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, no single task has been finalised during the first 18 months of the project. The table below shows the planned timing of the work in different tasks:

Task	Title	Month 1-6	Month 7-9	Month 10-12	Month 13-15	Month 16-18	Final delivery month
1.1	Risk factors for *Campylobacter* colonization in broilers						36
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						48
1.4	Distribution of *Campylobacter* sub-types in EU broiler production						44
1.5	Modelling in-house colonization in relation to environ. and bird welfare						42
2.1	Fly screens add-on to biosecurity						44
2.2	Phage therapy						48
2.3	Vaccination						48
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						48
4.1	Risk assessment						46
4.2	Data collection and compilation						46
4.3	Economics						48
4.4	Cost-effectiveness of interventions at farm and comparison with interventions post farm						48
4.5	Future data needs						48
5.1	Best Practice Manual for production of *Campylobacter*-free chickens						44
5.2	Specific targeted learning programmes for proficiency in implementing the "Best Practice Manual for production of *Campylobacter*-free chickens"						48
5.3	Voluntary Certification Programme						48

Still, there are some milestones and deliverables planned to be finalised during these first 18 months of the project. 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 (M1-36)

The work of Task 1.1 results in Deliverables 1.1.1 (M3), 1.1.2 (M12) and 1.1.3 (M36).

Questionnaire

A questionnaire (deliverable 1.1.1) was drafted, in collaboration with all involved institutions. Questions were selected based on previous experience from questionnaire surveys carried out in broiler productions. The questionnaire was developed in English and translated into Danish, Dutch, Norwegian, Polish and Spanish. Great emphasis was put on focusing the questions on factors previously pointed out as possible risk factors and on making the questionnaire as user friendly as possible.

In Poland and the Netherlands conventional broiler farmers were randomly selected among all broiler farms within both countries. In the UK, questionnaires were sent to UK broiler farms from three major production companies, representing the majority of poultry production in the UK. Due to the very different climate within the different geographical regions of Spain, it was decided to include only farms within the region of Catalonia. In Denmark and Norway, questionnaires were sent to all conventional broiler farmers, since the number of farms in these countries is quite low. A minimum of 200 questionnaires was sent out in each of the six participating countries, but in some countries more questionnaires were sent out in order to increase the number returned. Table 1 shows the number of questionnaires distributed and returned as well as how the questionnaires were distributed/ returned.

Table 1. Number of distributed/returned questionnaires and response rate

Country	Questionnaires	Number of questionnaires		Response rate (%)
		distributed	returned	
Denmark	Sent to producers/returned by mail	205	119	58.0
Norway	Sent to producers/returned by mail	309	183	59.2
Netherlands	Sent to producers/returned by mail	550	254	46.2
Poland	Filled out by veterinarian	250	249	99.6
Spain	Filled out by university staff	200	200	100
United Kingdom	Distributed and returned via companies	200	121	60.5

A total of 1,714 questionnaires were sent out in the end of 2010/beginning of 2011 and 1,126 questionnaires were returned from December 2010 to October 2011 (overall response rate approximately 66%). All questionnaires were sent to DTU where all data were entered into a common database. The final validated dataset consisted of 1,105 questionnaires (21 were excluded because these were from organic farms, from farms that had ceased the broiler production, or returned with all questions unanswered).

Preliminary results of the questionnaire survey were presented at the International Workshop on Campylobacter, Helicobacter and related organisms in Vancouver 28 August-1 September 2011-(CHRO 2011). The poster from CHRO 2011 has been published on the CamCon website.

The report on broiler production Across Europe (deliverable 1.1.2) is currently being drafted and expected to be finalised ultimo 2011. The results of the survey provided insight into a number of variables connected to management and biosecurity on the participating broiler farms. Some examples are given below:

- The annual production of broilers per year varied tremendously among the participating farms, from 826 to 20,840,020 broilers per year.
- Overall, farms in DK, the NL and the UK had more houses, a higher number of crops per year and a higher stocking density than farms in Norway, Poland and Spain. However, the largest farms, with respect to number of houses were observed in Poland, while the largest production, in terms of average number of birds raised per year, was reported by the UK.
- Differences in use and compliance with quality assurance programs varied. However, all the applied quality assurance (QA) schemes share many features. In some countries, it is mandatory for all broiler farmers to comply with a specified QA schemes while in other countries farmers comply with such schemes on voluntary basis.
- As part of their biosecurity measures, almost all of the participating farms indicated having an anteroom and/or a physical barrier between the entrance area of the broiler houses and the broiler flocks. The practice of having dedicated footwear and tools for each broiler house varied between countries, and was more common among farms in DK, NO and PL than among those in ES, the NL and the UK.
- With the exception of farmers in PL, almost all farms reported using an all in/all out system with a mean downtime between each crop varying from eight to 19 days. The use of partial depopulation of flocks were reported in all participating countries, but is used more often in ES, the NL, PL and the UK than in DK and NO.
- There are differences in pest controls, ventilation systems, sources of water and use of additives to the water, and presence of other animals on the farms.

With the exception of a recent Baseline survey carried out by the European Commission, describing a limited number of potential risk-factors, this is the first comprehensive standardized questionnaire survey carried out simultaneously in several European countries. The data generated by the survey have provided new insights into the broiler production in the participating countries. Some of the observed differences in management may reflect differences in strategies applied for reducing *Campylobacter* in broilers in the participating countries. Besides being published in an own report, the results of this survey will also be used, together with climate data and information on *Campylobacter* status of broiler flocks from a subset of the participating farms in a risk factor analysis aiming to identify external risk factors for flock colonization.

Farm study
This part of Task 1.1 will be performed slightly different in different countries:

UK: Caeca sampling commenced in June 2011 and to date, 24 batches of caeca have been sampled from 17 different farms at first thinning. Twelve of these were positive for *Campylobacter*. Questionnaires and batch health and management data has been collected and stored for analysis alongside the caecal prevalence data later in the study.

Spain: All 20 farms from 6 poultry companies in Catalonia have been recruited, and at least two flocks per farm have been studied to date, with a total of 46 flocks already analyzed. In most of the farms, caecal samples from both first and final depopulation have been cultured. Most of the flocks have been *Campylobacter* positive (both at thinning and at final depopulation), while eight flocks have been negative at first and final depopulation; also, few flocks were negative at thinning but positive 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.

Denmark and Norway: Retrospective prevalence data is being collected from poultry companies or from databases for the equivalent number of farms and climate data collected to cover the same period.

Poland: NVRI has organized the training for the veterinary inspectors who are involved in the sampling procedure. The caecal samples are being taken from broiler flocks at the slaughterhouse level. Altogether, 29 farms have been selected all over Poland for sampling at slaughter. Until now (November 15) 102 broiler flocks have been sampled for the presence of *Campylobacter*. From 4 farms, 2 sets of samples have been obtained (from 2 slaughtered flocks), from 9 farms – 3 sets of samples, from 13 farms – 4 sets of samples, and from 3 farms – 5 sets of samples. The samples (n = 102 pooled samples) have been analysed for the presence of *Campylobacter* by the use of culture and PCR methods. It was found that 86 (84.3%) were positive for *Campylobacter*. The majority of the isolates (59, 68.6%) were identified as *C. jejuni* whereas the remaining 27 isolates (31.4%) were found to be *C. coli*. The further samples are currently being examined and *Campylobacter* isolated and identified. Furthermore, NVRI are collecting and adding to a database all required information connected with farms, bird batch slaughters and weather conditions.

The Netherlands: Due to the recently detected ESBLs on poultry farms there was in 2011 a huge pressure on the boiler industry. For that reason we have waited to approach farms till 2012 and ask them for a year-long sampling.

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 (M9), 1.2.2 (M26), 1.2.3 (M46) and 1.2.4 (M46).

At the start of the project, several methods were considered for use in the longitudinal sampling, including both culture and PCR-based methods. After evaluation and comparison of different culture methods, a direct PCR-based method using boot socks was adopted. The boot sock method was originally developed by colleagues in Denmark for studies on broilers but had not been evaluated fully for use. The method involved walking the length of the broiler house several times in order to cover as much ground as possible, whilst wearing a pair of pre-moistened sterile fabric boot socks. Any *Campylobacter* in the faeces and/or litter is taken up onto the sock and can be detected by PCR after a simple kit-based DNA extraction is performed. This method has proven to be sensitive and also allows rapid detection of *Campylobacter,* less than 24 hours after sample collection.

Longitudinal study protocols were finalised after input by all relevant participants, and longitudinal sampling in the UK and Spain commenced in May/July 2011 following recruitment of farms and workshops being held for farm managers.

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 16 flocks have been followed longitudinally (ranging from 1 to 4 crop cycles per farm so far), of which 10 flocks have become *Campylobacter*-positive. The average age at which flocks become positive was 19.7 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 four of the ten 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.

Spain: All 5 farms from 3 major Spanish broiler producers were recruited. To date, 2 farms on the 3rd crop (1^{st} and 2^{nd} crop finished) and 3 farms on the 2^{nd} crop (1^{st} crop finished) have been studied. Caecal contents and cloacal swabs have been taken from at least 1^{st} crop of all 5 farms, and compared to determine if cloacal swabs could replace caecal samples. Since there were no differences, caecal samples have been replaced by cloacal swabs.

All flocks, but one have been positive for *Campylobacter*. The earliest detection of a *Campylobacter* positive bird in a flock was at 14 days old; the latest detection, at 34 days old. Usually samples from environmental boot sock (boot sock leading up to the house and in ante-room) have been negative. Three out of five farms have 2 houses, two of them have been positive in the second house at the same time as the study house, and the third one was always negative.

As in the UK, 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.

Sampling is on-going and the first year data will be analysed in May 2012 as stated in deliverable 1.2.2. A paper on the two-year longitudinal study is expected to be produced after May 2013 (deliverable 1.2.3.).

Task 1.3 Importance of flies in transmission of *Campylobacter* to broiler flocks (M16-48)

The work of Task 1.3 results in Deliverables 1.3.1 (M24) and 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. Flies were stored in cooler boxes and killed back in the laboratory by either chilling (UK) or CO_2 (Spain) before 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.

In UK, the four farms were visited 6 - 9 times between 7 July and 7 September; between 155 and 317 flies captured per farm. Totally 909 flies were cultured; 2 were found positive on different farms, one blow fly *Calliphora vomitoria* with *C. lari* and one fly of the family Heleomyzidae with *C. coli.* Newcastle assisted with identification of flies.

In Spain, the five farms were visited 2-5 times between 7 July and 14 October obtaining between 32 and 193 flies per farm (batches of 2 - 51 flies per visit). Totally 486 flies captured of which 478 were cultured. Six house flies *M. domestica* from three different samplings were found *Campylobacter* positive; both *C. jejuni* and *C. coli* have been isolated.

To conclude, the *Campylobacter* carriage rate of flies captured in UK and Spain in summer of 2011 have been found low and fly species preference for *Campylobacter* is so far inconclusive.

To investigate the distribution and amount of insects entering the broiler house from the surroundings, the following work has been performed so far:

UK: Four study farms have been selected to partake in the insect community study in the UK, and measurements of the ventilation systems in the chosen study houses have been taken and recorded. Further progress on this task has been halted by the fact that the majority of the ventilation inlets on UK broiler houses are located on the roof of the house. Smaller inlets are available on the side walls of the houses, but these are currently not used with enough frequency to be useful in this task. Discussions are on-going with UK broiler companies to try and resolve this issue. Sample collection is expected to start in summer 2012.

Spain: Traps have been installed twice per flock, and left for one week. The samples are stored in the fridge awaiting identification.

Task 1.4 Distribution of *Campylobacter* sub-types in EU broiler production (M10-45)

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 will collect isolates prospectively as part of task 1.1 and will each select 150 isolates for MLST again to be equally distributed over the year and from different crops/batches.

Norway is collecting retrospective data as part of task 1.1 and will select 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 Multi-locus sequence typing were agreed. Molecular strain typing is expected to commence before May 2012.

In addition for this task, a literature review is currently being conducted to inform on what data is available in the literature across Europe for comparison with the findings of the MLST work to be conducted here and to look at available data.

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) and 1.5.2 (M42).

The Newcastle group have been developing and testing the underlying modelling methodology for assessing risk in the longitudinal studies of *Campylobacter* infection in broilers. Specifically, we have been writing the algorithms that are necessary to allow us to investigate the interdependence of key risk factor variables in the Structural Equation Modelling (SEM) models that underpin the risk analyses. Here we have had to reconcile the binary nature of our response variables with an analytical approach, which assumes bivariate normality amongst predictor variables. Since many of our output variables are categorical or ordinal in nature, we have developed bootstrapping routines for the SEM analyses. These have been developed and tested.

Significant Results

Task 1.1: The results of the questionnaires survey provided new and detailed insight into a number of variables associated with management and biosecurity practices in the broiler production within the participating countries. With the exception of a recent baseline study carried out by the European Commission, describing a limited number of potential risk-factors, this is the first comprehensive standardized questionnaire survey carried out simultaneously in several European countries. Some of the observed differences in management may reflect differences in strategies applied for reducing *Campylobacter* in broilers within the participating countries.

Task 1.2: Frequent meetings have taken place with poultry producers in both the UK and Spain to agree farm access, sampling protocols and data collection. There has been much concern, particularly in the UK about confidentially and the release of data without company agreement.

A total of sixteen flocks have been sampled longitudinally in the UK. Of these, ten were positive for *Campylobacter* before slaughter. The average age at which flocks become positive was 19.7days, 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. A total of eight flocks have been sampled longitudinally in Spain. All flocks but one have been positive for *Campylobacter*. The earliest detection of *Campylobacter* to date has been at 14 days of age and the latest at 34 days.

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 four of the ten 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.

Deviations and corrective actions

Deliverable 1.1.2 has been delayed due to a number of obstacles connected to the collection of data. Broiler farmers from several countries did not want to participate unless they could remain anonymous. This issue was discussed and resolved by allowing farmers in the dataset with a unique identification number and the first two digits of the zipcode (for adding climate data). There were also legal aspects associated with the questionnaire survey in one participating country. These were legal issues were resolved in summer 2011. Thus, the process of collecting data was delayed, and therefore also drafting the report on broiler production across Europe (D 1.1.2).

Task 1.2.1: Annex 1 state that for each flock, 30 birds will be culled and the pooled caeca cultured for *Campylobacter* once per week for the first three weeks of each flock, then increasing to every 3-5 days until the flock becomes positive. Due to concerns raised by the farmers and production companies in the UK about practicality, cost and welfare of this method, an alternative sampling protocol was developed. The new protocol involves sampling the flocks using a non-invasive bootsock technique, which has been found to be extremely sensitive in detecting *Campylobacter* colonization in a flock. The frequency of sampling was increased to every day of the flock life, after *Campylobacter*-positive flocks were detected as early as 9 days of age. This will enable more accurate estimation of the time at which *Campylobacter* enters a flock.

Additionally, Annex 1 states that any flocks which remain negative will continue to be sampled after partial depopulation. After discussions between all participants, it was decided that due to potential masking effects of partial depopulation on other risk factors for colonization, all sampling will cease at first full or partial depopulation.

The use of gauze swabs to test surfaces in the house before stocking, and sampling of other domestic animals on the farms were excluded from the final protocol due to constraints upon time and resources. Instead, houses are samples prior to stocking of chicks by using boot swabs.

These deviations from Annex 1 will have no significant impact on other tasks or on available resources or planning.

Longitudinal sampling in the UK and Spain commenced 12 months later than originally planned. This was due to protocol issues as described above and additional problems concerning communication and co-operation from production companies. These issues were resolved and sampling commenced in May 2011. This will have a small impact upon the timescale of deliverable 1.5: Modelling in-house colonization of birds in relation to environment and bird welfare, as data will not be available for modelling until May 2013.

Task 1.3.2: Sample material are lacking from 2011 in UK due to difficulties with mounting of capture bags in the ventilation channels of the selected houses due to the farmers on those farms not opening the side vents during a flock cycle, unless the temperature was very high. Appropriate trap material for roof inlets was not available, and will probably not be applicable to the large roof inlet area both due to the required larger size of trapping bags and to the risk of 'spinning' the bags due to turbulence of the air flow. This has been discussed with the poultry company in question and is being resolved and sampling of the side inlet channels should be possible in 2012.

Number of missing sample portions of 2011 for input in the data analysis will be compensated for in the design of the 2012 study to secure sufficient data for the insect community analysis. The missing data influence on the data input for task 1.5, however should be compensated for by the 2012 sampling to be in right time for milestones of task 1.5 (M 1.5.1 and 1.5.2) and deliverables (D 1.5.1 and 1.5.2) to be achieved in due time.

There have been no other deviations.

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, both 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 (M7-45)

The work of Task 2.1 results in Deliverables 2.1.1 (M12), 2.1.2 (M13), 2.1.3 (M14), 2.1.4 (M15), 2.1.5 (M39), 2.1.6 (M40) and 2.1.7 (M44).

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.

The Task is divided in five 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

For fulfilling the objective of subtask 1.2.1 considerable effort has been executed during 2010 and 2011 comprising the task leader's repeated visits to UK, and Spain and partners 10 and 3's activities for building up contacts within each national broiler business. Several meetings with the larger broiler companies of the two countries have taken place to build up interest and network and to inspect farms for suitability to fly screens. Emphasis has also been placed on communication and information at meetings, by email correspondence and by seminars for relevant groups of persons within the UK and Spanish broiler business. The seminars were held at DTU in Denmark – also comprising farm visits to Danish farms having functioned with fly screens since 2004 or 2006.

However, despite considerable activity, recruitment of the planned 6 study houses had not succeeded within the first year (by April 2011) in either UK or Spain. Therefore changed study designs in both UK and Spain were approved at the first Annual Meeting (14 April 2011). Thus, in UK it was decided to reduce the number of fly screened farms to one farm and allocate some of the thereby saved budget to intensified sampling on the Defra project. In Spain, missing historical *Campylobacter* data and lack of house-level biosecurity prevents a timely start of the task, besides for functionality test on one farm was encouraged. Deviations from the CamCon timeline are described in more details under 'Deviations and corrective actions'.
Due to the below described obstacles with fulfilling the objective of subtask 2.1.1 start of the subsequent subtasks has been delayed accordingly.

Task 2.2 Phage therapy (M1-48)

The work of Task 2.2 results in Deliverables 2.2.1 (M24), 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.

Although isolated new phages were able to lyse virtually all relevant field isolates (see below) some in vitro generated phage resistant mutants from both institutes were persistent resistant to all present available *Campylobacter* phages. The occurrence and relevance of these in vitro resistant strains under

field conditions are at present unknown but urges both institutes (CVI, UMinho) to continue isolation of new phages continuing the duration of this project.

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
To determine the host-range of *Campylobacter* phages, from available collections or newly isolated phages, CVI established a panel of *Campylobacter* strains which were relevant to EU countries. This panel consisted of 54 strains from the Campynet collection (CNET) combined with 12 reference strains from NTCC. Table 2 shows the lytic spectrum of newly isolated phages from CVI.

In parallel, UMinho team analysed *Campylobacter* phages that were isolated within the scope of other projects (CEB-IBB's phage collection). These phages were screened against a panel of food and clinical isolates of *C. coli* and *C. jejuni* strains provided by University of Bristol (UK), Universidade Católica (Portugal) and by CVI (Table 1). From these phages, only phage 3A was selected for the subsequent subtasks since it has been already characterized (including its sequence), present broad lytic spectra and has already demonstrated its efficacy in vivo[1,2].

Table 1: Lytic spectra of the isolated phages against *C. coli* and *C. jejuni* strains of CVI

Strains	Phages																														
	1A	2A	3A	4A	5A	6A	7A	8A	11A	4B	5B	6B	7B	8B	9B	10B	1C	2C	3C	4C	5C	6C	3D	4D	5D	6D	7D	8D	9D	11D	12D	1E
64	-	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	+	+	+	+
89	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
95	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+
94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
65	+	+	+	+	+	-	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+
22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
83	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
96	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+
76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
77	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	-	+	+	-	+	-	-	+	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
19	+	+	+	+	+	-	-	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
88	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+	+	+	+	+	-	+	-	+	-	-	-	+
82	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
93	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
91	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+

Clear lysis areas were marked as (+), turbid lysis areas are represented as (+/-) and absence of lysis was marked as (-)

Table 2. Lytic spectra of *Campylobacter* phages to CNET/NTCC panel of *C. coli and C. jejuni* strains (+++ clear lysis, ++ bull's eye, weak lysis, - no lysis)

		Phage																					
Strain		001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	027
NCTC	12658	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	++	++	++	+++	+++	++
	12659	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	++	++	++	++	++	++
	12660	+++	+++	+++	+++	+++	++	++	++	++	++	++	+++	+++	++	++	++	++	++	++	++	+++	++
	12661	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	12662	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
	12663	-	-	-	-	-	++	++	++	++	-	++	++	-	-	-	-	++	++	-	++	-	++	-
	12664	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	++	++	++	+++	+++	++
	12665	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	++	++	++	++	+++	++
	12666	-	-	-	-	-	+++	+++	+++	+++	++	+++	++	-	-	-	+++	++	++	+++	++	++	++
	12667	-	-	-	-	-	++	++	++	++	++	++	+++	+++	-	-	+++	+++	++	++	++	+++	++
	12668	-	-	-	-	-	+++	+++	++	+++	+++	+++	+++	-	-	-	+++	+++	++	+++	++	++	+++
	11168	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
CNET	001	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	++	++	++	++	+++	++
	003	+++	+++	+++	+++	+++	+++	++	++	++	++	++	+++	+++	+++	+++	++	++	++	++	+++	+++	++
	005	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	++	-	++	-	-	++
	008	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	++	+	++	+	+	++
	010	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	-	-	++	-	+++	-
	012	+++	+++	+++	+++	+++	++	+++	++	++	++	++	++	+++	++	+++	++	++	+	++	+++	+++	+++
	015	-	-	-	-	-	+++	++	++	++	++	++	+++	-	-	-	++	++	-	++	++	-	+++
	017	-	-	-	-	-	++	+++	+++	++	++	++	++	-	-	-	+++	+++	++	+++	++	+++	+++
	020	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	022	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+++	+++	++	+++	++	+++	++
	024	-	-	-	-	-	+	++	++	++	++	++	++	-	-	-	++	++	+	++	-	+	+
	025	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	+	++	-	+++	++
	026	-	-	-	-	-	++	++	++	++	++	++	+++	-	-	-	++	++	-	++	-	-	++
	027	-	-	-	-	-	-	++	++	++	++	++	++	-	-	-	++	++	-	++	-	-	++
	028	++	++	++	++	++	++	+++	+++	++	+++	+++	+++	++	++	++	+++	+++	++	+++	++	++	++
	029	++	++	+++	++	++	++	++	++	++	-	++	++	++	++	++	++	+	-	++	-	++	++
	031	+++	+++	+++	+++	+++	++	++	++	++	++	++	+++	+++	+++	+++	++	++	++	++	+++	+++	++
	033	++	++	+++	+++	++	++	++	++	++	-	++	++	++	++	++	++	-	-	++	-	++	+
	035	-	-	-	-	-	-	-	-	-	-	-	++	-	++	+++	++	-	-	++	-	+++	+
	038	-	-	+	+	+	++	++	++	++	++	++	++	+	+	+	++	++	++	++	++	++	++
	040	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++
	041	++	++	++	++	++	++	++	++	++	+++	++	++	++	++	++	+++	++	++	+++	+++	+++	++
	044	-	-	-	-	-	-	++	++	++	++	++	++	-	-	-	++	++	++	++	-	-	++
	045	++	++	++	++	++	++	++	++	++	++	++	++	+++	++	+++	++	++	-	++	-	++	++
	047	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	+++	+++	+++	++	++	++	++	+++	+++	++
	049	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	+	++	++	+++	+++	++
	052	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	+++	+++	+++	++	++	+	++	++	+++	++
	055	+++	+++	+++	+++	+++	++	++	++	++	+	++	++	+++	+++	+++	++	++	+	++	+++	+++	++
	056	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	++	-	++	-	-	+
	058	-	-	-	-	-	++	++	++	++	++	++	++	-	-	+	++	++	++	++	++	++	++
	060	-	-	-	-	-	+++	+++	++	++	+++	+++	+++	-	-	-	+++	+++	+++	+++	+++	++	+++
	062	-	-	-	-	-	++	++	+++	++	+++	++	++	+	-	+	+++	+++	++	+++	++	++	++
	063	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	+++	+++	+++	++	++	-	++	+++	+++	++
	064	+	+	+	+	-	++	++	+++	++	+++	++	++	-	-	+	+++	+++	+++	+++	+++	+++	+++
	066	-	-	-	-	-	+++	+++	+++	++	+++	++	+++	-	-	-	+++	+++	++	+++	++	++	++
	069	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	++	++	++	++	++	++
	071	-	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	-	-	-	+++	+++	+++	+++	+++	+++	+++
	073	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	-	++	-	++	+
	075	++	++	++	++	++	-	-	-	-	-	-	-	++	++	++	-	-	-	-	-	++	-
	076	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	+++	+++	+++	++	++	++	++	+++	+++	++
	083	-	-	-	-	-	++	+++	++	++	++	++	++	-	-	-	++	++	-	++	-	-	++
	085	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	++	++	++	++	+++	++
	090	-	-	-	-	-	++	++	++	++	-	++	+++	-	-	-	++	++	-	++	-	-	++
	092	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	+	-	++	-	-	++
	093	+++	+++	+++	+++	+++	+++	+++	++	++	-	+++	+++	+++	+++	+++	++	++	-	++	+++	+++	++
	096	+++	+++	+++	+++	+++	++	++	++	++	++	++	++	+++	+++	+++	++	++	++	+++	++	+++	++
	099	-	-	-	-	-	++	++	++	++	-	++	++	-	-	-	++	++	++	++	++	++	++
	100	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	++	+++	++	++	++	+++
	103	-	-	-	-	-	+++	++	++	++	++	++	++	-	-	-	++	++	++	++	++	++	++
	104	-	-	-	-	-	++	++	+	+++	++	+++	++	-	-	-	++	++	+	++	-	-	++
	105	+++	+++	+++	+++	+++	+++	++	++	++	++	++	++	+++	+++	+++	+++	+++	++	+++	+++	+++	++
	107	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	++	++	++	-	++	++
	109	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	++	-	++	-	++	+
	111	-	-	-	-	-	++	++	++	++	++	++	++	-	-	-	++	++	-	++	-	+++	++
	113	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+	++	+	++	++
Host-range type		1	1	2	2	3	4	5	6	5	7	5	8	9	10	11	8	12	13	8	14	15	16

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. 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 (see Table 2).

The UMinho team isolated phages from poultry samples using the enrichment procedure previously described[3] (Figure 1). The isolated phages were screened against the formerly mentioned *Campylobacter* strains (Table 1) and the phages that showed the broadest lytic spectra (phages 3C, 4D, 1E) were selected for further characterization (Subtask 2.2.4).

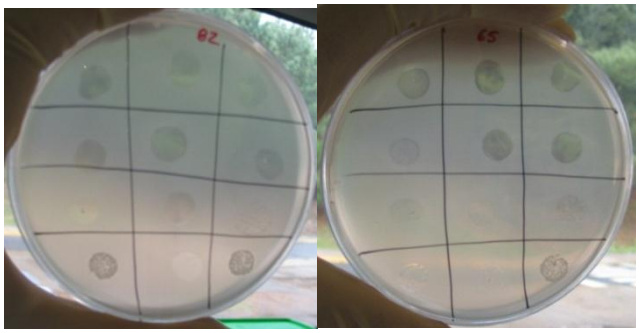

Figure 1. Phage halos in the “lawns” of bacteria 82 and bacteria 65

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 is currently optimizing the phage production in liquid broth.

The rate of phage production is logarithmic and proportional to the rate of cells growth and therefore the optimization of *Campylobacter* hosts growth in liquid media is also being addressed. Therefore during these 18 months period, the selected phages were screened against a panel of *Campylobacter* strains in order to select the host that yield better phage growth. Nevertheless, until the moment the maximum phage titer obtained was around 10^7PFU/ml in liquid media. A *Campylobacter* strain that has been described as having a faster growth will be provided by Utrecht University to Minho University and their performance evaluated. Different conditions such as media, incubation time, and agitation will be also assessed in the following months.

The UMinho team assayed different methods of purification/concentration already described for other phages. This is especially important since *Campylobacter* phages are highly sensitive to aggressive methods on account of their size, and it is still unknown if they “survive” to most of the purification/concentration methods. The methods used were Precipitation with Polyethylene Glycol (PEG) in the presence of high salt, purification by Isopycnic, centrifugation through CsCl, equilibrium gradients, purification by centrifugation through a glycerol step gradient and purification by pelleting/centrifugation. Nevertheless, the maximum concentration achieved for *Campylobacter* phages was 10^9PFU/ml. Although some of these methods are very valuable to concentrate and purify phages for molecular analysis, they cannot be used to purify large volumes of phage, as it is intended for therapeutic purposes.

UMinho also performed some phage adsorption assays in order to determine which factors may contribute to the *Campylobacter* phage adsorption and for the increase of phage titers.

Subtask 2.2.4: Characterization of phages
As a tool for identification, CVI developed a method to characterize *Campylobacter* phages by AFLP. Essentially, the same AFLP conditions (primers, ligation, protocol) were used as described for typing *Campylobacter* strains (Duim et al., 1999. Appl. Environ. Microbiol. 65,2369-2375), but to isolated phage DNA without its bacterial background DNA we used a dedicated phage DNA isolation kit (Norgen, Biotek Corp. Canada) preceded by a DNAse/RNAse treatment of phage particles. Figure 2 depicts the first results obtained by comparing AFLP patterns of phages and their host strains showing clustering between phages. Further typing of phages is in progress as well as phage DNA sequencing for some phages.

Figure 2. AFLP patterns of two phages and their respective host strains

The selected phages isolated by UMinho were screened against a panel of food and clinical isolates of *C. coli* and *C. jejuni* strains provided by University of Bristol (UK) and Universidade Católica (Portugal). In these particular strains the four phages showed similar lytic spectra and formed mostly turbid halos (Table 3).

Table 3: Lytic spectra of the selected phages against *C. coli* and *C. jejuni* strains

Strains	Phages			
	3A	3C	4D	1E
A11	T	T	T	T
2141	T	T	T	T
12668	T	T	T	T
P3	T	T	T	T
A17	-	-	-	-
1381	-	-	-	-
8909	T	T	T	T
A12	-	-	-	-
37	T	T	T	T
22	-	-	-	-
29	-	-	-	-
A15	+	+	+	+
2140	+	T	T	+
25	-	-	-	-
A2	+	+	+	-
21	-	-	-	-
8908	-	-	-	-
A4	-	-	-	-
A3	+	+	+	+
3820	T	T	T	-
8024	T	T	T	T
24	-	-	-	-
17565	T	T	T	T
27283	-	-	-	-
25594	T	T	T	-
11168	T	T	T	T
18940	T	T	T	-
28113	T	T	T	T
32711	T	T	T	+
18710	T	T	T	-
24030	T	T	T	-
28193	-	-	-	-
18724	-	-	-	-
27	T	T	T	T
24829	T	T	T	T
23	+	+	+	+
12658	-	T	T	-
8911	-	-	-	-
8910	T	-	-	-
P4	+	+	+	+
8907	T	T	-	-
12660	T	T	T	-
17572	+	+	+	-
12268	T	T	T	T
19	-	-	-	-
18709	T	T	T	-
24831	T	T	T	T

Clear lysis areas were marked as (+), turbid lysis areas are represented as (T) and absence of lysis was marked as (-)

Due to the low titers normally found in *Campylobacter* phages, it was needed to optimize the SDS-PAGE procedure in order to obtain the protein profiles of the selected phages. The results show that phages have different although similar protein profiles (Figure 3). This was expected since *Campylobacter* phages have been reported as highly conserved.

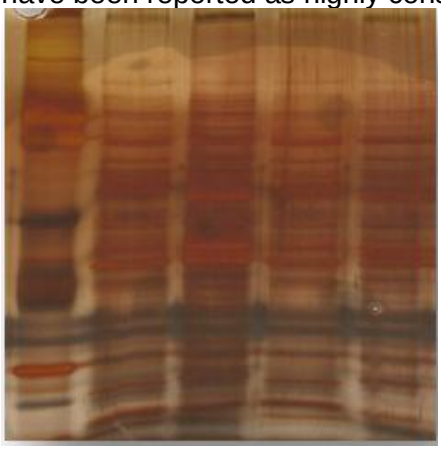

Figure 3. Agarose gel (10%) stained with silver stain: Ladder (lane 1); Phage 1E (lane 2); Phage 3C (lane 3); Phage 3A (lane 4); Phage 4C (lane 5)

Additionally and in order to check if the selected phages were able to cross-lyse the phage resistant variants of each other, a total of 14 *Campylobacter* in vitro resistant mutants were generated. The selected

phages were screened against these mutant strains. However none of the selected phages were able to lyse these strains. Therefore in order to check if the phages isolated by the other WP2 partners were able to lyse these mutants, there was an exchange of biological material. Accordingly, UMinho sent to CVI a total of 4 *Campylobacter* phages (previously selected), 45 *C. coli* and *C. jejuni* strains belonging to the CEB-IBB UMinho collection and 14 *Campylobacter* strains that are in vitro-induced resistant to the selected phages.

Phages supplied by the CVI were tested against the 14 *Campylobacter* phage-resistant mutants but, none of these phages was able to lyse these strains. On the other hand, UMinho selected phages were tested against Wageningen *Campylobacter* phage-resistant strains and did not lyse these strains.

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 4. 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																													

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 is still ongoing in order to isolate new phages from sewage, faeces of broiler chickens and wastewater from broiler houses and slaughterhouses. Subtask 2.2.4 will also progress depending on the results obtained from subtask 2.2.2. It is expected by the end of the 24 month project period, that these subtasks are accomplished and that a group of phages is selected for composing a phage cocktail. The subsequent tasks (2.2.5, 2.2.6, 2.2.7) planned to be performed in following years of CamCon project will then be addressed.

References
1. Carvalho C, Gannon B, Halfhide D, Santos S, Hayes C, Roe J, Azeredo J. 2010a. The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of Campylobacter coli and Campylobacter jejuni in chickens. BMC Microbiology 10: 232.
2. Carvalho CM, Kropinski AM, Lingohr EJ, Santos SB, King J, Azeredo J. 2011. The genome and proteome of a Campylobacter coli bacteriophage vB_CcoM-IBB_35 reveal unusual features. Virology JournalIn Press.
3. Carvalho C, Susano M, Fernandes E, Santos S, Gannon B, Nicolau A, Gibbs P, Teixeira P, Azeredo J. 2010b. Method for bacteriophage isolation against target Campylobacter strains. Letters in Applied Microbiology 50: 192-197.

Task 2.3 Vaccination (M1-48)

The work of Task 2.3 results in Deliverables 2.3.1 (M24), 2.3.2 (M24), 2.3.3 (M36) and 2.3.4 (M48).

The objectives of this Task are to determine the immune response against a *C. jejuni* subunit vaccine and *C. jejuni* whole cell vaccine and to test the protection of this vaccine after challenge of chicken with *C. jejuni*. The first towards meeting these objectives involve 1; the production of recombinant flagellin and, as a control, the non-modified flagellins, 2; assessment of the purity of the product, 3: vaccination of chicken with the vaccine and collection of serum samples, 4; to set up and validation an ELISA to evaluate the chicken immune response, and 5; to test the immune response against the various vaccine components. When needed, the vaccine dose and delivery method need to be optimized. The same approach is followed for the *C. jejuni* whole cell vaccine. Once an appropriate antibody response is obtained, challenge experiments in which are exposed to different homologous and heterologous strains will be performed to assess the efficacy or protection.

The basis of the subunit vaccine is a modified *C. jejuni* flagellin with intrinsic adjuvant activity. Two potential subunit vaccines have been designed that differ in degree of protein glycosylation.

In the first period of the project large batches of the production strains have been grown to produce sufficient subunit vaccine consisting of glycosylated and non-glycosylated re-engineered bacterial flagellin. The products were verified by gel electrophoresis and Western blotting using flagellin-specific antisera. In the next step the vaccines were administered in ovo. Embryonated eggs of SPF chicken were injected with the subunit vaccine and serum samples were collected at Day 9, Day 15 and Day 21 after immunization. Each vaccine group and control group consisted of 10 chicken.

In order to be able to analyze the humoral immune response against the vaccine components, we had to develop an ELISA using the different candidate vaccine components as antigens. Different amounts of subunit vaccine were coated on ELISA plates and a variety of blocking buffers were tested for their ability to reduce non-specific binding. The ELISA was validated using sera from infected chicken. After the appropriate blocking conditions and amount of antigen had been established, reliable detection of chicken antibodies directed against the bacterial flagellins was achieved.

Subsequent ELISA analysis of the serum samples derived from the chicken immunized with the different subunit vaccines showed that the vaccine generated an antibody response. This was found for both the vaccine consisting of glycosylated flagellins and for the non-glycosylated product. The immune response however, was quite variable among the different chicken within the same group. Antibody titres ranged from 1:4 to 1:512 after single immunization. Comparison of the average antibody responses of the different chicken groups showed that the antibody titer was highest at Day 11. At the second time point (Day 15), mean titres had had slowly decreased. In the third week after vaccination, a further sharp decrease in flagellin-specific antibody levels was observed. This decrease in antibody titres may indicate that antibodies are mainly produced in the first two weeks after vaccination, at least with the vaccine dose used. After this period, antibody production may not keep up with the dilution of antibodies in serum due to the rapid growth of the animals in the first weeks of life. With regard to the possible difference in response towards the different *C. jejuni* subunit vaccines, it was noted that there were small differences in antibody response against the engineered flagellin of the vaccine strain versus the positive control (native flagellin). In general the antibody response towards glycosylated flagellin vaccine was higher than the response towards non-glycosylated vaccine, but these differences were not significant.

The positive results of the immunization studies led us to move forward to ensure the quality of the vaccine components and to improve the stability of the vaccine production strain. A key aspect of our subunit vaccine approach is the combination of antigen and adjuvant in a single molecule. To exclude that other contaminants with possible adjuvant activity (such as LPS or DNA) interfered with the results, we

applied the previously developed chicken Toll-like receptor reporter system. This method involves the single expression individual Toll-like receptors in cultured cells and allows monitoring the activation of these adjuvant receptors by single bacterial ligands. With this method minute amounts of LPS, DNA or lipoproteins that may contaminate the vaccine preparations can be detected. Analysis of the purity of the used subunit vaccines using the chicken Toll-like receptor reporter system revealed differences in contamination between the different vaccines. However, minimal amounts of contamination were found after optimization of the flagellin purification procedure. In the next chicken immunization experiment which is scheduled in the next reporting period, the improved subunit vaccine will be administered.

During the production of the vaccine components, it was noted that production of the glycosylated product was repeatedly unstable. To improve the stability of the production of the engineered flagellin and its degree of glycosylation, we are in the process of moving the flagellin gene from the thus far used expression plasmid onto the bacterial chromosome. This next generation vaccine strain is expected to provide more stable and more homogenous glycosylation of the flagellin than after plasmid-encoded flagellin production.

In the coming period, a second chicken immunization experiment will be carried out using the improved C. jejuni subunit vaccine in more different formulations. Furthermore, the first whole cell vaccine will be prepared and tested in chicken. To reduce the need of animals in the future *C. jejuni* challenge of immunized animals to assess the level of protection of the vaccine, we will attempt to develop in vitro methods that allow prediction as to whether the antibodies that are generated in the chicken protect against colonization. Good correlates of protection do not exist for *C. jejuni* but would be a great asset to reduce the number of animal experiments in the future.

Significant Results

Although the on-farm implementation of fly-screens is delayed for Spain and the UK, there have been very informative discussions with integrations in both countries. For the UK the CamCon project can be strengthened through a close collaboration with a DEFRA project.

New bacteriophages have been isolated and exchange of phages and strains between the two groups makes it possible to compare the results of the two collections.

The first in ovo vaccination has been performed with a potential vaccine.

Deviations and corrective actions

Task 2.1: Fly screens add-on to biosecurity

UK: Broiler study houses (n = 28, on 12 farms) were recruited primo 2011 for another UK project on interventions against Campylobacter, nationally financed from DEFRA and also comprising fly screen intervention. As no houses at that point was recruited for CamCon, and the budget for 6 CamCon houses were anyway deficient, it was decided that the DEFRA project should share the future results of these 12 farms (and 12 control farms) with CamCon. In return CamCon should allocate part of the budget for intensified sampling of the chicken flocks raised in the DEFRA houses, as the DEFRA budget for sampling chicken flocks on the contrary was limited to sampling for two flock cycles only. The two projects will then share results of the fly screen intervention, on the premise that this agreement is approved by the funders of the DEFRA project (DEFRA).

Further it was decided at the Annual Meeting 14 April, that CamCon should still carry out one farm to demonstrate the feasibility of fly screens, by recording airflow, energy consumption, dust build up, time consumption etc. on one 2 house farm. A farm was recruited for that by July 2011 and preparatory work with the netting design and construction was started, but stopped again shortly.

Spain: A problem concerning the fly screen task was quickly noticed, that no data are available in Spain on the historical *Campylobacter* prevalence of flocks. Therefore the fly screen study has to wait for data to be achieved over a longer period (one year) in a number of farms (the 20 farm study). First thereafter, a potential fly screen intervention will be justified at all.

Another challenge identified in Spain by visiting potential study farms is that biosecurity practice in Spain is currently limited to farm level, not house level, which is insufficient for controlling of *Campylobacter*. Training activities targeted against the update of biosecurity to house level in Spain is therefore necessary before fly screen intervention can take place. Recently (21 Sept 2011), DTU has hosted a seminar concerning biosecurity for several representatives of Spanish broiler companies. Experience from this seminar confirmed too, that a training program for farmers in Spain is absolutely necessary before a benefit of fly screens can be expected. Furthermore, Mogens Madsen – task leader of WP5 and responsible for the planned training and learning programs to be produced in CamCon , has meet 27 Oct 2011 with partner 10 to target in more details the need for training of the Spanish farmers. The conclusion from that meeting is, that an advanced starting point for the work planned in task 5.1 - The best practice manual - will be beneficial and a precondition for launching the planned task 2.1 in Spain.

A functionality-test farm was recruited too in Spain to test the screen under the Spanish conditions. There has however been very limited feedback from the respective broiler company, so work has per end of this reporting period not started. Another Spanish broiler company has however expressed a true interest in cooperating with CamCon, but a more concrete consent has not been obtained as per 31 Oct 2011.

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 (M24), 3.1.2 (M24), 3.1.3 (M24) and 3.1.4 (M24).

Two devices for air sampling and subsequent detection of *Campylobacter* have been emphasized and compared in 4 broiler flocks to sock sampling as conventionally practiced in broiler houses in Denmark. The monitoring was combined with the profiling of airborne particle size distribution using a AeroTrak™ (TSI inc.) optical particle counter. One air sampling device is a “sniffer” based on the integrated lab on chip (ILOC) technology with a capacity of 120 ml/min (sampling for 15 min). The ILOC technology was previously shown to be superior to sock sampling in broiler houses (Olsen et al., 2009, AEM 75: 2074-2078) and to other air sampling methods for the monitoring of foot-and-mouth disease virus in the breath of cattle (Christensen et al., 2011, J Vir Methods 77: 44-48). However, technical problems with the ILOC devices combined with a bankruptcy of the inventor of ILOC and another focus of the company who took over the IPR of the technology resulted in inconsistent data in the present project. In conclusion, a strategy to implement a semi-automatic device for semi-continuous monitoring for Campylobacter is presently being reconsidered.

The other device tested in the project is a Sartorius Airport MD8 Air Sampler allowing the sampling of 2 m^3 of air. This approach gave highly reproducible results and gave positive results prior to - or concurrent with positive results of sock sampling. In conclusion, air is a most feasible sample matrix for the monitoring of *Campylobacter* in broiler houses. Among the technology platforms available at present, the Sartorius Airport MD8 is considered the most robust and user friendly device for air sampling.

A method to quantify *Campylobacter* in air has been developed. The method is based on the Sartorius Airport MD8 Air Sampler sampling 750 l of air through a gelatine filter with a pore size of 3 µm and diameter of 8 cm. Air borne particles are absorbed in the gelatine. A method for DNA extraction from the gelatine filters was developed and a standard curve for quantification was established based on a 10-fold dilution series of an overnight liquid culture of *Campylobacter jejuni*. Based on the standard curve, a detection limit was also defined, and data on the concentration of air borne Campylobacter in broiler houses were collected.

A manuscript is being prepared.

Task 3.2 Feasibility of real-time monitoring of *Campylobacter* in broiler flocks (M1-36)
The work of Task 3.2 results in Deliverables 3.2.1 (M36) and 3.2.2 (M48).

As air was found to be most reliably revealing the colonization with *Campylobacter* in broiler flocks an approach of semi-continuous quantitative monitoring based on air samples is feasible for study purposes. However, commercially available automatic or semi-automatic devices to make such an approach cost-efficient in broiler production are still needed.

Further studies will be 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 of monitoring in air will be tested in Poland and England and will be made available for the other work packages.

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
It was concluded that air in broiler houses is a most suited target for the sampling and detection of *Campylobacter*.

Methods to sample and quantify *Campylobacter* in air were developed.

Deviations and corrective actions
The progress in WP3 is on schedule.

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 (M1-48)
The work of Task 4.1 results in Deliverable 4.1.1 (M46).

In agreement with the planning, the development of the risk assessment has started. A conceptual model, which identifies modelling approaches and data needs, has been presented and discussed during the annual meeting. Pending the availability of results from the other WPs, the development of a virtual risk assessment model has started, which will allow an exploration of the potentials of the model, and clarify these to the project partners. This is of particular importance to maintain a good alignment between this WP and WPs 1-3.

Task 4.2 Data collection and compilation (M13-48)
The work of Task 4.2 results in Deliverable 4.2.1 (M46).

In agreement with the planning, the data collection and compilation has started. During a workshop at the annual meeting in Copenhagen in April, the plans and data needs for tasks 4.1, 4.3 and 4.4 have been explained. Data collection is started by sending out a specific data request to the CamCon partners, which resulted in an appropriate overview of the available data. Additional data will be obtained within the other WPs, at this stage the results of the questionnaire in WP1 (Task 1.1) are of particular interest.

Task 4.3 Economics (M13-48)
The work of Task 4.3 results in Deliverable 4.3.1 (M48).

In agreement with the planning, the research in the area of economics has started. A conceptual model, which identifies modelling approaches and data needs, has been presented and discussed during the annual meeting. Specific data requests related to costs of interventions at the farm in the selected participating countries have been sent out to the partners, and data have been received, as explained for task 4.2. 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 (M31-48)
The work of Task 4.4 results in Deliverable 4.4.1 (M46).

This task will be started in M31.

Task 4.5 Future data needs (M43-48)
The work of Task 4.5 results in Deliverables 4.5.1 (M46) and 4.5.2 (M48).

This task will be started in M31.

Significant Results

A successful workshop communicating data needs and data quality in WP4 has been held during the annual meeting 2011.

Deviations and corrective actions

The progress in WP4 is on schedule.

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 (M13-45)
The work of Task 5.1 results in Deliverable 5.1.1 (M44).

Consultations have been carried out with WP1 in the design of farm questionnaire. In order to secure relevant and necessary data and information that are to be used later in the knowledge dissemination activities carried out in WP5.

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 44, and will incorporate up-to-date information and knowledge created through the research activities in the other work packages of CamCon. However, as 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. For this reason the WP5 Leader met with partner 10 in Spain in October 2011in order to target in more details the need for training of the Spanish farmers. The outcome of the meeting was that a basic best practice manual based on our present knowledge on biosecurity measures needed to reduce Campylobacter introduction would be beneficial and a precondition for launching the planned task 2.1 in Spain.

This work will be carried out in the coming months and will be presented to potential broiler companies and cooperating farmers under Task 2.1, and will serve as a test case for creating the final version of the Best Practice Manual to be delivered in the last part of CamCon.

Task 5.2 Specific targeted learning programmes for proficiency in implementing the "Best Practice Manual for production of *Campylobacter*-free chickens" (M13-48)
The work of Task 5.2 results in Deliverables 5.2.1 (M42) and 5.2.2 (M48).

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 (M48), and work has not really started yet, except for a preliminary exercise with a video production, with the program running for a test period on the platform provided by the subcontractor (Conzentrate) of WP5.

Task 5.3 Voluntary Certification Programme (M37-48)
The work of Task 5.3 results in Deliverable 5.3.1 (M48).
Work has not started on this task yet, which is according to plans.

Significant Results

According to plan, the work in WP5 depends on inputs from the other WPs in this project, in particular WPs 1, 2, and 3, and has not really started yet. However, in preparation for work to be carried out later in the project under Task 5.1, it has been decided to prepare a basic best practice manual based on our present knowledge on biosecurity measures needed to reduce *Campylobacter* for use with test farms in Spain participating in WP2 trials. Also, in preparation for work with learning programmes to be performed later in Task 5.2, a preliminary exercise has been carried out with a video production, with the program running for a test period on the platform provided by the subcontractor (Conzentrate).

Deviations and corrective actions

The progress in WP5 is on schedule.

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)
The Financial assistant and the Project assistant were appointed in May 2010 and presented (and approved) at the kick off meeting. Both are affiliated to same Institute as the Coordinator (the Norwegian Veterinary Institute).

Task 6.3 Project web site established (M4)
The structure and the content of the web page was discussed with the WP leaders and other project participants during the summer 2010, and a test page was launched for testing. After adjustments, the web page was "officially opened" 18 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 was developed by the Coordinator and Project assistant and sent to the WP leaders for approval in November 2011. In December 2011, the approved plan was placed on the project's internal web pages. It will be revised later in the project.

Task 6.5 Reports of project's meetings
The Minutes from all meetings have been sent out to all participants and to the Advisory Board (and the Scientific Officer in the Commission) as soon as they have been approved. All Minutes have also been placed on the project's internal web page.

CamCon started with a kick-off meeting at DTU in Copenhagen 18 May 2010, 18 days after the official starting day. In 2011, the Annual meeting was held at DTU in Copenhagen 14 – 15 April. All participants except one were attending the kick off meeting, while it was impossible to participate for two institutions in the Annual Meeting in 2011. The Coordinator will make sure to emphasize the decision in the Consortium Agreement that all institutions should be present at Annual Meetings, in the preparations for the next Annual Meeting.

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. A few physical meetings have also been held within WPs.

The next Annual Meeting will be held at DTU in Copenhagen 24 – 25 April 2012.

Task 6.6 Regular reports to the European Commission (M20, M38, M50)
The first report is delivered on time.

Other management tasks
The Executive Board have had regular meetings, both in connection with the kick off meeting and the Annual Meeting. In addition, there have been - as part of the quarterly meeting questions to the WP leaders and to all the participants if there are any managerial problems or issues for discussions. No potential problems have ben reported.

Every 6 months, the Financial assistant or his co-workers have been asking the administrative contacts in each participating institution for financial status reports, mimicking the reporting requirements for the periodic reports. These have been compiled and evaluated by the Coordinator. Not every institution has delivered such reports, 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 project is developing reasonably well in relation to the actual plan. Only a few milestones are delayed, especially in the Task 2.1, dealing with implementing and evaluating fly screens for prevention of *Campylobacter*. This Task has run into difficulties, which were hard to foresee; unwillingness of farmers to believe in the concept, and suspicion from their side whether fly screens are practical at all. See further description in the Chapter on WP2 for explanations on how these difficulties have been dealt with. Even if it's delayed, the plan as it stands now will give valuable insight into, and knowledge about the possibilities to use this prevention method also in climates warmer than in Denmark.

Milestones in Task 1.1 are also delayed, mainly due to a bit optimistic planning. Having to deal with the prolonged summer vacations across Europe did make it difficult to get responses on drafts and implement them in a few weeks' time. Questionnaires were also not returned on time. Still, the Task has developed nicely, and a report from the questionnaire study is due in a few weeks.

Coordination and communication activities

The project as such was presented at CHRO 2011 in Vancouver by the Coordinator, and the Task leader for Task 1.1 presented results from the questionnaire study. The project as such has also been presented by the Deputy Coordinator at the General Assembly of a.v.e.c (Association of Poultry Processors and Poultry Trade in the EU countries). By this, the aims of the project have been communicated quite widely, both in the scientific community and to the industry.

As described elsewhere, the problems with identifying farms in UK for fly screening, the Annual Meeting 2011 discussed and decided that we should explore the possibility of collaborating with a newly funded Defra project. The possibilities and details of this collaboration are currently under discussion.

The Task on vaccination (Task 2.3) is collaborating with other vaccine projects like

Changes in Consortium or legal status of the beneficiaries

None.