dak)?	Ja/nee	Indien ja, 1 punt	
5.11	Vindt vliegenbestrijding plaats in het voorlokaal?	Ja/nee	Indien nee, 1 punt	
5.12	Wordt het binnenkomen van insecten tegen gegaan (door bijvoorbeeld een luchtgordijn)?	Ja/nee	Indien nee, 1 punt	
5.13	Wordt specifiek aandacht besteed aan het bestrijden van insecten/eieren in gaten en kieren van vloeren en wanden?	Ja/nee	Indien ja, 1 punt	
5.14	Is het bedrijf vrij van hobby pluimvee?	Ja/nee	Indien nee, 1 punt	

Indien een vraag op het bedrijf niet van toepassing is, dient deze te worden overgeslagen. Het is hierbij van belang dat de formule wordt aangepast, zodat de berekening in het Campas blijft kloppen.

Annex 3

Housing system	Number of compartments	Number of pools per sample collection round
Patio 1	3	21
Conventional 1	1	7
Patio 2	3	21
Conventional 2	1	7
Patio 3	3	21
Conventional 3	1	7
Patio 4	2	14
Conventional 4	1	7
Total	**24**	**105**

Annex 4

Flytrap number	Location of flytraps in Patio system per compartment method 1	Location of flytraps in Patio system per compartment method 2	Location of flytraps in conventional housing system
1	Left row 1st level front	1st level front left	Front left
2	Left row 2nd level middle	1st level front right	Front middle
3	Left row 3rd level back	2nd level middle left	Front right
4	Left row 4th level last quarter (3/4)	2nd level middle right	Middle left
5	Left row 5th level first quarter (1/4)	3rd level back left	Middle middle
6	Right row 1st level front	3rd level back right	Middle right
7	Right row 2nd level middle	4th level last quarter left	Back left
8	Right row 3rd level back	4th level last quarter right	Back middle
9	Right row 4th level last quarter (3/4)	5th level first quarter left	Back left
10	Right row 6th level first quarter (1/4)	5th level first quarter right	