

## Core of the report for the period

### 1 Project objectives for the period

The overall strategy of the work plan was to begin with investigating industrial chicken farms in geographically distinct regions of the EU to identify risk factors and epidemiology associated with *Campylobacter* colonization. The next step was to study the impact of intervention methods such as preventing flies as vectors, phage-therapy and vaccination in a selected number of farms. Novel air sampling techniques was developed for monitoring and quantification, but also to gain epidemiological insight. A risk assessment was also performed. At the final stage a certification programme is proposed to producers and regulators. In order to ensure technical integration of the WPs, harmonized protocols for sampling and methodologies was produced at the beginning of the project.

Due to this strategy, many Tasks was finalised during the last two years of the project. The table below shows the planned timing of the work in the different Tasks:

Task	Title	Covered in 1 <sup>st</sup> periodic Report (1-18)	Covered in 2 <sup>nd</sup> periodic Report (19- 36)	Month 36-47	Month 48-60	End month
1.1	Risk factors for <i>Campylobacter</i> colonization in broilers					60
1.2	A longitudinal study of broiler flocks in the UK and Spain					46
1.3	Importance of flies in transmission of <i>Campylobacter</i> to broiler flocks					60
1.4	Distribution of <i>Campylobacter</i> sub-types in EU broiler production					46
1.5	Modelling in-house colonization in relation to environ. and bird welfare					42
2.1	Fly screens add-on to biosecurity					60
2.2	Phage therapy					60
2.3	Vaccination					60
3.1	Development of methods of quantification of <i>Campylobacter</i> in air					24
3.2	Feasibility of real-time monitoring of <i>Campylobacter</i> in broiler flocks					48
3.3	Report on future research needs					60
4.1	Risk assessment					60
4.2	Data collection and compilation					60
4.3	Economics					60
4.4	Cost-effectiveness of interventions at farm and comparison with interventions post farm					60
4.5	Future data needs					60
5.1	Best Practice Manual for production of <i>Campylobacter</i> -free chickens					57
5.2	Specific targeted learning programmes for proficiency in implementing the "Best Practice Manual for production of <i>Campylobacter</i> -free chickens"					60
5.3	Voluntary Certification Programme					60

## 2. Work progress and achievements during the period

### WP1 Epidemiology

The specific objectives for WP1 are as follows:

- To examine external risk factors for flock colonization in different areas of Europe.
- To examine the role of climate and geography in determining flock colonization rates.
- To determine the role of farm management factors and their interaction with climate and geography in determining colonization rate.
- To determine the roles of the in-house environment and bird health and welfare in colonization.
- To examine the distribution of *Campylobacter* sub-types in EU broiler production in relation to climate, geography/region, husbandry and farm management.
- To use Structural Equation Modelling to investigate the pathways of risk of colonization arising from environmental factors, management and flock welfare to quantify and integrate risks across the different potential routes to colonization in different areas of the EU.

### Summary of individual task progress

#### Task 1.1 Risk factors for *Campylobacter* colonization in broilers (M1-60)

The work of Task 1.1 results in Deliverables 1.1.1 (Submitted), 1.1.2 (Submitted), 1.1.3 (M36 – Delayed – will be two papers).

By combining retrospective data on the *Campylobacter* status of broiler flocks, from national surveillance programmes in 2010 and 2011, with the data obtained from a questionnaire survey, a risk factor analysis was carried out on data from Norway and Denmark. *Campylobacter* data were obtained from 107 Danish and 173 Norwegian farms. The *Campylobacter* status of more than 5200 flocks and more than 40 explanatory variables were included in the analysis.

An increased risk was associated with country; i.e. Danish broiler flocks were more frequently colonized by *Campylobacter* than Norwegian flocks. Furthermore, the age of the broiler house, density of birds, level of biosecurity (anteroom and barrier at entrance), length of downtime and type of drinkers were all found to be associated with the risk of the broiler flocks becoming colonized by with *Campylobacter*.

The preliminary results were presented at 17th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms (CHRO) 2013, September 2013. Furthermore, a manuscript has been drafted, and submitted to the Preventive Veterinary Medicine April 2015.

The risk factor analysis expanded to include *Campylobacter* data from the 20-farm study from the Netherlands, Poland, Spain, and the UK, as well as climate factors, to further investigate differences among different countries and different climatic regions of the EU. This risk factor analysis included data from more ca. 6000 broiler flocks.

Also here, an increased risk was associated with country. In descending order, broiler flocks were more frequently colonized in Poland, Spain, UK, the Netherlands, Denmark and Norway. There was an association between temperature and colonization of flocks, i.e. the number of positive flock increased with increasing temperatures. Furthermore, the conclusions from the first study (Norwegian and Danish data) were supported by this study. Factors associated with biosecurity on the farm were again identified as risk factors. Age of house, anterooms and barriers in all houses, designated tools for each house, as well as length of downtime and the types of drinkers were found to be associated with the risk of the broiler flocks becoming colonized by with *Campylobacter*.

The analyses have been completed and a manuscript is in preparation and to be submitted soon.

### Task 1.2 A longitudinal study of broiler flocks in the UK and Spain (M10-46)

The work of Task 1.2 results in Deliverables 1.2.1 (Submitted), 1.2.2 (M26 - Delayed), 1.2.3 (M46 - Delayed), 1.2.4 (M46 - Delayed).

*UK:* The sampling of the eight broiler farms from two major UK poultry producers finished in the summer of 2013, the completion of the longitudinal study was delayed by approximately one year due to difficulty in recruiting broiler farms. All data was collected from the broiler production companies and farms and provided in full to Newcastle for analysis in 2014. A paper on the two-year longitudinal study in UK and analysis is currently being drafted.

*Spain:* The sampling of the five broiler farms was completed in October 2014 and the full dataset has been sent to Newcastle for analysis.

A paper on the two-year longitudinal study in Spain is in preparation.

### Task 1.3 Importance of flies in transmission of *Campylobacter* to broiler flocks (M16-60)

The work of Task 1.3 results in Deliverables 1.3.1 (Submitted), 1.3.2 (M48 - Delayed).

UK: 1.3.1 & 1.3.2. No further work on these tasks were carried out in the P3 reporting period besides for work with publications.

Main findings in UK have been:

- An estimate of 0.3% of the UK flies carried *Campylobacter*
- Flies carried cattle and broiler associated STs, responsible for human disease
- Flies may spread *Campylobacter* from cattle to broiler farms
- Flies come close to broiler house ventilation inlets in large numbers, with a considerable risk that they enter the broiler house and transmit *Campylobacter* to the chickens.

A publication has been drafted and is being submitted currently to Applied and Environmental Microbiology.

#### SPAIN:

##### 1.3.1:

*Musca domestica* (house fly) was the most frequent (89.8%) fly species captured and the only species from which *Campylobacter* was isolated. The prevalence of positive flies detected by culture was 1.7% with a peak in September, where 31.8% of all the positive flies were found. By PCR (detection from Bolton enrichment broths), overall prevalence was 10.5% (mainly *M. domestica*, but also *Ophyra* sp., *Calliphora* sp. and *Fannia canicularis*), with a peak of 32.18% of positives in August. Most of the broiler flocks became *Campylobacter* positive around the same time or just after detecting *Campylobacter* in the sampled flies. Also, same PFGE profiles were found among *Campylobacter* isolates from flies and broilers during the same rearing cycle. Thus, flies especially *M. domestica*, near broiler houses constitutes a considerable risk for infection of broilers with *C. jejuni* and *C. coli*.

##### 1.3.2:

Insects harvested from farm ventilation were subject to taxonomical sorting to family level. Overall 7003 insects from 17 orders were captured, with *Diptera* order (n=5215) being the most abundant (74.5%). Total captures per farm ranged from 72 to 2741. Identification efforts have been focused on those families of this order identified as *Campylobacter* carriers in task 1.3.1: 0.14% of *Diptera* belonged to families *Muscidae*, *Fannidae* and *Calliphoridae* while the remaining bulk of captured *Diptera* belonged to other families.

Concerning the ability of flies to transmit *Campylobacter* between sources and farms, a behavioral laboratory study conducted with a Danish and a Spanish housefly population observed a flight range of around 10,000 meters within 24 hours. So flies may easily spread *Campylobacter* to broiler farms within that range, although the flight range in external environment may be less.

A publication is in the pipeline and close to submission.

#### Task 1.4 Distribution of *Campylobacter* sub-types in EU broiler production (M10-46)

The work of Task 1.4 results in Deliverable 1.4.1 (M44 - Delayed).

A total of 457 isolates were typed at ULIV using MLST. These comprised *Campylobacter* isolates collected prospectively from Spain (n=160) and Poland (n=172) as part of task 1.1, of which full allelic profiles were obtained from 132 and 157 isolates respectively. Isolates were also obtained from retrospective collections of *Campylobacter* species from surveillance in poultry in Norway (n=174), with full allelic profiles obtained from 168 isolates. Strain typing by MLST was completed during 2014 and analysis of the sequence traces completed in early 2015. Five isolates produced complete sequence data using MLST but were excluded from analysis due to the presence of multiple *Campylobacter* species alleles in the sample.

The resulting sequence data were combined with retrospective MLST data provided by the UK (211 isolates), the Netherlands (158 isolates) and Denmark (185 isolates), giving a total of 1011 isolates to be included for analysis. All retrospective isolates had been selected from studies which took place between 2003 and 2011. All isolates selected were from broiler chickens, with one isolate selected per crop and equally distributed over the year to allow any seasonal effects on sequence type (ST) to be explored.

The combined dataset was analysed to give sequence types for all isolates, and clonal complexes were available. The frequency of occurrence of each ST was determined and Principal Component Analysis applied in order to show the frequency of occurrence of STs, while allowing for the bias which would result from using geographically and temporally separated datasets.

PCA showed a large degree of diversity amongst the STs from different countries, with isolates from the UK being most divergent from the other countries, and Spain and Poland being most similar to each other.

A draft manuscript to publish the results of task 1.4 is in preparation, while additional analysis is being considered.

#### Task 1.5 Modelling in-house colonization of birds in relation to environm. and bird welfare (M10-42)

The work of Task 1.5 results in Deliverables 1.5.1 (M40 - Delayed), 1.5.2 (M42 - Delayed).

We used generalised linear models to identify factors that significantly contributed to the risk of colonization of UK flocks by *Campylobacter*. The variation explained by the parsimonious model was model  $\bar{R}^2 = 0.481$ . Models were compared using AIC and the parsimonious model identified using information Theory (Burnham and Anderson (2002)).

The infection of adjacent production facilities (CSA) was a significant predictor of increased risk in the study flock (Z-value = 4.779, P-value = 1.76E-06). External air temperature was a significant risk factor of colonization by *Campylobacter* (Z-value = 0.363, P-value = 0.018). There was a significant increase in the probability of infection according to stocking density/welfare system employed during production, where flock-cycles produced at higher stocking densities were significantly more likely to become colonized by *Campylobacter* than flock-cycles produced at lower densities (Z-value = 2.813, P-value = 0.005) (Table 2). In addition, a greater than average down-time (ADT) between successive flock-cycles significantly decreased the risk of colonisation (Z-value = -2.882, P-value = 0.004). Internal temperature of the study building was marginally significant with regard to modifying the risk of colonization by *Campylobacter* (Z-value = 1.665, P-value = 0.096). The type of drinking water provider also affected the underlying risk of colonization by *Campylobacter*, where the risk of colonization associated with drinking cups and nipples was significantly greater than drinking water providers that did not provide cups (Z-value = 2.144, P-value = 0.032). The infection status of the previous flock-cycle within a farm, the hours of sunshine and rainfall in the month of depopulation did not significantly alter the risk of colonization and were removed from the final model.

### Significant Results WP1

**Task 1.1:** An increased risk for *Campylobacter* colonization was associated with country; i.e. Danish broiler flocks were more frequently colonized than Norwegian flocks. Furthermore, the age of the broiler house, density of birds, level of biosecurity (anteroom and barrier at entrance), length of downtime (reduced downtime being protective) and type of drinkers were all found to be associated with the risk of the broiler flocks becoming colonized by with *Campylobacter*.

Furthermore, an increased risk of *Campylobacter* colonization was also associated with other countries. In descending order, broiler flocks were more frequently colonized in Poland, Spain, UK, the Netherlands, Denmark and Norway. There was an association between temperature and colonization of flocks, i.e. the number of positive flock increased with increasing temperatures. Factors associated with biosecurity on the farm were again identified as risk factors; age of house, anterooms and barriers in each house, designated tools for each house, as well as length of downtime and the types of drinkers were found to be associated with the risk of the broiler flocks becoming colonized by with *Campylobacter*.

In conclusion, the results of Task 1.1 have provided the first risk factor analyses of comparable data from six European countries and have provided an insight into the risk factors across countries. There is a clear effect of country and temperature. Furthermore, a number of specific biosecurity measures have been identified as important risk factors for *Campylobacter* colonization of broiler flocks.

**Task 1.2:** In total of 54% of flocks (n=109) (13-15 crops per farm) were positive for *Campylobacter* before slaughter. The average age at which flocks become positive was 23 days, with the earliest detection of *Campylobacter* to date being at 9 days of age, which is significantly earlier than has been previously reported in the literature. No farm remained consistently negative throughout, with some seasonality evident. In Spain, in total 60% of flocks (n=63) were positive for *Campylobacter* before slaughter. None of the farms remained consistently negative throughout, with the earliest detection of *Campylobacter* at 7 days of age.

**Task 1.3:** Main findings in UK have been: A low prevalence estimate of 0.3% of UK flies was found to carry *Campylobacter*, with positive flies carrying cattle and broiler associated STs, responsible for human disease. Although the prevalence of positive flies was very low, large numbers of flies were found to be associated with broiler house ventilation inlets, therefore representing a considerable risk that they enter the broiler house and transmit *Campylobacter* to the chickens.

Main findings in Spain: *Musca domestica* (house fly) was the most frequent (89.8%) fly species captured, in contrast to the UK, where very few *M. domestica* were caught on broiler farms. The prevalence of positive flies detected by culture was 1.7% with a peak in September, where 31.8% of all the positive flies were found, with only *M. domestica* positive by culture. However, PCR detection found an overall prevalence of 10.5% (mainly *M. domestica*, but also *Ophyra* sp., *Calliphora* sp. and *Fannia canicularis*), with a peak of 32.18% of positives in August. Most of the broiler flocks became *Campylobacter* positive around the same time or just after detecting *Campylobacter* in the sampled flies, with evidence of the same strains being found in both flies and broilers during the same rearing cycle. Thus, flies especially *M. domestica*, near broiler houses in Spain constitutes a considerable risk for infection of broilers with *Campylobacter* species.

**Task 1.4:** Strain typing by MLST demonstrated a large degree of diversity amongst the STs from different countries, with isolates from the UK being most divergent from the other countries, and Spain and Poland being most similar to each other.

**Task 1.5:** Generalised linear models identified factors that significantly contributed to the risk of colonization of UK flocks by *Campylobacter*. The infection of adjacent production facilities (CSA) was a significant predictor of increased risk in the study flock (Z-value = 4.779, P-value = 1.76E-06). External air temperature was a significant risk factor of colonization by *Campylobacter* (Z-value = 0.363, P-value = 0.018). There was a significant increase in the probability of infection according to stocking density/welfare system employed during production, where flock-cycles produced at higher stocking densities were significantly more likely to become colonized by *Campylobacter* than flock-

cycles produced at lower densities (Z-value = 2.813, P-value = 0.005). In addition, a greater than average down-time (ADT) between successive flock-cycles significantly decreased the risk of colonisation (Z-value = -2.882, P-value = 0.004). Internal temperature of the study building was marginally significant with regard to modifying the risk of colonization by *Campylobacter* (Z-value = 1.665, P-value = 0.096). The type of drinking water provider also affected the underlying risk of colonization by *Campylobacter*, where the risk of colonization associated with drinking cups and nipples was significantly greater than drinking water providers that did not provide cups (Z-value = 2.144, P-value = 0.032). The infection status of the previous flock-cycle within a farm, the hours of sunshine and rainfall in the month of depopulation did not significantly alter the risk of colonization and were removed from the final model.

#### Deviations and corrective actions

**Task 1.2.1:** Longitudinal sampling in the UK and Spain was not completed till June and October 2014 respectively, and this impacted upon the timescale of deliverable 1.5. However, Newcastle had the models in place and ready to run once the full dataset was available.

There have been no other recent deviations.

#### Use of resources WP1

No large deviations from the planned use of resources. However, several participants have adjusted their budget between consumables and person cost compared to the budget to optimize the project. Details on the economical reporting are presented in the Final Report.

## WP2 Intervention strategies

The specific objectives for WP2 are as follows:

- To implement and evaluate the effectiveness of fly control in two different countries on farms with a high standard of biosecurity.
- To develop and test user friendly and cheap fly nettings.
- To further develop, evaluate and test phage therapy, at experimental and in field conditions.
- To further develop, evaluate and test vaccination with new generation vaccines.

### Summary of individual task progress

#### Task 2.1 Fly screens add-on to biosecurity (M7-60)

The work of Task 2.1 results in Deliverables 2.1.1 (Submitted), 2.1.2 (Submitted), 2.1.3 (Submitted), 2.1.4 (Submitted), 2.1.5 (M56 - Delayed), 2.1.6 (M56 - Delayed), 2.1.7 (M58 - Delayed).

The objective of this Task is to investigate if fly screen will prove useful under different geographical, climatic and production conditions. The precondition for success is farms with a specified high level of biosecurity.

Adjustments of project plans in both UK and Spain were approved at the first Annual Meeting (14 April 2011) and later formalized in 'CamCon Annex 1 Amendment no. 1' and in a collaboration agreement with a Defra project.

The Task is divided in six Subtasks.

Subtask 2.1.1 Identification and recruitment of study and control farms

Subtask 2.1.2 Establishment of fly screens at each study farm

Subtask 2.1.3 Daily management of study farms

Subtask 2.1.4 Testing of study farms and control farms for *Campylobacter*

Subtask 2.1.5 Data analysis

Subtask 2.1.6 Institution of biosecurity at house level in Spain and test of fly screens as add on to biosecurity in Spain

UK. Results and the corresponding raw dataset from the CamCon sampling period (summer 2012 to summer 2013) of the UK farms of the Defra-CamCon cooperation have been provided by Sept13 to the project leader of the Defra project to be analysed together with the rest of data obtained at the three interventions conducted under the Defra project (acidification of drinking water, biosecurity, fly screen). It is agreed that the project leader of the Defra-project, is responsible for integrating the CamCon data into the Defra data for the purpose of a shared publication. In short, the flock prevalence was extremely high (70-80% positive flocks at slaughter) during the entire CamCon sampling period, with no reduction even during the winter period.

SPAIN. During this reporting period, the 18-farm study starting by July 2013 has been conducted according to plans described in the Amendment to Annex 1, but with more broiler cycles than planned.

Much effort has been done during the reporting period to institute a sufficient upgrade of the on-farm biosecurity, by providing education material for training of Broiler Company veterinarians (meetings) and farm personnel (farm visits, posters for anterooms). Furthermore, advice about practical installations (benches, specific tools, etc.) required on the farms for the upgrade of biosecurity was carried out in cooperation with the broiler company and farmers. For weekly control of the current upgrade and farmer compliance, we have developed and provided support tools (the farm checklists) to the broiler company.

To get the farmers and all farm personnel motivated and all farms up and running at a sufficient biosecurity level required actually nearly a year (from July13 to June14) which was more time than planned in the original 18-farm design with only two initial rotations and two rotations with biosecurity in full place. However results by June14 showed that there was a reductive effect of the

biosecurity instituted. The reduction was evaluated to be sufficient to proceed to establish flyscreens on the biosecurity + flyscreens group.

A pilot study of two 2-house farms, with flyscreens at one house each was conducted during the 2<sup>nd</sup> reporting period of CamCon. In particular the ventilation functionality had been surveyed and approved by the broiler company and the farmers. Experience from the pilot was utilized for construction and mounting of flyscreens between July14 and Oct14 t in 5 more houses (now total 7 houses with flyscreens). So the three experimental groups in the 18-farm study, i.e. control group, biosecurity group, biosecurity + flyscreens group were running concurrently from Oct14 and forward. Final results of the influence of flyscreens can however not be expected until after end of CamCon since most of the period with all screens in place has been cold/winter and outside of the insect season. Therefore, to be able to recognize the final effect of the flyscreens CReSA/UAB has taken responsibility to extend the 18-farm study until at least Dec 15.

Furthermore, from summer 2014, sampling of flocks on farms with upgraded biosecurity in two separate houses has been expanded to comprise both houses not only the initially selected study house. The purpose is to recognize an eventual delay in the within farm spread from house to house.

#### Results by April 15:

Results given below are all monitored at day 34 of age, and before thinning and/or slaughter.

*Biosecurity:* Effect of the biosecurity practice: Preliminary results point to a positive effect of the implementation of biosecurity, as a reduction from 56% positive flocks in the introductory 2 rotations to 36% positive flocks through the following 4 rotations (from Jan14 to Sep14) when biosecurity procedures were currently implemented. For comparison, the respective figures for the control group were 58% positive in the introductory 2 rotations, and 61% during the following 4 rotations.

*The flyscreens:* So far, and only based on preliminary data from the first 3 cycles of the houses with flyscreens obtained from September 2014 to March 2015 there seems to be an additional positive effect of the fly screens. More data is however urgent to show the final influence of the screens, and that will not be possible to obtain until November 2015.

*Season:* The most recent rotations, cycles 7-9 during winter, mainly Dec14-Mar15, with all three groups of the 18 farm study running a probably effect of season has been observed in the control group, as the flock prevalence dropped from 60% to 41% positive.

*Within farm transmission:* Furthermore, the sampling of the additional houses in those farms with 2 houses, where both houses have biosecurity, further suggests that the biosecurity practice delay house to house transmission. Thus, four flocks from different farms each, have been observed to remain negative in one of the houses for a remarkable long period (2-3 weeks) although the other house on the farm was positive at a much earlier point (2<sup>nd</sup> or 3<sup>rd</sup> week) of the rotation. This is in contrast to experience gained in the longitudinal study, where house to house transmission usually took place within a week.

Results given above are based on raw data. Proper statistical treatment will be instituted Nov 2015 when all data are collected.

Overall we expect that the outcome of the 18-farm study will confirm our hypothesis, that the implemented biosecurity practice can reduce the yearly flock prevalence of *Campylobacter* in Spanish broiler houses, and that fly screens may add further to the effect of the implemented biosecurity practice.



### Task 2.2 Phage therapy (M1-48)

The work of Task 2.2 results in Deliverables 2.2.1 (Submitted), 2.2.2 (M48 - Delayed) and 2.2.3 (M48 - Delayed).

The main goal of this task is to go further than the proof of principle that phage therapy by oral administration of lytic phages to broilers is feasible for the control of *Campylobacter*. The aim now is to translate previous results from experiments under controlled conditions to more realistic field conditions. As part of this task (subtasks 2.2.1/2) a cocktail of phages was produced with a host-range broad enough to ensure the killing of most *Campylobacter* strains. Both CVI and UMinho have finished this subtasks and a mixture of 4 phages (CVI: Cje 4, 12, 16, 21 or Uminho: 3D, 3E, 6E, New) has served as a phage cocktails in trials.

#### Subtask 2.2.1: Analyse the two combined sets of bacteriophages for optimum host spectrum

This task finished in the First Reporting Period.

#### Subtask 2.2.2: Isolate new phages

Until the end of the project the isolation of new phages has been undertaken since still a few isolated or *in vitro* induced resistant *Campylobacter* strains remained in the *Campylobacter* strains collection of CVI/Uminho. With the end of the project this task was finished recognizing that the established phage cocktail for trials had a very broad, but not complete, host-range for *Campylobacter*.

#### Subtask 2.2.3: Small-scale phage production

Phage production protocol has been established by using T-flasks with high surface area. In this case, the maximum titre obtained was  $1.69E+09$  PFU/ml. It was notice that the period that the plaques were left shaking at 4 °C with SM buffer is an important factor. When the plaques stayed shaking for three days the title was  $1E+10$  PFU/ mL. This last system was chosen to produce phages for the *in vivo* trials and has successfully been carried out, thereby finishing this task.

#### Subtask 2.2.5: Efficacy of phages under experimental conditions

Different experimental conditions for phage therapy of naturally infected broilers were tested (treatment group n=10), varying inoculum size ( $10^5$ - $10^9$  PFU/animal) and daily vs. one time (day 35) phage application. Results were highly variable but never showed a statistical significant reduction of *Campylobacter* more than as was found for a daily treatment with  $10^9$  PFU/animal that only showed a temporary reduction of 1 logs, similar in pattern to the temporary 2-3 logs reduction demonstrated by Wagenaar *et al.* ([Vet Microbiol.](#) 2005 Aug 30;109(3-4):275-83), which encompass experimental infected broilers with a susceptible *Campylobacter* strain and lytic phage.

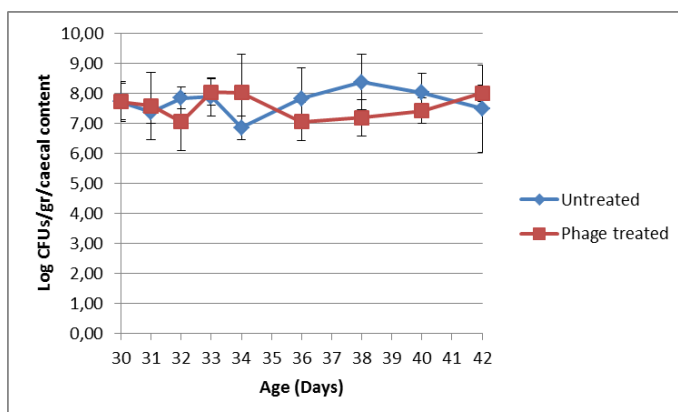


Fig. *Campylobacter* culturable cells of phage treated (daily orally  $10^9$  PFU) vs untreated broilers.

#### Subtask 2.2.6: Establish a system for the production of the phages

Phage production was optimized in previous tasks and according to the results the highest yield of production was obtained in solid cultures. It is harder to scale-up solid cultures than liquid cultures, 500 cm<sup>2</sup> tissue culture plates were used to produce high amounts of phages to be used in the large field trials (subtask 2.2.7).

*Subtask 2.2.7: Trial under field conditions*

No statistical reduction in *Campylobacter* loads were found in the previous reported field trials. The strong biological variation in the field had led to standard deviations greater than 100% of the average. In order to reduce the variability among samples a final field trial was performed and the number of samples collected were greater than in the previous trials. In addition to that, 2 administrations of the phage cocktail in 2 consecutive days were performed (Figure 1). The results, although with a lower standard deviation, showed no decrease of *Campylobacter* loads after phage application.

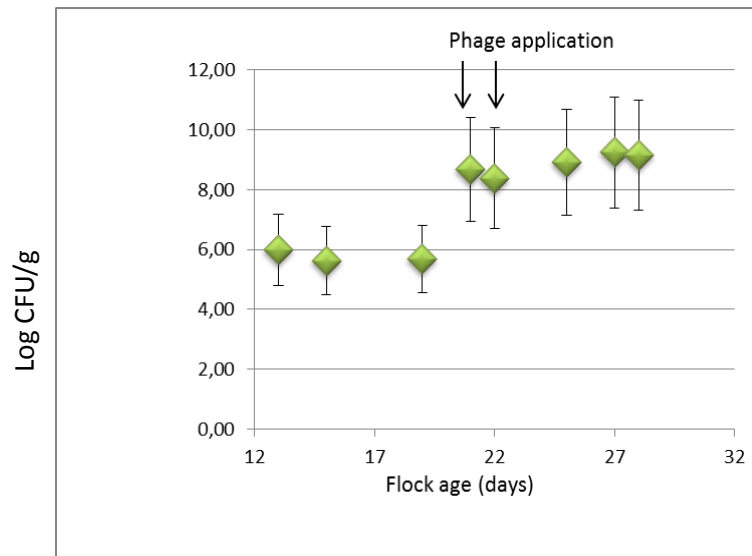


Figure 1. *Campylobacter* culturable cells present at the caecal content over time. The amount is expressed in cfu per g of caecal content and the values are averages of 20 birds sacrificed at each time point.

**Extra-Task**

In order to understand the failure of phage therapy in the tested conditions 3 hypothesis were tested:

- i) Phage inactivation due to emergence of resistance
- ii) Phage inactivation due to GI tract environmental conditions (namely pH)
- iii) Phage inactivation by the presence of other microorganisms ruled by quorum sensing

## i) Phage inactivation due to emergence of resistance

To study acquired resistance of *Campylobacter* a parent strain was compared to an *in vitro* induced resistant daughter strain. After whole genome sequencing 30 SNPs were identified that need further analysis and confirmation. No sequence differences were found between parent and daughter strain for CRISPR-arrays and the hypervariable Cj1412 genes as both are involved in acquired resistance to phages.

## ii) Phage inactivation due to GI tract environmental conditions

CVI tested their phage cocktail in response to two physical parameters known to influence efficacy of phage lysis: pH and bile salts. It was shown that over a pH-range of ~3 to ~8 lytic phages Cje 4, 12, 16, 21 were insensitive with respect to lytic potential. Furthermore the phages also remained equally lytic in the presence of 0.1 % deoxycholate (DOC), which is regarded as a bile salt known to trigger phenotypical changes in *Campylobacter* (Malik-Kale *et al.*, J. Bacteriol. **April 2008** vol. 190 no. 7 **2286-2297**)

## iii) Phage inactivation by the presence of other microorganisms ruled by quorum sensing

To test the influence of the GI tract microbiota on phage efficacy, *in vitro* assays were performed in which the phage activity was measure in mixed cultures of *Salmonella* and *E. coli*. The results revealed that in the presence of *Salmonella* and/or *E.coli*, phage replication ability reduced approximately 3 logs.

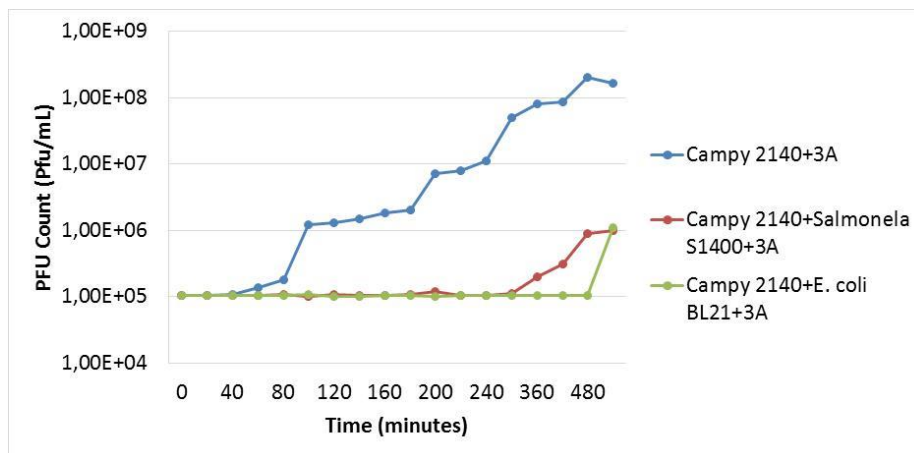


Figure 2: Phage replication in the presence of *Campylobacter* and *Campylobacter* and *Salmonella* or *E. coli* measured in PFU/mL

To further exploit these results, phage infection ability was performed in the presence of supernatants of mixed cultures of *Campylobacter* and *Salmonella* or *E. coli*

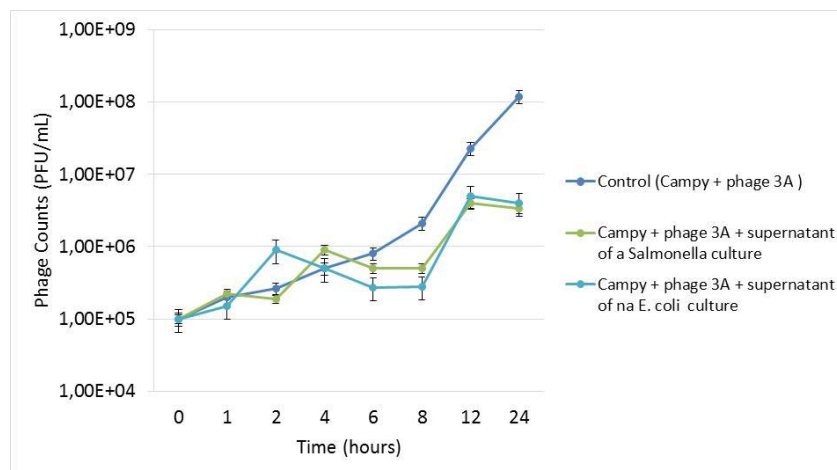


Figure 3: Phage replication in the presence of *Salmonella* and *E. coli* supernatants measured in PFU/ml

The phage titre was 2 logs lower in the presence of supernatants of *Salmonella* and *E. coli* cultures, meaning that metabolites secreted by these species might have inhibited phage interaction with *Campylobacter*, which can probably be quorum sensing molecules. Further studies on the metabolome of these cultures and the effect of QS in phage replication need to be done in order to prove this hypothesis. Nevertheless the in vivo failure of phage therapy might be attributed to the presence of other species in the GI tract of the chickens

Conclusion

Although lytic *Campylobacter* phages, can be discovered and *in vitro* broad spectrum phage cocktails can be produced, in field no statistical significant reduction of *Campylobacter* by oral administration in broilers has been demonstrated.

### Task 2.3 Vaccination (M1-48)

The work of Task 2.3 results in Deliverables 2.3.1 (Submitted), 2.3.2 (Submitted), 2.3.3 (M36 - Delayed) and 2.3.4 (M48 – ready for submission).

We previously designed a candidate *Campylobacter* subunit vaccine with intrinsic adjuvant activity for use poultry and demonstrated that embryonated eggs express the TLR receptor that is the target of the adjuvant. During the first 18 months of the project, we successfully produced and purified the candidate vaccine, immunized 18-day embryonated chicken eggs with the engineered flagellin subunit vaccine, and analysed the generated antibody response using a newly developed ELISA. In the 2<sup>nd</sup> period (M19-36) we reported *in ovo* experiments with glycosylated, a non-glycosylated flagellin and a whole cell vaccine. The vaccines induced immune responses (IgG and to a lesser extend IgM and IgA). However, these responses were not protective against a challenge. There was a dose dependent immune response and therefore for future experiments a higher dose will be tested. In addition, the experiments will be extended with glycosylated recombinant subunit vaccine (NHC). Administration of a higher vaccine dose required optimal production of the antigen. During the production of the vaccine components, it was noted that the production of the glycosylated product was repeatedly unstable: only in rare cases batches produced a reasonable amount of antigen but most batches failed to produce any glycosylated protein. Until now the antigen was present on a plasmid and this was most probably the reason that the production was instable.

To improve the stability of the production of the engineered flagellin and its degree of glycosylation, we moved the flagellin gene from the thus far used expression plasmid onto the bacterial chromosome. This so-called “next generation” vaccine strain was expected to result in a more stable and homogenous production for glycosylation of the flagellin. Despite the successful insertion of the vaccine-encoding gene into the bacterial chromosome, the yield of glycosylated vaccine was not improved. For the secretion of flagellin *Campylobacter* uses additional proteins (so called chaperones proteins). Currently, chaperones for *Campylobacter* flagellin are used to optimize the production, secretion, and purification of the glycosylated vaccine antigen. The immunizations are foreseen in the second half of 2015 and therefore after the finalization of the project. As this immunization was at the start not identified as a specific deliverables, all the deliverables and milestones were met during the project. Vaccine development was seen as a long term activity for the control of *Campylobacter*.

Conclusion: a series of *in ovo* experiments during the project have provided more insight in the immune response of the possibilities of vaccination with a *Campylobacter* antigen with intrinsic adjuvant activity. The need for glycosylation of the subunit-vaccine was confirmed during the project and essential fundamental knowledge of the immune response of chickens against *Campylobacter* was obtained; this is/will be published and is available for the scientific community.

### Significant Results

Results from UK have confirmed the anticipation, already set in the original application, that high level biosecurity is crucial to get benefit of fly screen. To meet such level of biosecurity in Spain took much more effort and time than expected. Although the study in Spain will not be final at the end of CamCon, preliminary results of biosecurity and fly screens seem promising by April 2015.

Although lytic *Campylobacter* phages, can be discovered and *in vitro* broad spectrum phage cocktails can be produced, in field no statistical significant reduction of *Campylobacter* by oral administration in broilers has been demonstrated.

*In ovo* vaccination of embryonated chicken eggs with a novel subunit vaccine and whole cell vaccine with natural adjuvant activity have been demonstrated to generate antibodies against *C. jejuni* that can be detected to up to 23 day after hatching. Experiments to test the efficacy of the vaccine in protection against *C. jejuni* colonization have not yet been successful, but this may change when larger quantities of glycosylated protein vaccine can be produced.

### Deviations and corrective actions

Several deviations and corrective actions have been necessary to conduct task 2.1. This is described in detail in the 'Amendment no. 1' of 9 Nov 2012. In short, the work planned in UK was joined into the Defra project, both during the original Defra period and as a 9 months continuation at the end of the original Defra period. The reasons and plans for cooperation with the Defra project were described in the P1 report.

A further delay was faced during the 3<sup>rd</sup> reporting period, due to the more than expected time needed to get biosecurity in Spain implemented to a sufficient degree. So, task 2.1 cannot be ended until November 2015. The study will however be undertaken by partner CReSA after end of the CamCon period.

### Use of resources WP2

No large deviations from the planned use of resources. However, several participants have adjusted their budget between consumables and person cost compared to the budget to optimize the project. Details on the economical reporting are presented in the Final Report.

## WP3 Development of detection methods and monitoring regimes

The specific objectives for WP3 are as follows:

- To get an estimate of the quantities of airborne *Campylobacter* in broiler houses.
- To develop a detection method suited for quantification of airborne *Campylobacter*.
- To provide sample material for typing activities of WP1.
- To assess the feasibility of a real-time monitoring approach.
- To identify appropriate technologies to solve this objective.
- To develop a document on future research needs.

### Summary of individual task progress

#### Task 3.1 Development of methods of quantification of *Campylobacter* in air (M13-24)

The work of Task 3.1 results in Deliverables 3.1.1, 3.1.2, 3.1.3 and 3.1.4 (all Submitted).

The majority of scientific work was done in the First Reporting Period. All Deliverables were submitted during the Second Reporting Period.

#### Task 3.2 Feasibility of real-time monitoring of *Campylobacter* in broiler flocks (M1-48)

The work of Task 3.2 results in Deliverable 3.2.1 (Submitted).

A manuscript focusing on assessment of airborne particle size distribution under various farming conditions and ventilation systems and quantification of airborne *Campylobacter* in relation to airborne particle size distribution has been published. The approach has been tested in Poland and Denmark.

Low-cost monitoring of *Campylobacter* in poultry houses using enumeration by air sampling and quantitative PCR (2014). Søndergaard, M.S., Josefsen, M.H., Löfström, C., Christensen, L.S., Wieczorek, K., Osek, J., & Hoorfar, J. *Journal of Food Protection* 77, 325-330.

#### Task 3.3 Report on future research needs (M42-48)

The work of Task 3.3 results in Deliverable 3.3.1 (Submitted).

A manuscript has been published: Monitoring *Campylobacter* in the poultry production chain - From culture to genes and beyond (2015). Josefsen, M.H., Bhunia, A.K., Engvall, E.O., Fachmann, M.S.R., & Hoorfar, J. *Journal of Microbiological Methods* 112, 118-125.

### Significant Results

Air sampling on filters, coupled with qPCR, was able to detect *Campylobacter* colonization before it could be detected in boot swabs and was found to be a promising future technique for monitoring of *Campylobacter*.

### Deviations and corrective actions

No deviations or corrective actions in the present period.

### Use of resources WP3

No large deviations from the planned use of resources. Details on the economical reporting are presented in the Final Report.

## WP4 Risk assessment and economics

The specific objectives for WP4 are as follows:

- Define and communicate data needs and data quality for risk assessment and economics.
- Collect and compile data for risk assessment and economics.
- Develop microbiological risk assessment model for primary production in different geographical regions in Europe based on results from WP1 and WP2.
- Estimate the relative decrease in public health risk consequential to interventions at farm.
- Estimate costs of interventions at farm in selected participating countries (the Netherlands, Denmark, Norway, the UK, Spain and Poland).
- Estimate cost-effectiveness and cost utility of interventions at farm.
- Compare the cost-effectiveness of interventions at farm with interventions post-farm.
- Communicate results on the most cost-effective interventions to guide risk management decisions.
- Identify and communicate future data needs.

### Summary of individual task progress

#### Task 4.1 Risk assessment (M1-58)

The work of Task 4.1 results in Deliverable 4.1.1 (M58 - Delayed).

In agreement with the planning, the development of the risk assessment has continued. The progress and plans have been presented and discussed during the annual meeting 2014, the results were presented at the final meeting 2015.

The risk assessment has been split in two separate activities, that required their own approach and have resulted in two different research papers, one more than originally promised.

The first activity relates to interventions at the farm that result in a reduction in flock prevalence. This study was primarily based on the risk factor study performed in WP1. The risk factor study has identified which farm related risk factors do have a significant impact on the *Campylobacter* flock prevalence in the different countries involved in CamCon. These risk factors have been translated into practical control measures (interventions). Based on the collected data and the risk factor study, the effect of implementation of these interventions in the six different countries has been analysed. The results are summarized in the table below. The interventions that are predicted to have a significant effect are “building an anteroom and barrier in all houses”; “reduction of the downtime to less than ten days between flocks, together with rodent control and disinfection”; “building new houses if houses are > 15 years of age (houses with anteroom and barrier, using nipples without cups and with designated tools per house)”; “apply drinkers with nipples without cups”; “have designated tools in all houses < 15 years old”.

Three additional interventions were included in the analysis. The results of WP1 did not allow an analysis of these interventions due to data limitations in the 20 farms study of WP1. Still, they are considered effective in the literature. Their effects have been analysed on the basis of previous research done by EFSA (a ban on thinning and reduction of the slaughter age to < 35 days) and by Bahrndorff et al. (2013) (fly screens applied to new houses in Denmark). The results of the studies on fly nets performed in WP2 were not available on time to be included in the analyses.

The percentage of farms involved in the intervention reflects the farms that are amenable for the intervention: in those farms the intervention is not implemented already and it is possible to do it. If this percentage is large, a larger effect of the intervention may be expected.

The change in prevalence is calculated on the basis of the flock prevalence reported by EFSA on the baseline study 2008. From these results both the relative reduction in prevalence and the absolute reduction in prevalence can be derived. From previous risk assessment activities (e.g. Nauta et al 2009) it is assumed that the human health risk of campylobacteriosis from (domestically produced) broiler meat is proportional to the flock prevalence.

It can be seen that none of the interventions sticks out as the single solution. The highest effect is achieved for building new houses in Spain. The general insight that biosecurity is crucial stands, all effective intervention somehow imply an improvement of biosecurity and should preferably be applied in combination.

Intervention	from	already OK if	intervention	involved in intervention (% of all farms)						Campylobacter prev. after control <sup>2</sup>							
				DK	ES	NL	NO	PL	UK	DK	ES	NL	NO	PL	UK		
anteroom barrier in new houses	WP1	both physical barrier and anteroom in all houses < 15 yrs	build anteroom and barrier (assume not both = none) in all houses < 15 yrs	27.5	30.6	48.8	37.2	39.9	36.8	17.4	84.2	21.0	2.9	77.0	71.5		
down time	WP1	downtime <10 days	reduce downtime to < 10 days + rodent control in 30 % of these farms + disinfection (improved cleaning) between all crops at the end of downtime in 3 % of these farms	20.6	89.3	25	96.3	84.1	15.4	17.8	79.0	22.5	1.9	72.9	74.3		
new houses <sup>3</sup>	WP1	< 15 years of age	build new house if > 15 years + anteroom and barrier+ nipples without cups+ dedicated tools	42.2	66.3	34.4	30.5	49.0	48.7	14.9	38.1	17.9	2.4	76.0	55.5		
drinkers	WP1	nipples without cups	apply nipples without cups in all farms	85.3	81.1	50.8	68.9	67.8	78.6	14.5	80.3	20.9	2.5	75.1	67.0		
dedicated tools per house in new houses	WP1	each new house (<15 yrs) has its own tools (basic equipment)	have dedicated tools in each house < 15 yrs	2.0	25.5	32.8	7.9	8.2	36.8	18.9	82.4	20.3	3.1	78.7	67.3		
no thinning <sup>4</sup>	EFSA	no thinning applied	stop thinning	24.8	49.1	42.2	3.3	49.4	63.4	16.5	80.4	18.4	3.2	69.6	60.0		
lower slaughter age <sup>5</sup>	EFSA	35 days or less	slaughter at 35 days	85.0	94.6	85.3	5.1	97.4	80.2	17.2	75.5	19.0	3.3	66.5	66.9		
fly screens (in new houses)	WP2	applied for all houses	apply fly nets in houses < 15 years	33.0						16.6							
boot dips	WP1, ns <sup>6</sup>	boot dips available in all houses	start applying boot dips	not done						have no effect							
improved cleaning	WP1, ns	Disinfection between all crops	start disinfection	not done						combined with downtime							
rodent control	WP1, ns	Farmer applies rodent control > 6 times per year	start application of rodent control	not done						combined with downtime							

<sup>1</sup>Camcon data <sup>2</sup>based on EFSA baseline 2008 <sup>3</sup>the only intervention with different effects per country. <sup>4</sup> Using data and models from EFSA (2010; 2011). <sup>5</sup>current prevalence based on EFSA <sup>6</sup>ns = no significant effect in WP1 study <sup>7</sup> but p<0.05 from two different runs

The second activity relates to interventions that impact the *Campylobacter* concentration in the intestinal content of live chickens. The plan was to apply results of WP2 on the effectivity of vaccines and bacteriophages, but no quantifiable results were obtained in CamCon. Therefore this study more generally considers the question whether a reduction of *Campylobacter* concentration in the chicken caeca or chicken faeces at the entrance of the chicken processing plant can be translated to a reduction in human health risk of campylobacteriosis, by the use of a risk assessment model. Two approaches are compared: one where a linear regression of log transformed data on concentrations in the birds caeca and on concentrations on skin samples of the same flocks are analysed, and one where a previously published risk assessment model is applied. Results are illustrated by the graphs below.

Roughly the results may seem similar (with a considerable risk reduction predicted with a 1 log reduction of the mean, i.e. between 45% and 80% relative risk reduction, depending on the assumptions and the country). However, in general it is not possible to derive a valid “rule of thumb” that allows one to simply translate a reduction in concentration in the intestinal content to a risk reduction. Reasons for this complexity are that different data sets may predict very different relations between concentrations in the faeces and in chicken skin, which is also predicted by the risk assessment model. Also, some assumptions in the risk assessment model (like the relation between concentrations in the faeces and on the carcass exterior) have an impact on the predicted effect, whereas there are no data available to analyse these assumptions. Possibly the variation in performance between slaughterhouses is too large to allow the derivation of a rule of thumb anyway.



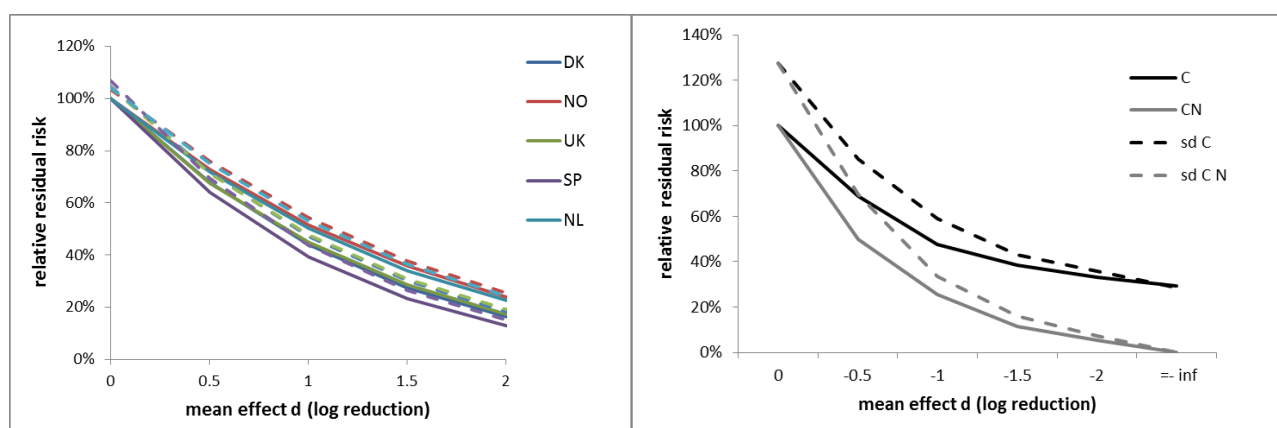


Figure: Relative residual risks after reduction of the mean concentration in broiler faeces by  $d$  log, estimated by analysis of a linear regression model using data from five countries involved in CamCon (left) and a risk assessment model (right).

#### Task 4.2 Data collection and compilation (M13-58)

The work of Task 4.2 results in Deliverable 4.2.1 (M58 - Delayed).

The data collected in this WP have been gathered and analysed and a report has been written that describes the data and some basic analyses of the data, which allowed us to use them in other tasks of WP4.

The report contains the following sections:

1. Literature survey on *Campylobacter* in the broiler chain
2. *Campylobacter* in caeca and on broiler carcasses in Norway
3. *Campylobacter* in broiler caeca in the UK
4. Interpretation of *Campylobacter* data from pooled caecal samples, Spain
5. *Campylobacter* in Poland
6. The impact of thinning and slaughter age (data analysis to support risk assessment)
7. The impact of fly screens (data analysis to support risk assessment)
8. Data on costs

#### Task 4.3 Economics (M13-60)

The work of Task 4.3 results in Deliverable 4.3.1 (M60).

In agreement with the planning, the research in the area of economics has been finalized. The progress and plans have been presented and discussed during the annual meeting 2014.

The results of the study have been described in a research paper. A model was developed to analyse the impact of average technical and economic farm performance on the costs of *Campylobacter* control measures on broiler farms in Denmark, the Netherlands, Norway, Poland, Spain and the United Kingdom. Results (see the table below) show that costs of *Campylobacter* control measures on broiler farms vary significantly between countries. This is caused by differences in general cost levels between countries and by differences in average technical farm performance. For studies to correctly estimate cost-effectiveness of *Campylobacter* control measures across countries, differences in average technical and economic farm performance between the countries must be considered.

	Average costs per intervention measure (€/100 chickens housed/year/farm without measure)					
	Denmark	Spain	Netherlands	Norway	Poland	UK
Discontinue thinning	48.41	35.56	39.05	58.08	28.26	34.81
Anteroom with hygiene barrier	0.11	0.34	0.16	0.76	0.12	0.29
New houses	126.90	97.76	57.45	174.73	73.10	164.69
drink nipples without cup	20.48	16.46	18.10	25.91	14.93	19.04
Slaughter at 35 days <sup>1</sup>	61.55	118.38	74.13	-	35.64	118.13
Maximum downtime ten days <sup>1</sup>	18.39	2.08	12.69	-	-21.98	14.69
Dedicated tools	0.12	0.26	0.18	0.27	0.26	0.13
Fly screens <sup>2</sup>	0.83	-	-	-	-	-
<sup>1</sup> not estimated for Norway (average delivery at 33 days; legal maximum number of broilers per year)						
<sup>2</sup> only estimated for Denmark (effectiveness only provided for Denmark)						

#### Task 4.4 Cost-effectiveness of interventions at farm and comparison with interventions post farm (M31-58)

The work of Task 4.4 results in Deliverable 4.4.1 (M58 - Delayed).

The results of task 4.1 and task 4.3 have been combined into a cost effectiveness analysis of interventions at farm. The basis of this work is the interventions listed in the table shown for task 1 and the cost associated to them. By including published campylobacteriosis incidences in the different countries involved in CamCon, and published values for the attributable fraction of chicken meat, a factor to translate incidence to DALY (disability adjusted life years) and trade data between countries, costs and cost effectiveness of interventions could be analysed.

It is found that the differences between different countries are large, which makes it hard to draw general conclusions on the cost effectiveness of control measures. Still, some conclusions can be drawn.

- The control measures “building an anteroom + barrier” and “designated tools for each house” are cheap and result in a significant risk reduction
- In some countries (especially Denmark and Norway) the costs of control measures are much higher than in others. These are the countries where the *Campylobacter* prevalences are already relatively low.

#### Task 4.5 Future data needs (M43-60)

The work of Task 4.5 results in Deliverables 4.5.1 (M60) and 4.5.2 (M60).

The major outcomes of the WP and future data needs that are identified are summarized in two reports, the two deliverables of this task.

The major outcomes are the conclusions of tasks 1, 3 and 4

The future data needs that have been identified are based on the discussions. In general, it is difficult to formulate “generic” data needs, as they depend on the question at hand.

#### Significant Results

Significant risk factors at the farm have been identified and translated to practical control measures. They have been analysed for their effectivity in reducing the flock prevalence in the six countries involved in CamCon. The differences between countries are considerable and none of the interventions is much better than the others.

It is not possible to derive a simple rule of thumb to relate reduction in *Campylobacter* concentration in the intestinal content of chickens to reduction in concentration on the skin of human health risk. The reason for this is a combination of the existing variability in concentrations and processes, and the uncertainty of their impact.

Costs of control measures vary between countries, which is caused by differences in general cost levels between countries and by differences in average technical farm performance.

The cost effectiveness study, again, shows large variation between countries. Results were obtained and presented. Cheap interventions that results in significant risk reduction at population level are the use of separate designated tools per farmhouse and building an anteroom and barrier.

#### Deviations and corrective actions

None

#### Use of resources WP4

No large deviations from the planned use of resources. However, several participants have adjusted their budget between consumables and person cost compared to the budget to optimize the project. Details on the economical reporting are presented in the Final Report.

## WP5 From science to industry

The specific objectives for WP5 are as follows:

- To create a Best Practice Manual in a user friendly format to aid in the production of low-risk *Campylobacter* chickens in EU.
- To upgrade skills of key parts of the broiler business.
- To provide the primary production level with a web-based state-of-the-art educational tool.
- To build up distribution channels for the E-learning programme from top to bottom of the broiler business pyramid.
- To provide the European Commission and other regulatory bodies with a Best Practice Format that could be used for quality assurance and certification of broiler production
- To provide the poultry industry with a Certification Programme that may be used as a tool aiding company policies for improvement of *Campylobacter* reduction.

### Summary of individual task progress

#### Task 5.1 Best Practice Manual for production of *Campylobacter*-free chickens (M13-56)

The work of Task 5.1 results in Deliverable 5.1.1 (Submitted).

The specific objective of this task is to create a Best Practice Manual to aid in the production of low-risk *Campylobacter* chickens in the EU. Due to specific needs in WP2, task 2.1, work in Spain with upgrading of biosecurity practices for fly screen test houses has served as a test case for creating the final version of the Best Practice Manual. This initial work has included the production of biosecurity posters with easily comprehensible drawings and illustrations, and two illustrated Power Point presentations, one with step-by-step instructions for correct entry and exit from poultry houses and one covering biosecurity control points included in the check list used.

Further work on the Best Practice Manual has been carried out since the last report by incorporating existing and new knowledge from the CamCon project partners on *Campylobacter* prevention and control. By the incorporation of up-to-date information and knowledge created through the research activities in the other work packages of CamCon, and by aligning the Best Practice Manual with the other educational products from this Work Package, the Best Practice Manual has now been completed.

As biosecurity forms a central part in the procedures for *Campylobacter* prevention at poultry farms. The manual aims at highlighting and explaining biosecurity procedures in a way that can be implemented directly by the poultry industry. It is important to stress that the manual cannot stand alone, but should be integrated in existing producer guidelines and quality programs for poultry meat production.

The Best Practice Manual was presented at the CamCon stakeholder seminar on April 14<sup>th</sup> 2015, and is now available at [www.camcon-eu.net](http://www.camcon-eu.net). In addition to this, the manual has been distributed as hard copies together with the Draft Certification Program (see Task 5.3) according to the distribution plan (see task 5.2). As the Best Practice Manual has been temporally dependent on completion of work in the other CamCon work packages the task has been completed with some delay, but within the project period.

#### Task 5.2 Specific targeted learning programmes for proficiency in implementing the "Best Practice Manual for production of *Campylobacter*-free chickens" (M13-60)

The work of Task 5.2 results in Deliverables 5.2.1 (Submitted) and 5.2.2 (Submitted).

The specific objectives of this task are to establish a web-based educational tool, and to build up distribution channels for the resulting E-learning programme to the broiler industry.

As previously reported we had to terminate the cooperation with our original subcontractor, Conzentrate. Following this, and with the approval of the Commission, we initiated negotiations for a new subcontractor with two experienced companies and selected one of these – Context – and entered into an agreement and production plan that could be accommodated within the remaining budget.

Obviously, the change of subcontractor has caused delays in the work on this deliverable, but we have been very satisfied with the cooperation and outputs delivered by the new subcontractor, and all learning modules and a web-based test have now been produced within a tight time plan. They will be available in an English and a Spanish version.

The comprehensive E-learning program on *Campylobacter* and biosecurity may be used by poultry producers and poultry advisors to train and educate farm staff. It goes systematically through the risk factors for *Campylobacter* introduction at farm and house level in primary poultry production, and underlines the importance of biosecurity procedures in the control and prevention of *Campylobacter*.

A distribution plan creating distribution channels for the educational products has been completed. The distribution list includes all major European poultry companies as well as poultry producer organisations and EU and national competent authorities. In addition, the E-learning program was demonstrated at the CamCon stakeholder seminar on April 14<sup>th</sup> 2015, and is now available at [www.camcon-eu.net](http://www.camcon-eu.net). The task has been completed with some delay, but within the project period.

#### Task 5.3 Voluntary Certification Programme (M37-60)

The work of Task 5.3 results in Deliverable 5.3.1 (Submitted).

The Voluntary Certification Program is based on outputs from Task 5.1, and has been aligned to the E-learning program developed in Task 2. The Draft Certification Program provides poultry companies and independent auditing bodies with a complete list of measures that should be implemented and checked regularly in order to ensure and document a uniform quality production of broilers with a reduced risk of *Campylobacter*. The task has been completed according to plan (April 2015).

#### Significant Results

The dissemination and communication of up-to-date scientific knowledge on *Campylobacter* control and prevention to stakeholders in the poultry industry has been achieved through the production of educational material and learning tools. These comprise a Best Practice Manual, a Draft Certification Program and a web-based E-learning Program on *Campylobacter* and biosecurity as a high level of biosecurity at house level is essential for successful reduction of *Campylobacter* in primary poultry production.

The Best Practice Manual aims at highlighting and explaining how these procedures can be implemented directly by the poultry industry while the Draft Certification Program provides poultry companies and independent auditing bodies with a complete list of measures that should be implemented and checked regularly in order to ensure and document a uniform quality production of broilers with a reduced risk of *Campylobacter*. The comprehensive e-learning program on *Campylobacter* and biosecurity may be used by poultry producers and poultry advisors to train and educate farm staff in support of this.

#### Deviations and corrective actions

The progress in WP5 has been satisfactory, and all milestones and deliverables have been achieved within the project period.

#### Use of resources WP5

No large deviations from the planned use of resources. Details on the economical reporting are presented in the Final Report.

### 3. Project management during the period

#### Consortium management tasks

##### Task 6.1 Consortium Agreement signed data needs (M0)

The Consortium Agreement was signed by all participants before the project started.

##### Task 6.2 Management support team appointed (M2)

At NVI several staff members are supporting the Coordinator regarding financial and administrative aspects of the project.

##### Task 6.3 Project web site established (M4)

The web page was launched October 2010. The page is regularly updated, both the public domain and the participants pages.

##### Task 6.4 Plan for the use and dissemination of foreground (M50)

A draft Communication plan approved by all participants was placed on the project's internal web pages in December 2011. It was revised during the Third Period. In addition, a list of publications/tentative publications coming from the project is being maintained in the Minutes for the Quarterly Meetings. Reference to published papers is placed on the project's public web pages.

##### Task 6.5 Reports of project's meetings

The Minutes from all meetings are sent out to all participants and to the Advisory Board (and the Scientific Officer in the Commission) as soon as they are approved. All Minutes are also placed on the project's internal web page.

##### Annual Meetings:

- Kick-off meeting at DTU in Copenhagen May 2010
- Annual Meeting at DTU in Copenhagen April 2011
- Annual Meeting at DTU in Copenhagen April 2012
- Annual Meeting at CHRO in Aberdeen, September 2013
- Annual Meeting at CRESA in Barcelona, April 2014
- Final Meeting at DTU in Copenhagen, April 2015

In addition to the Annual Meetings, Quarterly Meetings have been held. After agreement with the project participants, these have mainly been held as "email-meetings" or Skype meetings and in two instances it was decided that the process around the drafting of the 1<sup>st</sup> and 2<sup>nd</sup> Periodic Report should replace ordinary Quarterly Meetings. A few physical meetings have also been held within WPs.

##### Task 6.6 Regular reports to the European Commission (M20, M38, M60)

All three Periodic Reports and the Final Report were delivered on time.

#### Other management tasks

The Executive Board have had regular meetings in connection with the Annual Meetings. In addition, there have been - as part of the Quarterly Meetings - questions to the WP leaders and to all the participants if there are any managerial problems or issues for discussions.

The major task in the third period was the Request for Amendment sent in September 2014 and approved in October 2014. The amendment was a change in a Subcontractor in WP5.

The Financial assistant asked the administrative contacts in each participating institution for a financial status report midway between 1<sup>st</sup> and 2<sup>nd</sup> Periodic Report and again midway between 2<sup>nd</sup> and 3<sup>rd</sup> Periodic Report, mimicking the reporting requirements for the Periodic Reports. These have been compiled and evaluated by the Coordinator. Not every institution delivered the report, but this procedure has at least made the participants aware of the requirements for the Periodic Report.

### Project planning and status, problems and their solution and impact

The main problem with CamCon was to find farms willing to participate in the Task 2.1 (fly screens). A large amount of work was done to overcome this problem during the Second Reporting Period. After that, the project has developed reasonably well in relation to the updated plan.

All Deliverables was delivered before the end of the Project, however, some of them in a “preliminary form, i.e. describing a draft Abstract and a planned process (who, when, where) for publication.

### Coordination and communication activities

The coordination of the project has gone according to plan with regular meetings.

A tentative list of scientific publications from CamCon was established early in the project, and has been updated regularly. This list is attached to the Minutes from all Quarterly Meetings.

The majority of Participants are closely in contact with the poultry industry, and CamCon is presented at various meetings and in other ways to the industry.

During the last month of the Project, a Stakeholder Seminar, presenting all results in CamCon, was held in Copenhagen. The meeting was in collaboration with the EMIDA project CamChain. A total of 66 participants registered for the meeting, of which more than 25 were from the industry.

### Changes in Consortium or legal status of the beneficiaries

The Subcontractor in WP5 was changed during the last year of the project. The new Subcontractor has delivered according to plans.