Core of the report for the period

1. Project objectives for the period

The overall strategy of the work plan is 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 is 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 will be developed for monitoring and quantification, but also to gain epidemiological insight. A risk assessment will also be performed. At the final stage a certification programme will be proposed to producers and regulators. In order to ensure technical integration of the WPs, harmonized protocols for sampling and methodologies will be produced at the beginning of the project.

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

Task	Title	Covered in 1st periodic Report (Month 1-18)	Month 19-24	Month 25-30	Month 31-36	End month
1.1	Risk factors for *Campylobacter* 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 *Campylobacter* to broiler flocks					60
1.4	Distribution of *Campylobacter* 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 *Campylobacter* in air					24
3.2	Feasibility of real-time monitoring of *Campylobacter* 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 *Campylobacter*-free chickens					57
5.2	Specific targeted learning programmes for proficiency in implementing the "Best Practice Manual for production of *Campylobacter*-free chickens"					60
5.3	Voluntary Certification Programme					60

Some Deliverables and Milestones are still planned to be finalised during the 1st and 2nd Reporting Period. These are described more in detail in the Tables in Chapter 3 and in Chapter 2 describing each Work Package more in detail.

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).

By combining retrospective data on the *Campylobacter* status of broiler flocks, from national surveillance programmes in 2010 and 2011, with the data obtained from the above questionnaire survey, a risk factor analysis has been carried out on data from Norway and Denmark. The *Campylobacter* data were obtained through existing surveillance programs for *Campylobacter.* Farm data from 107 Danish and 173 Norwegian farms, *Campylobacter* status from approximately 5560 flocks, and 44 explanatory variables were included. 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, 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*.

An abstract describing the result of this study has been submitted for presentation at 17th International Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO) 2013, September 2013. Furthermore, manuscript is in draft, to be submitted to a peer-reviewed journal during summer 2013.

The next step will be to extend the model to include data 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.

UK: Caeca sampling commenced in June 2011 and to date, 229 batches of caeca have been sampled from 20 different farms at first thinning. One hundred and two (44.5%) of these were positive for *Campylobacter,* with evidence of seasonality amongst positive flocks. Questionnaires and batch health and management data has been collected and stored for analysis alongside the caecal prevalence data later in the study.

Spain: Caeca sampling started in June 2011. To date, 287 batches of caeca from either first and/or final depopulation have been sampled from 20 farms. Two hundred and four (70.9%) of these have been positive for *Campylobacter*. Seasonality has been observed, with a wide period of warm months and high *Campylobacter* prevalence. Most of the flocks have been *Campylobacter* positive both at thinning and at final depopulation. Also, questionnaires with information related to farms, batch health and management, as well as weather conditions are being collected from all sampled flocks and stored for a risk factor analysis together with the caecal prevalence data.

Poland: The sampling in Poland is now complete on the 30 farms which were located around different parts of Poland from February 2011 to May 2013. In total, 225/275 flocks at final depopulation were positive for *Campylobacter*, with 137 flocks (60.9%) positive for *C. jejuni* and 88 (39.1%) for *C. coli*.

The Netherlands: To date, of the 24 farms being sampled, 43/134 (32.1%) flocks were positive for *Campylobacter*, with 0-100% of the flocks per farm colonized.

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), 1.2.4 (M46).

UK: Eight broiler farms from two major UK poultry producers were selected, and one study house selected on each farm. To date, a total of 96 flocks have been followed longitudinally (ranging from 11 to 14 crop cycles per farm so far), of which 48 flocks (50.0%) have become *Campylobacter*-positive. The average age at which flocks become positive was 23.7 days, with the earliest detection of *Campylobacter* to date being at 9 days of age. None of the eight farms have remained consistently negative throughout. The highly sensitive boot sock method allowed detection of *Campylobacter* in some cases where it was not detectable by culture, and as a result of this, *Campylobacter* has so far been detected in broiler flocks much earlier than anticipated.

As well as daily sampling of the eight study houses, the surrounding houses and the surrounding environment of the study house on each farm has been sampled weekly. In most cases, the surrounding houses have been found to become *Campylobacter*-positive around the same time as the study house. The surrounding environment on the farms (the path leading to the study house, and the ante-room of the study house) has been found to be *Campylobacter*-positive in only nine of the 48 positive flocks to date. Data relating to geographical location, local climate, on-farm management practices and welfare indicators have been collected for every flock studied, in order to identify and quantify potential risk factors for task 1.1 and 1.2. Sampling is planned to finish in October 2013, making two years of longitudinal sampling on all farms.

Data to March 2013 have been received by Newcastle, so that mathematical models can be designed in preparation for the analysis, which will take place as soon as all results are obtained, during October 2013. A paper on the two-year longitudinal study in the UK is expected to be preparation by October 2013 (Deliverable 1.2.3).

Spain: Five broiler farms from five major Spanish poultry producers were recruited, and one study house selected on each farm. A total of 49 flocks have been followed longitudinally (ranging from 9 to 11 crop cycles per farm so far), of which 29 flocks (59.2%) have become *Campylobacter*-positive. None of the five farms have remained consistently negative throughout and the earliest detection of *Campylobacter* has been at 12 days of age. As for UK, in Spain *Campylobacter* has been detected in broiler flocks earlier than expected, due to the use of the highly sensitive boot sock method.

At each weekly visit at the five farms, besides sampling the study houses, samples from the surrounding houses and the surrounding environment of the study house on each farm have been obtained. Only six of the environmental samples (path leading to the study house and the ante-room of the study house) have been found to be *Campylobacter*-positive in the 49 studied flocks. In those farms having more than one house (2 house-farms), at least in two flocks the additional house has been *Campylobacter*-positive. Additional data have been collected for every flock studied, in order to identify and quantify potential risk factors for task 1.1 and 1.2. The two years of longitudinal sampling on all five farms is planned to finish by October 2013.

A paper on the two-year longitudinal study in Spain is expected to be in preparation by the end of 2013.

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).

To investigate the carriage rate around broiler houses, flies have been captured live around four UK broiler houses and five houses in Spain in summer of 2011. Four study farms were included in both the fly carriage rate study and the insect community study in the UK, rather than the full 8 farms due to the logistics in reaching the other four farms, which were based a considerable distance from ULIV, requiring a 3.5 hour drive each way. Flies were taxonomically determined to family or species. Thereafter, flies were conventionally cultured for presence of *Campylobacter*, including pre-enrichment in Exeter or Bolton broth followed by incubation on CCDA plates.

Subtask 1.3.1

UK: The second year of fly sampling was repeated during June to August 2012 in the UK on the four broiler farms in North Wales. However, flies were subject to killing with CO_2 and then flies of similar species/families were placed in batches of 10 flies for culture. In total, 1293 flies representing 28 different families were cultured in 127 batches from flies collected from the broiler farms and four batches (3.2%) of flies from three broiler farms were positive for *Campylobacter*. *C. jejuni* only was isolated from two of the positive batches and *C. jejuni* and another *Campylobacter* spp. were isolated from the other two batches. One positive batch contained fly species of the family Calliphoridae, whilst the other three positive batches contained a variety of species from the families Calliphoridae, Fanniidae, Muscidae and Scatophagidae (see table x). The broiler flocks on these farms were all positive for *Campylobacter* spp. when positive flies were detected. These isolates were typed using multi-locus sequence typing and represented sequence types common in poultry flocks, ST-1701 (CC-45), ST-45 (CC-45), ST-2599 (CC-45) and ST-48 (CC-48). An abstract on this has been submitted and accepted for poster presentation at CHRO, Aberdeen September 2013.

Table: Batches of flies (*Diptera*) from broiler farms testing positive for *Campylobacter* spp..

Farm (Batch ID)	Flock Age (days) At Sampling	Flies Present in Positive Batch	*Campylobacter* spp. isolated from Flies	Flock Age At First *Campylobacter* Positive (days)	*Campylobacter* spp. isolated from Flock
B (ID#1)	27	1xAnthomyiidae, 1xDolichopodidae, 1xFanniidae, 1x*Muscina stabulans*, 3x*Phaonia* sp., 2x*Polietes lardarius*, 1xPsychodidae, 1xScatophagidae, 1xUnidentified sp.	*C. jejuni*	21	*C. jejuni*
C (ID#2)	21	3xAnthomyiidae, 1xFanniidae, 1xMuscidae, 1x*Phaonia* sp., 3xScatophagidae, 1xSciaridae	*C. jejuni*	21	Other *Campylobacter* sp.
D (ID#3)	26	8x*Calliphora vicina*, 1x*Calliphora vomitoria*, 1x*Phormia terranovae*	*C. jejuni* and other *Campylobacter* sp.	13	Other *Campylobacter* sp.
D (ID#4)	26	1x*Calliphora vicina*, 5x*Fannia canicularis*, 1x*Phaonia* sp., 1xScatophagidae, 2x*Stomoxys calcitrans*	*C. jejuni* and other *Campylobacter* sp.	13	Other *Campylobacter* sp.

Spain: Individual flies were collected in all 5 farms, from June to November 2012, covering 3 to 4 flocks per farm. Overall, fly sampling has been carried out in sixteen flocks from 5 farms. Two samplings per farm and flock have been performed. Flies were cultured for *Campylobacter* individually. *Campylobacter* was isolated from flies in 8 out of 16 sampling occasions. At least in one flock in each farm *Campylobacter* has been isolated from flies. In one farm, *Campylobacter* positive flies have been detected by culture in all 3 sampled flocks. Some *Campylobacter* positive flies have been detected before or at the same time as *Campylobacter* has been isolated from broiler flocks or detected by PCR from boot socks. *Musca domestica* (house fly) was the most frequent fly species captured and the only species from which *Campylobacter* was isolated. Besides *Campylobacter* isolation from flies, PCR detection of *Campylobacter* from the enrichments in Bolton broth has also been attempted. PCR-positive flies were detected in 14 out of 16 sampling occasions, and belonged mainly to the species *M. domestica*. Also few *Ophyra* sp. (black garbage fly), *Calliphora* sp. (blow fly) and *Fannia canicularis* (lesser house fly) were *Campylobacter* PCR-positive. Most of the broiler

flocks became *Campylobacter* positive around the same time or just after detecting *Campylobacter* in the sampled flies.

Subtask 1.3.2

UK: For the fly community study, it was not possible to trap flies via the ventilation system, as the production company would not agree to this. To analyse the dipteran species diversity on the study farms, two malaise traps were set up at each live fly sampling event for approximately 2 hours. To assess the species diversity on the broiler farms and to survey the numbers and species of flies accessing the broiler houses through the ventilation inlets, the malaise traps were stood perpendicular to the house wall, at a right angle to the insect flight line. Seventy percent ethanol was used in the collecting vessel as a killing agent and as a preservative until specimens could be identified under a microscope in the laboratory. In total, over 20 sampling periods, 1771 insects were collected, including 1644 flies representing 28 families. The range of Diptera caught in one sampling session ranged from 0-612. On the broiler farms, 1356 (82.5%) flies were associated with livestock, dung or carrion, of which 25 (1.5%) were typical filth flies of the families *Calliphoridae*, *Fanniidae* and *Muscidae*.

Spain: At each of the study houses from the five broiler farms of the longitudinal study in 1.3.1, two interception traps have been placed in two windows to capture all insects entering the broiler house. Traps have been installed twice per flock, and left for one week. The same procedure as in 2011 to capture insects has been used. A total of 7245 insects from 17 orders have been captured, being the most abundant the *Diptera* order (n=5583). The range of *Diptera* caught in one sampling session was 0 to 1306.

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).

Due to a lack of MLST data available for some partner countries, the budget available for typing should mainly be used to gather data on sequence types associated with broiler production in Norway, Spain and Poland. For the UK, data are available from previous studies and the Netherlands and Denmark are currently establishing what MLST data is available.

Spain and Portugal collected isolates prospectively as part of task 1.1 and each selected more than 150 isolates for MLST, which were equally distributed over the year and from different crops/batches.

Norway collected retrospective data as part of task 1.1 and selected more than 150 retrospective isolates from the last few years, with one isolate selected per crop and equally distributed over the year to allow any seasonal effects to be demonstrated.

Criteria for selection of isolates to be examined by MLST were agreed and a total of 459 isolates have been received by ULIV. During 2012 and 2013 Spain sent 113 isolates to ULIV, Norway sent 174 and Poland sent 172. Molecular strain typing by MLST commenced in March 2012 and sequencing of all 459 isolates is expected to be completed by June 2013. Analysis of the sequence data is ongoing and is expected to be completed in July 2013.

In addition for this task, a literature review is currently being conducted to inform on what data are available across Europe for comparison with the findings of the MLST work to be conducted here and to analyse such data in a European context. At present, a first draft manuscript has been completed and sent to colleagues for comment. Submission of the manuscript is expected before July 2013.

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), 1.5.2 (M42).

At the last reporting stage we had completed development of the modelling framework for the statistical modelling of *Campylobacter* spread in broiler flocks. This is effectively based on a three tier modelling approach using Generalised Linear Mixed Models (GLMM) and Structural Equation Modelling (SEM). Data to be analysed with these models are still being collected and we are awaiting completion of the weather and environmental data before moving forward. In the meanwhile we have developed a stochastic spatial epidemiological model of *Campylobacter*. This we hope to parameterise and then test using data from the completed CamCon data sets. Again we are waiting on data provision from other partners.

Significant Results

Task 1.1: Risk factor analysis on retrospective data on the *Campylobacter* status of broiler flocks, from from Norway and Denmark demonstrated 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, 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*. These results have been submitted for presentation at 17th International Workshop on Campylobacter, Helicobacter and Related Organisms (CHRO) 2013, September 2013 and a manuscript is in draft, to be submitted to a peer-reviewed journal during summer 2013.

Task 1.2: A total of ninety six flocks have been sampled longitudinally in the UK. Of these, 48 were positive for *Campylobacter* before slaughter. The average age at which flocks become positive was 23.72 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. None of the farms have remained consistently negative throughout. Additionally, there appears to be evidence of seasonality but this need to be confirmed by more formal statistical analysis. In Spain, from the 5 study farms, a total of 49 flocks have been sampled longitudinally, with 29 positive (59%). None of the farms have remained consistently negative throughout. The earliest detection of *Campylobacter* has been at 12 days of age.

In both the UK and Spain, there has been extensive sampling of the farm environments. The surrounding houses and the surrounding environment of the study house on each farm have been sampled weekly. In most cases, the houses have been found to become *Campylobacter*-positive around the same time as the study house. The surrounding environment on the farms (the path leading to the study house, and the ante-room of the study house) has been found to be *Campylobacter*-positive in only nine of the 48 positive flocks to date in the UK. In Spain, samples from the environment (boot sock leading up to the house and in ante-room) have usually been negative. Data relating to geographical location, local climate, on-farm management practices and welfare indicators have been collected for every flock studied in both countries, in order to identify and quantify potential risk factors for task 1.1 and 1.2.

Task 1.3: Although the overall prevalence of *Campylobacter* positive flies were found to be low in both UK and Spain, flies must anyway be considered a risk factor for infection of broiler flocks during rearing due to the large potential for ingress of flies to broiler houses in both countries. *C. jejuni* was the most frequently isolated *Campylobacter* species among the flies. In UK, where flies were cultured in pools of ≈10 and determined to family level, the positive pools comprised mainly the families *Calliphoridae* (blow flies), *Fanniidae* (lesser house flies), *Muscidae* (including the common house fly) and *Scatophagidae* (dung flies). In Spain, where flies were individually cultured and determined to species level, all culture positive flies were common houseflies, but PCR detected positives also among *Calliphora* spp. (blow fly) and *Fannia canicularis* (lesser house fly) and *Ophyra* sp. (black garbage fly).

Deviations and corrective actions

Task 1.2.1: Longitudinal sampling in the UK and Spain will not be completed till October 2013 and this will impact upon the timescale of deliverable 1.5. However, Newcastle have the models in place and ready to run once the full dataset is available: Due to the late start of task 1.2.1 in the UK, to ensure that a PDRA is employed for longer to process these samples, some of the remaining consumables budget has been used to support their salary.

Task 1.3 – UK: At the annual meeting in April 2012 (and reported in D1.3.1) it was decided to change the UK sampling for Task 1.3.1 from individual species determination of flies, to determination merely to family level and that flies were cultured in pools. In Task 1.3.2, it was decided to change the insect trapping in ventilation channels to trapping by malaise traps installed adjacent to the outer side of house walls and capturing flies near to the side inlets and therefore likely to access the broiler house.

There have been no other deviations.

Use of resources WP1

Participant	ULIV	NVI	DTU	UU	UNEW	CSA	NVRI
Planned person months total	55	10	20	2	14	32	6
Approx. actual person months used in Second Reporting Period	28	1	10	1	8	18	2

Due to the late start of task 1.2.1 in the UK, to ensure that a PDRA was employed for a longer period to process these samples, some of the remaining consumables budget has been used to support their salary. Furthermore, as the UK fly screen work (Task 2.1) was done in collaboration with a Defra-funded intervention project in Northern Ireland, involving 24 farms over two crop cycles which were sampled by ULIV, this required a lower input of man months to undertake this work, however this was off-set by the increased man months again necessary to continue processing the samples as part of Task 1.2.1, again due to the late recruitment of farms.

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 (M36 - Delayed), 2.1.4 (M40), 2.1.5 (M56), 2.1.6 (M56), 2.1.7 (M58).

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: Activities are currently running within the CamCon/Defra cooperation according to the Agreement of collaboration. The Defra study comprised three interventions to be tested succeedingly; 1st biosecurity, 2nd acidification of drinking water, and 3rd biosecurity together with fly screen. CamCon participate in the 3rd activity only. The Defra project comprised 28 broiler houses with fly screen and 26 houses without in the UK. CamCon agreed to contribute with expanded samplings during the original Defra project period (summer 2012) as well as a continued sampling period (Aug12 to May13) after end of the Defra study.

The expanded sampling (U_LIV) took place in the form of boot sock samples taken at day 20, day 28 and the day before the first depopulation event. The results from the two flock cycles (crop 1 and 2) sampled by U_LIV found that there was a significant difference in the *Campylobacter* prevalence between houses with screens (test houses) compared to those without (controls) at day 20 of sampling however by day 28 of sampling this effect had disappeared. It therefore appears that from the results of the two crop cycles the presence of the fly screens delayed the entry of *Campylobacter* earlier in the crop cycle (<20 days), but not later on.

The continued sampling (DTU) took place in the form of boot sock samples taken at day 20, day 28, the day before the first depopulation event and the day before final depopulation, total 1080 samples for Campylobacter PCR detection at DTU. By end of the CamCon P2 period, 906 samples are received at DTU. Preliminary results show, that no effect of fly screen could be realized during autumn 2012 in UK, most probably due to a lack of effective biosecurity. Furthermore the average flock *Campylobacter* prevalence in the Defra-continued study remained high all through winter 2012-13 (at 1st thin ~30% and at slaughter ~70%), also suggesting that general biosecurity management were insufficient against *Campylobacter* introduction.

Results communicated from the Defra projects in April 2013 revealed 'that biosecurity had proved no effect during the original Defra period', a conclusion which retrospectively is in severe conflict with the precondition for success set in CamCon, and probably will be reflected in the results from task 2.1 when final analyses of data are ready.

Spain: Priority for this reporting period has been on measures for implementing biosecurity practice in Spain which within CamCon is done in close cooperation with WP5. Furthermore, to carry out a pilot study of functionality of flyscreens on two different kind of ventilation constructions in Spain (transversal and a combination of transversal and tunnel). All stakeholders are included in the planning (the broiler integration, local veterinary consultants, two carpenter companies, a ventilation company, and Spanish farmers). Three major meetings/workshops have taken place in Spain (June, September and October 2012) with all partners (2, 5, 10) and stakeholders. Furthermore, several local meetings have taken place in Spain between CReSA and individual stakeholders.

Thus, main activities in Spain have been:
a) Recruitment and following motivation of stakeholders.
b) Providing information of house level biosecurity and training material for stakeholders.
c) Visiting and selecting potential study farms.
d) Establishing 2 pilot farms with fly screen.
e) Documenting ventilation functionality on the pilot farms (still ongoing at end of P2).
f) Design of the '18 farm study' (still ongoing at the end of P2).
f) Elaborating an agreement of cooperation with the broiler integration (still ongoing at the end of P2).

During this reporting period, all stakeholders' responsibilities were settled and work performed accordingly as agreed, all relevant information and training material from CamCon were delivered, the 18 farm study designed and described, and at the end of the P2 period 2 pilot fly screen farms are running and being thoroughly monitored for ventilation functionality. When the outcome of the pilot study has been evaluated for ventilation functionality, final selection of the farm types (either tunnel or tranversal) to participate in the '18 farm study' will take place (candidate farms are pre-appointed by the broiler company). The design of the 18 farm study will be amended with a clause, that the sufficiency of the biosecurity procedures must be consolidated by a significant reduction of the number of *Campylobacter* positive flocks produced during the winter period 2013-14. If this clause is not met, fly screens will not be installed in the last part of the 18 farm study (M 40) and the study limited to communicate data and experience with upgrading of the biosecurity.

The cooperation agreement with the broiler company for the '18 farm study' including the pilot study, has been worked out headed by CReSA. For the moment, the agreement is under consideration within the legal department of the broiler company.

Task 2.2 Phage therapy (M1-48)

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

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 (Cje 4, 12, 16, 21) is proposed to serve as a phage cocktail for further use. Medium and large scale production variables were improved and high volumes of the phage cocktail is being produced for the in vivo experiments.

Among all subtasks to be accomplished until the end of this project, the subtask 2.2.1 was the only one planned to be accomplish by the end of this 18 months period. In fact, UMinho and CVI teams finished this subtask and others subtasks (2.2.2, 2.2.3, 2.2.4, 2.2.5 to 2.2.7) are in progress.

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
The limited host-range of available phages (16 *Campylobacter* reference NTCC phages) as determined for subtask 2.2.1 were a starting point for the isolation of new phages. Moreover, as the *in vitro*-induced phage resistant *Campylobacter* strains from UMinho and from CVI were not susceptible to lysis by any of the phages belonging to the collections of both institutions, it was important to find phages capable of lysing these strains. Therefore both groups started isolating new phages. This parallel approach was chosen to increase the chance for isolating a broad scale of phages. From 150 environmental samples (broiler farms, pig farms, sewage) CVI was able to isolate 27 phages with 16 different host-range types as determined with mentioned *Campylobacter*-strains panel.

The UMinho team isolated phages from poultry samples using the enrichment procedure previously described[3] (Figure 1), and the phage resistant *Campylobacter* strains were used in the enrichment of samples. In total 16 samples of chicken and pig intestinal contents and faeces collected from different places, were used in the isolation method. The resistant strains IW32, 3AM2, 3AM5, 3AM6, 3AM7, 3AM8 and 3AM10 showed to have prophage and therefore were not included in the enrichment broth. In total, 12 new phages were isolated. From these phages, 3 were not selected for further experiments since they formed very turbid halos.

Figure. Phage halos in the “lawns” of bacteria 82 and bacteria 65

Subtask 2.2.3: Small-scale phage production
The phages that were selected in subtask 2.2.1 and 2.2.2 were produced on a small scale in liquid or solid media. Nevertheless as the ultimate goal is to produce phages in a large-scale, UMinho team has developed several attempts to improve phage production in liquid broth. Several modifications to conventional production protocols were tested and variables such as culture medium, multiplicity of infection, infection dose and infection timing and production system were optimized. A *Campylobacter* strain that has been described as having a faster growth was provided by CVI to Minho University in order to evaluate their ability to be used as a phage host in the production and to increase the phage titer. The phage propagation in the host bacteria and in this bacteria provided by CVI and MOIs tested are presented in table 1.

Table. PFU/mL of phage 1E using *C. jejuni* 2140 *and* CVI strain as hosts. Different MOI were tested and the quantification was performed every 24 hours.

	2140		**CVI strain**	
T (h)/MOI	**0,1**	**0,01**	**0,1**	**0,01**
0	1,99E+07	5,50E+05	1,82E+07	5,00E+05
24	4,70E+07	1,00E+06	**1,43E+08**	**9,20E+07**
48	1,80E+07	1,60E+06	2,56E+07	5,00E+06

These experiments were performed in T-flasks (750 cm^3) and the results demonstrated that the MOI that equals 0,1 is better in order to obtain higher titres of phage concentration. Different broths for the phage production were tested namely BHI, BHI plus salts (calcium chloride and magnesium sulfate), Muller-Hinton and NZCYM (see table 2). The best broth appears to be Muller-Hinton with the biggest title showed.

Table. Concentration of phage 1E using *C. jejuni* 2140 as host and T-Flasks of 750 cm^3. Different broth and MOIs were tested.

	BHI		**BHI + salts**		**Muller-Hiton**		**NZCYM**	
	0,1	1	0,1	1	0,1	1	0,1	1
0h	6,10E+05	3,94E+06	4,50E+05	4,02E+06	2,10E+05	**5,94E+06**	-	3,42E+06
24h	1,20E+05	7,40E+05	7,00E+04	6,40E+05	1,10E+05	**1,07E+08**	-	1,41E+06
48h	2,25E+04	1,40E+05	8,50E+03	5,40E+04	6,90E+06	**5,75E+07**	-	1,15E+06

These conditions were tested in 5 l reactors, in batch operation mode with controlled atmosphere (5% O2, 5% H2, 10% CO2, 80% N2). Fed-batch cultures with addition of fresh medium and bacteria suspension were also assessed. The maximum titre achieved in large fermenters was 1.53E+06 PFU/ml after 55 h of culture..Simultaneously phage production in solid medium using T-flasks with high surface area was also optimized. 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.

UMinho University performed the lytic spectra of the 9 newly isolated phages against their *in vitro*-induced resistant *Campylobacter* strains and against the Campy strains provided by CVI. The results showed that 6 of these phages were able to lyse a total of 50% of these strains. Therefore the evaluation of the lytic spectra of these 6 new isolated phages against the *Campylobacter coli* and *Campylobacter jejuni* strains from UMinho and CVI collection was performed in order to evaluate their lytic activity and their potential as candidates for the phage cocktail (Table 3,4). Moreover a total of 33 *Campylobacter* phages belonging to the IBB-CEB UMinho collection were also tested against the *in vitro*-induced phage resistant *Campylobacter* strains, and 2 phages were selected since they lysed 30% of the resistant strains. Three phages were selected to compose the cocktail.

Single-step growth experiments of the selected phages were performed in order to assess the latent period and burst size of a single round of phage replication. In general these phages showed high latent periods (between 50 to 85 min) and small burst sizes (between 8 to 25 virions per cell)

Table. Lytic spectra of the 9 new isolated phages and phages from the CEB-IBB collection against the in vitro induced resistant Campylobacter strains

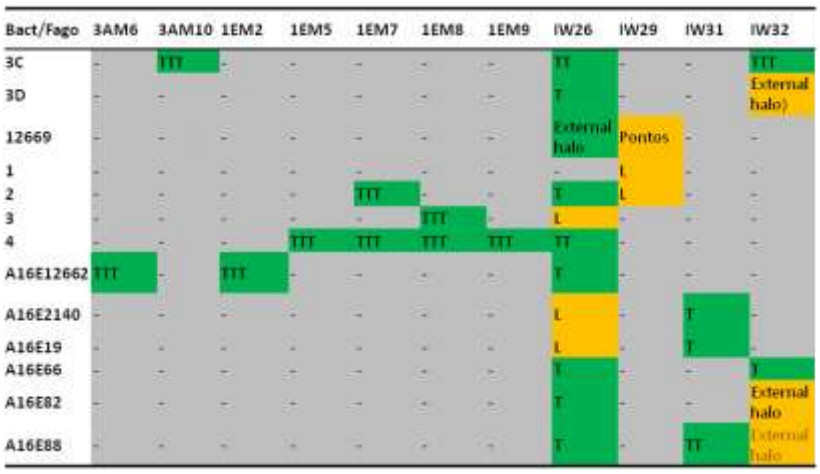
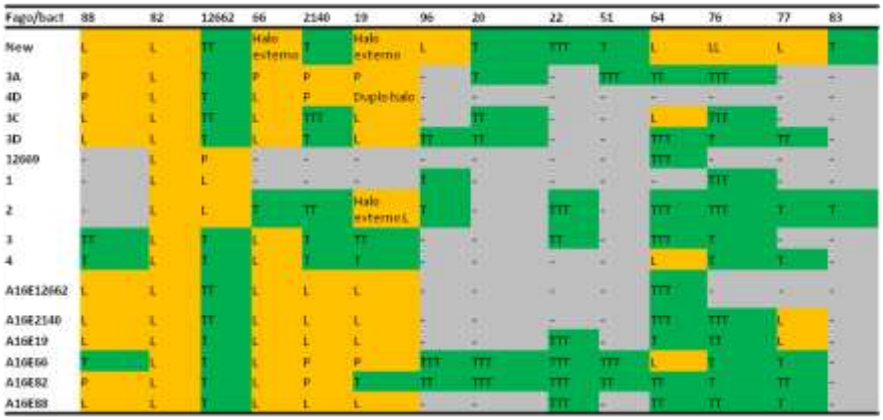


Table. Lytic spectra of the 9 new isolated phages and phages from the CEB-IBB collection against the Campylobacter strains provided by CVI



CVI performed some assays in which by in vitro induced mutagenesis it was possible to isolate several strains that were persistent resistant to a particular phage compared to the parent strain. As mentioned their relevance to in vivo conditions is unclear, nevertheless these strains are now in use for additional screening of new lytic phages. Next to mentioned in vitro induced resistance of *Campylobacter* strains we also observed changes in host-range phenotypes of *Campylobacter* strains with a particular knock-out mutation of a gene involved in the synthesis of the lipo-oligosaccharide cell wall layer (LOS) of Guilain-Barré associated *Campylobacter* strains (GBS). This cstII gene encodes the terminal sialylation of LOS. Strains with a knock-out cstII gene do not carry a terminal sialylic acid whereas wild type strains do. Table 4 shows that strains without a terminal sialic acid are more susceptible to phages than wild type GBS. This implicates a protection mechanism against phages by sialylation. These observations are part of a study carried out in collaboration with Rogier Louwen of the Department of Medical Microbiology and Infectious Diseases from the Erasmus MC in Rotterdam (NL) of which a paper is submitted for publication.

Table. Lytic profile of two GBS (GB11, GB19) and cstII knock-out mutants against two sets of phages (CVI, NTCC).

	CVI														NTCC														
Strain/phage	**Cje001**	**Cje002**	**Cje003**	**Cje004**	**Cje005**	**Cje006**	**Cje007**	**Cje008**	**Cje009**	**Cje013**	**Cje015**	**Cje016**	**Cje017**	**Cje019**	**669**	**670**	**671**	**672**	**673**	**674**	**675**	**676**	**677**	**678**	**679**	**680**	**681**	**682**	**683**	**684**
GB11	-	-	-	-	-	++	++	+	+++	-	-	++	+	+++	-	-	-	-	-	-	+++	+++	++	+	-	-	+++	+++	+++	+++
GB11Δ*cst-II*	+	+	++	+++	+++	+++	+++	+++	+++	+	+++	+++	+	+	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	++	+++	+++
GB19	-	-	-	-	-	++	++	+	++	-	-	++	-	+	-	-	-	-	-	-	+++	+++	+++	-	-	-	+++	+++	+++	+++
GB19Δ*cst-II*	+++	+++	+++	+++	+++	+++	+++	+	+++	+	+++	+	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	+	+++	+++

susceptible

resistant

Subtask 2.2.5: Efficacy of phages under experimental conditions
UMinho team performed some experiments in which Campylobacter coli and Campylobacter jejuni strains were isolated from the poultry farm and their sensitivity to a panel of phages previously isolated was checked. The previously selected phages were able to lyse these strains and thus were used for animal experiments.

Campylobacter phages previously administered to Campylobacter colonized chickens, showed sensitivity to the low pH of the chickens intestinal tract. Therefore in the field trials already performed sodium bicarbonate was given to the chickens before phage administration. Moreover, since phages were administered in food and in water, their viability maintenance was checked. The results show that in food 1 log reduction was observed after 4 hours, while in water no difference in phage concentration was observed.

Subtask 2.2.6: Establish a system for the production of the phages
This task has not started yet, but will start soon.

Subtask 2.2.7: Trial under field conditions
Two field trials were already performed in flocks of 10 000 animals. In the first trial the phage cocktail was incorporated in the food. Consequently the titre administered was10^8 pfu per 100 g of food in order to guarantee that each chicken would take 10^7 pfu. In the second trial, phages were incorporated in buffered drinking water that preserves phage activity for longer period and therefore demands lower phage concentrations. In both trials no Campylobacter reduction was observed following phage treatment (Figure 1).The next trail is planned for the end of May; in this trial more birds will be sacrificed in order to decrease the variability of the data, which is 50 to 30% of the average. Furthermore it is planned to do sequential phage administrations.

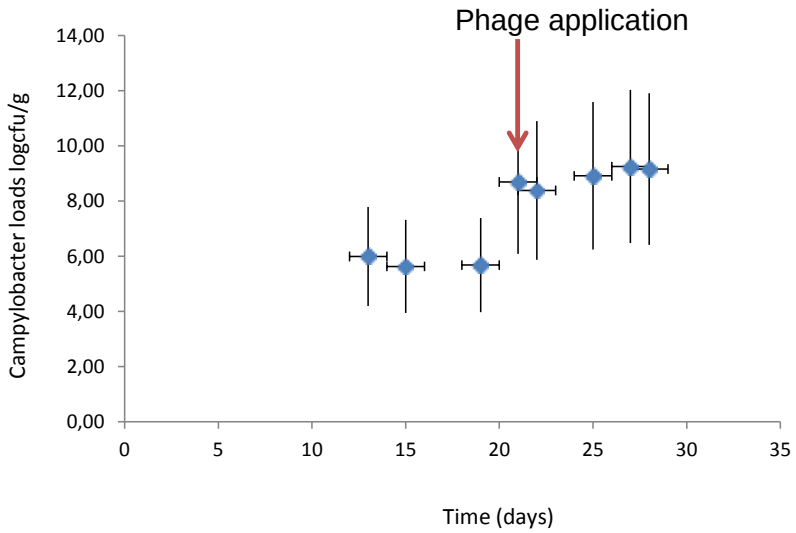


Figure. Campylobacter culturable cells present at the caecal content over time. The amount is expressed in cfu per g of caecal content and the values are averages of 10 birds sacrificed at each time point.

Conclusion
Overall it can be concluded that neither phages selected by UMinho or by the CVI were efficient in lysing phage-resistant mutants from the collections of both institutions. Therefore subtask 2.2.2 enables the isolation of new phages from sewage, faeces of broiler chickens capable of infecting resistant phenotypes. Phage characterization is still in progress (Subtask 2.2.4). The subsequent tasks (2.2.5, 2.2.6, 2.2.7) have started already (or will start shortly) and are in progress.

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).

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 this reporting period (M19-36), we moved on with a second *in ovo* vaccination experiment with glycosylated and non-glycosylated flagellin as vaccine following a similar immunization schedule (Fig. 1). Each vaccine group and control group consisted of 12 SPF chickens. Extensive testing of the immune response using the developed ELISA demonstrated again good antibody responses, but also high background responses for some chicken in the control groups. Western immunoblotting revealed that sera of part of the control chicken contained antibodies that reacted with *C. jejuni* flagellin and the flagellin vaccine antigen. This may indicate that part of SPF flock used as egg source may have been colonized with Campylobacter-like microorganisms, resulting in reactive maternal antibodies, and/or that, after hatching, chicken have been colonized with intestinal flora that elicit antibodies that cross-react with the vaccine antigen. As the presence of antibodies in the control group seriously hampered conclusions about the effect of *in ovo* vaccination, a third *in ovo* immunization experiment was planned using a different parent flock.

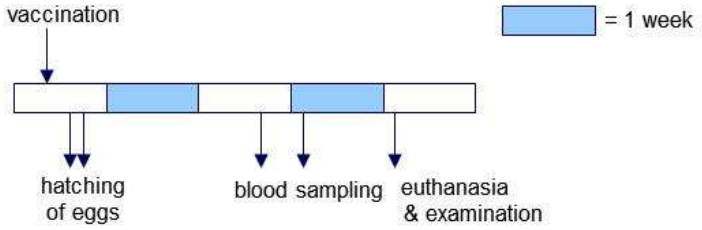


Figure. Standard immunization schedule for testing the engineered vaccines. *In ovo* vaccination is carried out on 18-day embryonated eggs.

Before performing the third *in ovo* vaccination experiment we produced new batches of non-glycosylated and gycosylated flagellin subunit vaccine, and prepared a *C. jejuni* whole cell vaccine for use as comparison. The recombinant vaccines were tested for purity using SDS-PAGE and for contamination with DNA and LPS using established TLR assays. The contamination with DNA and LPS was minimal for the vaccines produced in *Campylobacter*, whereas vaccines produced in *E. coli* were less pure. This information is important to assess the adjuvant potential of the vaccine and the contribution of LPS, DNA and flagellin to the establishment of the immune response. The functionality of the engineered flagellins with regard to folding and intrinsic adjuvant activity was validated using the previously established TLR5 assay and found to be as desired.

The third immunization experiment involved injection of eggs with the flagellin subunit vaccine, the whole cell vaccine, or the appropriate solvents. The immunization and subsequent handling followed the depicted schedule (Fig. 2). ELISA on collected serum samples demonstrated the presence of vaccine-directed antibodies after *in ovo* vaccination. Titration of the antibody reactivity against the vaccine antigen using ELISA demonstrated high serum immune responses (i.e. considerable above the already high background levels) for chicken immunized with the non-glycosylated flagellin-based vaccine (Fig. 2A) and for the whole cell lysate vaccine (Fig. 2B). The antibodies were mainly of the IgG class; serum IgM and IgA levels were very low, as can be expected for these antibody subtypes in the sera. In this experiment, we used SPF eggs and commercially available eggs to early predict the immunogenicity of the vaccine in non-SPF eggs, which is the target group of vaccination. Relatively similar immune responses were noted for chicken from SPF eggs and commercially available (non-SPF) eggs (Fig. 2). These results completed the Deliverables 2.3.1 and 2.3.2.

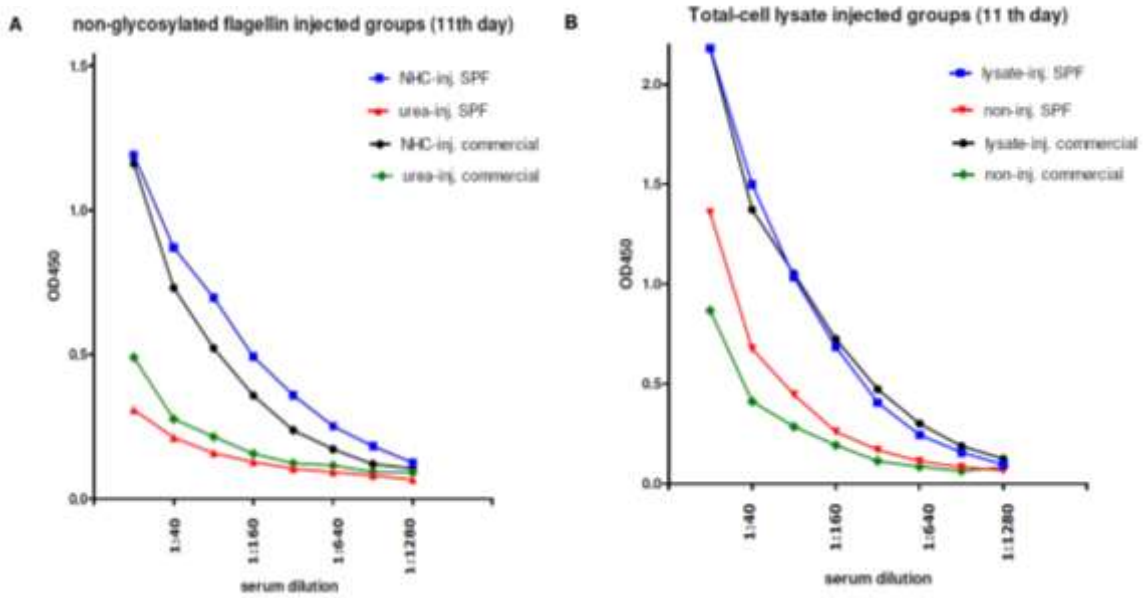


Figure. Immune response of SPF and commercially acquired chicken against (A) the subunit vaccine and (B) the whole cell *C. jejuni* vaccine (B) as determined by ELISA.

The establishment of a satisfactory immune response after *in ovo* vaccination with the subunit vaccine and whole cell vaccine paved the way to challenge experiments to test the level of protection induced by the vaccines. In preparation of the challenge experiment, we passaged *C. jejuni* strain 81116 in chicken to ensure optimal colonization of the laboratory-adapted strain in the chicken. Next we determined the optimal colonization dose with this strain in 18-day old chicken. This is important as a pilot experiment showed that a too high inoculation dose may overwhelm existing immunity and proper evaluation of the efficacy of the vaccine. The optimal colonization dose for our 23 day old-chicken was determined to be 4x10E4 CFU for the passaged *C. jejuni* strain 81116.

The next step was to perform the challenge experiment with vaccinated and control animals. Hereto four groups of embryonated eggs were injected (as depicted in Fig. 1) with the subunit vaccine, the whole cell vaccine, or the appropriate controls. Chicken were challenged with *C. jejuni* at day 23 after hatching. Sera as well as mucosal washings were collected for determination of immunoglobulin titers and intestinal mucosal sIgA values.

Results suggest that most animals were colonized with *C. jejuni*, although bacterial overgrowth with highly resistant intestinal flora prevented exact enumeration of colonization. Analysis of serum titers with ELISA indicated high antibody response against the whole cell vaccine in the vaccinated groups but, as noted earlier, also for chickens in the (non-immunized) control groups. Preliminary analysis of mucosal IgA levels suggest a strong IgA response in the vaccinated groups only.

The seemingly poor efficacy of the vaccine in this challenge experiment may indicate the need to increase the vaccine dose or to include a vaccine boost after hatching to counter the dilution of antibodies by the strong growth of the chicken in the first weeks of life. These results imply that Deliverable 2.3.3 has not yet given the expected results. In the 4th year of the project it is scheduled to further investigate the efficacy of the vaccines (Deliverable 2.3.4). However. as we expect that challenge experiments for subunit vaccines and whole cell lysates can be run in parallel, the delay in D.2.3.3. may well be compensated in the next period. Obviously, we don't know what the outcome will be. Once in the next vaccination study an appropriate protection is obtained, the vaccines will then be administered to a new group of animals to define the level of protection against different homologous and heterologous *C. jejuni* strains.

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.

The field experiments with phages have been performed.

In ovo vaccination of embryonated chicken eggs with a novel subunit vaccine and whole cell vaccine 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 are still in progress.

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. During P2 this was further formalized in a Collaboration agreement. In Spain, the precondition of house level biosecurity could not be met, but had to be integrated in the amended study designs, also described in the P1 report.

Recently, the Defra-funded project has concluded that the intervention 'biosecurity' tested during the original study period, turned out to be unsuccessful. Retrospectively, this is in severe conflict with the assumed precondition for success of fly screen in CamCon.

Use of resources WP2

Participant	UU	DTU	ULIV	CVI-LEI	UMinho	CSA
Planned person months total	26	20	4	16	30	4
Approx. actual person months used in Second Reporting period	12	10	1	12	5	2

No large deviations.

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. In the Second Reporting Period, all deliverables were submitted.

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

The work of Task 3.2 results in Deliverables 3.2.1 (M36 - Delayed) and 3.2.2 (M48).

Studies in this Reporting Period have been 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. The approach has been tested in Poland and England. The results will be available through a research paper, which is almost ready for submission to Vet. Microbiol. The preliminary abstract of the paper is as follows:

Towards a low cost, semi-continuous, and quantitative monitoring of Campylobacter by air sampling in chicken flocks by M.S.R. Søndergaard , M.H. Josefsen, C. Löfström, L.S. Christensen, J. Osek, K. Wieczorek & J. Hoorfar

Campylobacter is one of the leading causes of acute diarrheal disease worldwide. It is therefore relevant to control and reduce levels of Campylobacter in broilers which are the main reservoir for human infection. This study describes an evaluation of a semi-continuous method for quantification of Campylobacter by air sampling in broiler houses. To identify control measures that would be universally applicable sampling was carried out in conventional broiler houses in Poland in addition to preliminary samplings in Denmark. Each measurement consisted of air samples on gelatin filters, standard boot swab faecal samples and particle counts. Sampling was conducted over an 8-week period in three flocks assessing the presence and levels of Campylobacter in boot swabs and air samples using culture and quantitative real-time PCR (qPCR). The detection limit for the air sampling coupled with qPCR was approximately 100 Campylobacter cell equivalents (CCE)/m3. The particle counts were used to analyse size distribution in airborne particles (0.3-10 µm) in the broiler houses in relation to bacterial distribution. No correlation between airborne Campylobacter and a specific particle size was found. Campylobacter was first detected in the flocks after 0 and 2 weeks using air sampling and boot swabs, respectively. All samples were positive for Campylobacter from week 2 and the rest of the rearing period with both methods, though 1-2 logs higher levels were found with air sampling. At week 8 the levels were approximately 10^4 and 10^5 CCE/sample for socks and air. In conclusion, 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.

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

The work of Task 3.3 results in Deliverable 3.3.1 (M48).

No specific progress to report.

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

The progress in WP3 is on schedule except for deliverable 3.2.3. (due April13). This deliverable is delayed by 3 months due to two key researchers being on maternity leave. The deliverable is expected to be submitted July 13.

Use of resources WP3

Participant	DTU	NVRI
Planned person months total	13	3
Approx. actual person months used in Second Reporting Period	4	2

No large deviations.

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

In general, the progress in WP4 has been limited in the past period as a consequence of the project extension, which put the milestones and deliverables one year forward.

Task 4.1 Risk assessment (M1-58)

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

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 2012. Pending the availability of results from the other WPs, the development of a virtual risk assessment model has continued. It will allow an exploration of the potentials of the model, and clarify these to the project partners, which is of particular importance to maintain a good alignment between this WP and WPs 1-3. The collected data and the results of the risk factor study performed in Task 1.1are of particular interest and will be of used to assess the effect of farm interventions

Task 4.2 Data collection and compilation (M13-58)

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

A data request has been send to the project partners related to (1) the available data on prevalences and distributions of concentrations of Campylobacter in broilers and broiler meat in the countries participating in the project, as well as the production data, and (2) the expected format of results of task 2.1 – 2.3, and replies have been obtained.

Next, the data collection for tasks 4.3 and 4.4 has continued. Among others, data are collected on the health burden in the six countries involved in the project and on the costs of interventions (for example the fly netting studied in WP2).

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 continued. The progress and plans have been presented and discussed during the annual meeting 2012. Pending the availability of results from the other WPs, the development of a virtual economic model has started, which will allow an exploration of the potentials of the model, and clarify these to the project partners.

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).

This task will be started in M43. The start has been postponed as a consequence of the project extension.

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).

This task will be started in M43.

Significant Results

No significant results were expected at this stage.

Deviations and corrective actions

Most milestones and deliverables of the WP have been postponed as a consequence of the project extension. The progress in WP4 is on schedule.

Use of resources WP4

Participant	DTU	CVI-LEI	NVI	ULIV	UU	CSA	NVRI
Planned person months total	36	12	1	1	1	1	1
Approx. actual person months used in Second Reporting Period	7	5	0	0	0	0	0

No large deviations. The majority of work will take place during the second half of the Project Period.

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 (M56).

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. The manual will be ready at month 56, and will incorporate up-to-date information and knowledge created through the research activities in the other work packages of CamCon. As a consequence, the final version of the Best Practice Manual is temporally dependent on completion of work in the other CamCon work packages.

However, as previously reported under WP2, task 2.1, it has proven essential to upgrade biosecurity practices for fly screen test houses in Spain in advance of initiating work here. This work also serves as a test case for creating the final version of the Best Practice Manual, and will be supported by easily comprehensible drawings and illustrations.

Consequently, further work on a basic best practice manual has been carried out since the last report. During working visits to Partner 10 in Spain in June and September 2012, drafts of biosecurity folders and posters were discussed with the commercial cooperating Spanish poultry company. A lot of specific comments and suggestions for alterations were received. Also, the content and design of an essential biosecurity check list for use on farm visits of the amended subtask 2.1.6 were discussed.

Following the visits, a second version of the biosecurity posters with amended and more detailed illustrations was produced. The illustrations and drawings will also be used for the production of a first version of the basic best practice manual. This work, as well as publishing of the check list will continue in 2013.

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 have also been produced. They were forwarded to Partner 10, to be used in an internal update meeting at Partner 10, for a planned meeting for veterinary technical staff of the commercial cooperating poultry company, and for dissemination to company farms at farmers' meetings in 2013 in advance of the planned biosecurity and fly screen studies in WP2.

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 (M54) and 5.2.2 (M60).

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.

The final delivery of this task will be at the end of the project period (M60) as it depends on inputs and research findings from the other work packages. Thus, and according to plans, activities have so far been restricted to more basal activities in order to explore the best technological and educational solutions for this task. Technological possibilities in particular have developed much since the start of the project with easier access to animation and video productions, and these advances will be included in the final E-learning product.

This preparatory work for the E-learning program has been initiated with visits to the Learning Lab of the Technical University of Denmark to obtain ideas and inspiration for the design and formulation of learning contents. The work will continue and be developed in cooperation with the subcontractor Conzentrate.

Task 5.3 Voluntary Certification Programme (M37-60)

The work of Task 5.3 results in Deliverable 5.3.1 (M60).

Work has not started on this task yet, which is according to plans. However, the development of the Best Practice Manual described under Task 5.1 will form the backbone of a certification programme, and will ensure the timely progress of this task during the coming reporting period.

Significant Results

Collaboration with the Spanish CamCon partner and a major poultry integration on a basic Best Practice Manual for production of *Campylobacter*-free chickens has resulted in the production of illustrated biosecurity folders and posters as well as the design of an essential biosecurity check list for use on broiler farms. Slide 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 have also been produced. This material will be further developed and integrated with new science-based knowledge generated in the CamCon project to form the backbone of the principal deliverables of this work package (Best Practice Manual and Voluntary Certification Programme).

Preparatory work for the E-learning program has been initiated in cooperation with the subcontractor Conzentrate in order to explore and identify the best technological and educational solutions for web-based education and E-learning programmes.

Deviations and corrective actions

The progress in WP5 is on schedule.

Use of resources WP5

Participant	DIA	NVI	DTU	ULIV	UU	CSA	NVRI
Planned person months total	16	1	5	1	1	0,5	0,5
Approx. actual person months used in Second Reporting Period	6	0	1	0	0	0	0

No large deviations. The majority of work will take place during the second half of the Project Period.

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 will be revised later in the project. 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 will be 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.

In addition to the Annual Meetings, Quarterly Meetings are 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 1st and 2nd Periodic Report should replace ordinary Quarterly Meetings. A few physical meetings have also been held within WPs.

The next Annual Meeting will be held in connection with CHRO (the major International Campylobacter Conference) in Aberdeen in September 2013.

Task 6.6 Regular reports to the European Commission (M20, M38, M60)
The 1st and 2nd Periodic Report is delivered on time. The Report on awareness and wider societal implications will be drafted and delivered towards the end of the project period.

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 second period was the Request for Amendment sent in November 2012 and approved in January 2013. The amendments were prolongation for the project with one year, and changes in some scientific tasks, especially regarding fly screens, where the problem of recruiting farms made it necessary to make some changes to the original plan. See more details in the description of WP 2.

The Financial assistant and his co-worker asked the administrative contacts in each participating institution for a financial status report midway between 1st and 2nd 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 so far has been the problem of finding farms willing to participate in the Task 2.1 (fly screens). A large amount of work has been done to overcome this problem, and solutions have been found both in UK and in Spain. The project had to be changed due to this, and the changes were included in an Amendment to the original plan. After the Amendment was approved, the project is developing reasonably well in relation to the updated plan.

Some milestones and deliverables are delayed, but several of them are expected within a few months.

Coordination and communication activities

Some “unplanned” administrative work was required regarding the collaboration with the Defra project and the Request for Amendment. Otherwise the coordination of the project has gone according to plan with regular meetings.

A tentative list of scientific publications from CamCon has been established, and is being updated regularly. This list is attached to the Minutes from the quarterly meetings.

There has been contact with the magazine “Word poultry” concerning publication of the results from the Questionnaire study later this year. 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.

Many of the participants have sent in Abstracts to the biannual Campylobacter conference (CHRO), which will be held in Aberdeen in September 2013, and various aspects of CamCon will be presented either orally or as posters. The Coordinator and one of the WP leaders will be Session Chairs at the Conference.

Changes in Consortium or legal status of the beneficiaries

None.