

Control of Campylobacter infection in broiler flocks through  
two-steps strategy: nutrition and vaccination

-CAMPYBRO-

FP7-SME-2013-605835

## *Campylobacter* challenge for EU poultry producers

Budapest, 30/10/2013



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## *Who is Campylobacter?*

- ☐ **Mobile bacili.**
- ☐ **Microaerophylic**
- ☐ **Termophylic (42° C)**
- ☐ **Two main species:**
  - ☐ *C. jejuni*
  - ☐ *C. coli*
- ☐ **Sensible to oxygen, heat, desiccation and disinfectants**



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## Project Proposal - Necessity



### 8.1. Chicken and broiler meat as sources of human campylobacteriosis

The results from different attribution approaches, as applied in different countries, confirm previous epidemiological investigations that poultry is a major, if not the largest, single source of human infection. The proportion attributed by microbial subtyping to chicken as a reservoir ranges between

- Campylobacteriosis is now the most frequently reported zoonotic illness in the EU. There is considerable underascertainment and underreporting, and the true number of cases of illness is likely to be 10-100 times higher than the reported number. Serosurveillance indicates that European citizens may be exposed to *Campylobacter* sufficiently to produce an immune response every 1-3 years. There may be not less than 2 million and possibly as high as 20 million cases of clinical campylobacteriosis per year in the EU27.

EFSA Journal 2010; 8(1):1437

It is estimated that there are approximately nine million cases of human campylobacteriosis per year in the EU27. The disease burden of campylobacteriosis and its sequelae is 0.35 million disability-adjusted life years (DALYs) per year and total annual costs are 2.4 billion €.

In 2009, *Campylobacter* continued to be the most commonly reported gastrointestinal bacterial pathogen in humans in the EU since 2005. The number of reported confirmed human campylobacteriosis cases in the EU increased by 4.0 % in 2009 compared to 2008. The increase was

Campylobacteriosis is largely perceived to be food-borne, with poultry meat as a major source.

EFSA Journal 2011; 9(4):2105

Despite all efforts during the past decade there is still no effective, reliable and practical intervention measure available to prevent or to reduce *Campylobacter* colonization in broilers (Lin, 2009). As a consequence, neither the

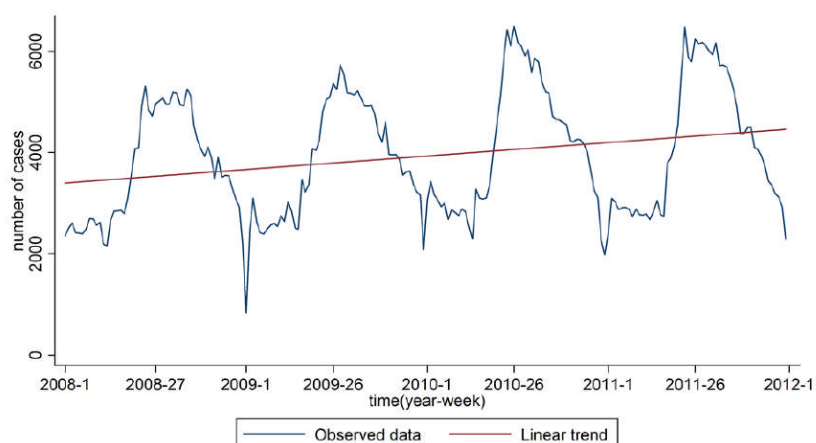
Hermans, et al., 2011

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## Project Proposal - Necessity



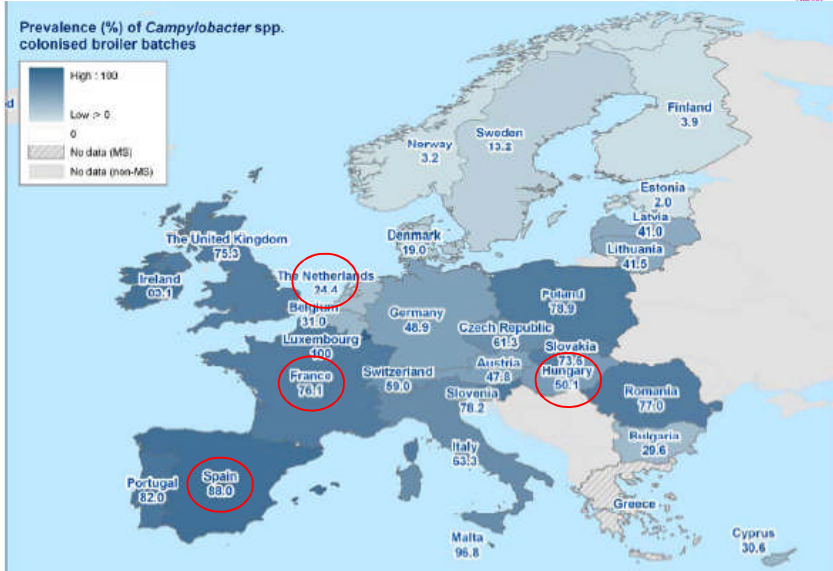
Figure CA1. Trend in reported confirmed cases of human campylobacteriosis in the EU, 2008-2011



EFSA Journal 2013,11(4):3129 4

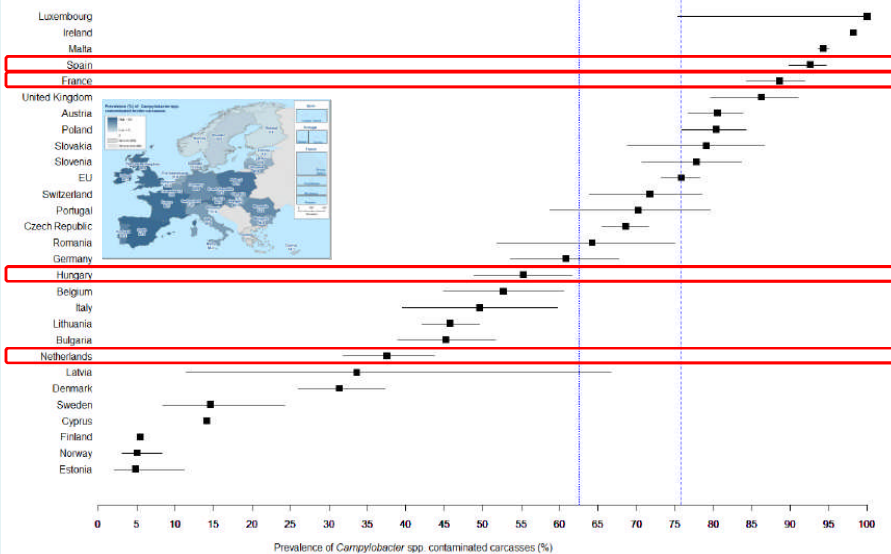


## Project Proposal - Necessity (batches)



EFSA Journal 2011; 9(4):2105

## Project Proposal - Necessity (Carcass)



EFSA Journal 2010; 8(03):1503



## Project Proposal - Impact

**Table 1:** Reported human campylobacteriosis in EU-27, Norway and Switzerland: 2010c)

Country	Population (million)	Reported human campylobacteriosis Cases	Incidence rate (per 100,000)	Country	Population (million)	Reported human campylobacteriosis Cases	Incidence rate (per 100,000)
Austria	8.319	4,301	51.7	Lithuania	3.366	762	22.6
Belgium	10.667	5,111	47.9	Luxembourg	0.484	439	90.7
Bulgaria	7.640	19	0.2	Malta	0.410	77	18.8
Cyprus	0.789	23	2.9	Poland	38.116	257	0.7
Czech Republic	10.381	20,174	194.3	Portugal	10.618	-	-
Denmark	5.475	3,470	63.4	Romania	21.529	2	0.0
Estonia	1.341	154	11.5	Slovakia	5.401	3,143	58.2
Finland	5.300	4,453	84.0	Slovenia	2.026	898	44.3
France	63.753	3,424	5.4	Spain	45.283	5,160	11.4
Germany	82.218	64,731	78.7	Sweden	9.182	7,692	83.8
Greece	11.214	-	-	The Netherlands	16.486	3,341	20.3
Hungary	10.045	5,563	55.4	United Kingdom	61.194	55,609	90.9
Ireland	4.401	1,752	39.8	EU-27	497.528	190,820	40.8
Italy	59.619	265	0.4	Norway	4.737	2,875	60.7
Latvia	2.271	0	0.0	Switzerland	7.593	7,877	103.7

Reduction associated with interventions in primary production is expected to vary considerably between MSs. Reducing the numbers of *Campylobacter* in the intestines at slaughter by 3 log<sub>10</sub>-units, would reduce the public health risk by at least 90%. Reducing the numbers of *Campylobacter* on the carcasses by 1 log<sub>10</sub>-unit, would reduce the public health risk by between 50 and 90%. Reducing counts by more than 2 log<sub>10</sub> units would reduce the public health risk by more than 90%. The risk

EFSA Journal 2011; 9(4):2105

- **≈ 9.000.000 human infections per year (UE-27).**
- **Total cost: 2.4 billions € per year.**

### High Economic Impact

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## UK

### A UK survey of *Campylobacter* and *Salmonella* contamination of fresh chicken at retail sale.

A UK-wide survey was undertaken by the Agency between May 2007 and September 2008 to determine *Campylobacter* and *Salmonella* prevalence on fresh chicken at retail. During the course of the survey 3363 samples were collected, with 3274 being acceptable for testing and microbiological examination using a presence/absence method for the detection of *Campylobacter* and *Salmonella*. *Campylobacter* enumeration tests were conducted on 927 samples, collected between April 2008 and August 2008.

The prevalence of *Campylobacter* in chicken at retail in the UK was 65.2%, based on the results from both methods combined, for the 927 samples tested.

A total of 1519 *Campylobacter* isolates were tested for their sensitivity to a series of antimicrobial drugs. Of these isolates, 197 (13.0%) were sensitive to all the drugs tested. This figure represents an increase in the frequency of antimicrobial resistance among *Campylobacter* isolated from retail chicken compared to that seen in the 2001 survey. There has also been an increase in resistance to the quinolones ciprofloxacin and nalidixic acid. The increase

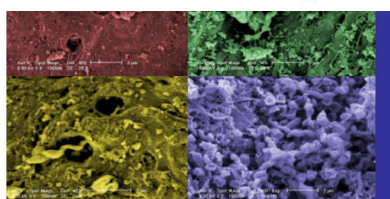
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## UK



Food security is an increasing priority for the UK Government and food safety is a key component of this. *Campylobacter* is the most common cause of food poisoning in the UK and is responsible for an estimated 321,000 estimated cases in England and Wales in 2008<sup>1</sup>, with over 15,000 hospitalisations and 76 deaths. *Campylobacter* accounts for a third of the cost of food-borne illness in England and Wales, estimated at £583 million in 2008. It is found mainly in poultry but also in red meat, unpasteurised milk and untreated water. Although it does not normally grow in or on food, it can transfer easily. Illness can arise from only a few bacteria in undercooked chicken, or in ready-to-eat foods that have been cross-contaminated from raw chicken. *Campylobacter* infections do not usually cause vomiting, but diarrhoea can be severe and bloody, with additional abdominal cramps.

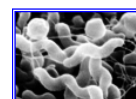


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## *Campylobacter* vs campylobacteriosis



- ❑ Poultry is one of the most important reservoirs for *Campylobacter* and constitutes a significant vehicle for the transmission of campylobacter to humans (Humphrey et al., 2007).
- ❑ Retail
  - ❑ UK: 46-77%
  - ❑ Ireland: 50%
- ❑ Most important risk factors for contracting *Campylobacter* infection are consumption of chicken, lettuce and eating in takeaways
- ❑ Chicken consumption showed a dose-response relationship, whereby more frequent consumption of chicken increased the risk of infection by 20% per time of consumption (Danis et al., 2009).



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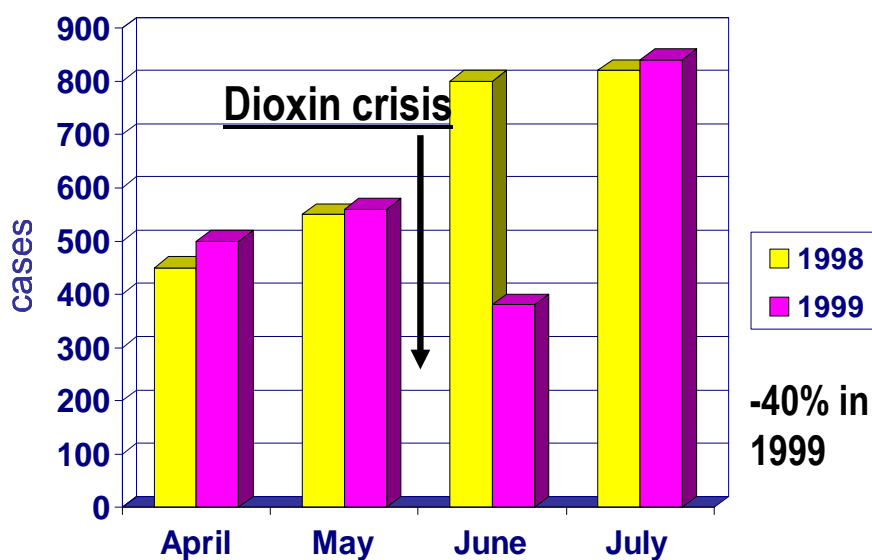
## Campylobacter vs campylobacteriosis



- ❑ A total of 31% of human clinical isolates can be related to retail chicken. A study clearly identified retail chicken as the single largest source of clinical campylobacter infection in Scotland (FSA Scotland, 2009).
- ❑ In Canada, a 30-year study revealed that 56% of outbreak cases of campylobacteriosis were associated with poultry (Ravel et al., 2009).

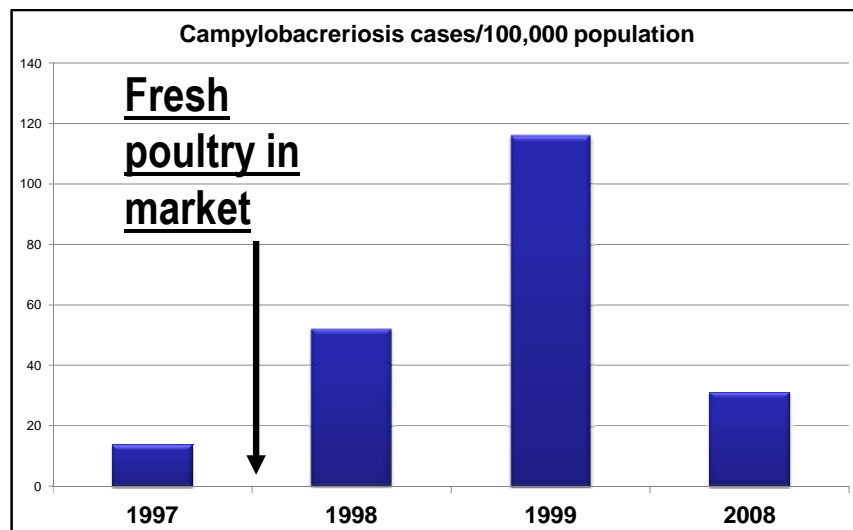
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## Belgium, 1999





## Iceland



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## Campylobacter vs campylobacteriosis

### How can I reduce the risk to consumers?

Reducing the contamination of broilers and the subsequent risk to human health can be achieved by reducing the concentration of Campylobacter in the intestines of broilers on-farm and reducing the concentration of Campylobacter on the surface of processed chickens in the slaughterhouse

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## Interventions



- ❑ Pre-harvest interventions
- ❑ Post-harvest interventions
- ❑ Labeling
- ❑ Education programs
  
- ❑ **NO VERTICAL TRANSMISSION**

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## Pre-harvest interventions



- ❑ Relationship between the concentration of *Campylobacter* in the intestines and the levels found on the surface of carcasses.
- ❑ It has been reported that *Campylobacter* levels in caeca are approximately 1 log<sub>10</sub> higher than faecal levels (Nauta et al., 2007).
- ❑ A positive correlation was found between high levels of colonisation in the caeca and concentrations on corresponding carcasses and portioned products (Reich et al. 2008)

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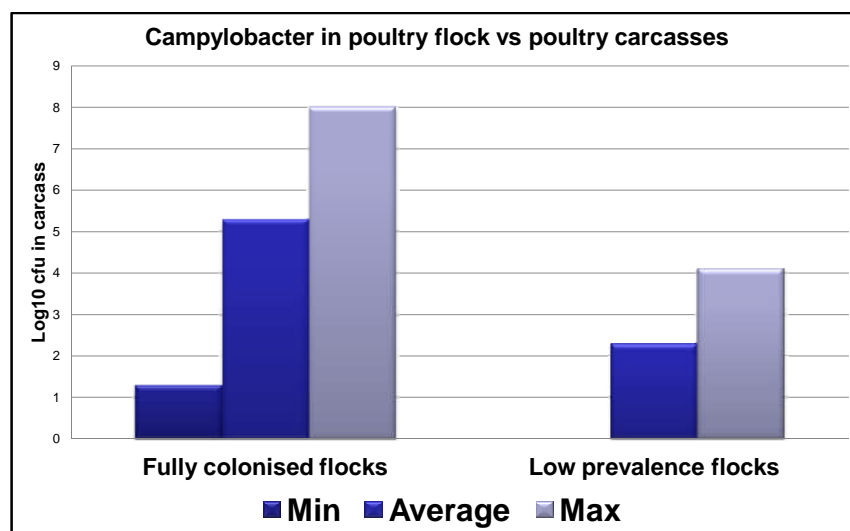
## Pre-harvest interventions



- ❑ Rosenquist et al. (2006) documented a correlation between campylobacter concentration in intestinal content and on chicken carcasses after defeathering.
- ❑ Mean concentrations in neck skin samples after defeathering are closely related to the mean concentration in the intestinal samples.
- ❑ Mean number of campylobacter/g in positive intestinal samples ranged from 4.74 log<sub>10</sub> to 8.2 log<sub>10</sub> cfu/g, compared to the mean number of *Campylobacter*/g in neck skin after defeathering which ranged from (1.90– 3.93 log<sub>10</sub> cfu/g).
- ❑ The concentration on neck skin (after defeathering) was 4.2 log<sub>10</sub> cfu/g less than levels recovered in the intestines

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## Pre-harvest interventions



Allet et al., 2007

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## Pre-harvest interventions



- ❑ A risk assessment simulation showed that the incidence of campylobacteriosis associated with consumption of chicken meals could be reduced 30 times by introducing a  $2 \log_{10}$  reduction of the number of *Campylobacter* spp. on chicken carcasses.
- ❑ Based on the Dutch risk assessment (Nauta et al., 2005), processing is expected to yield approximately a  $2 \log_{10}$  reduction from the initial *Campylobacter* concentration in faeces, prior to slaughter, to the final concentration on the dressed carcass.

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## Pre-harvest interventions



- ❑ Microbiological criteria
  - ❑ Ireland:  $4 \log_{10}$  cfu/g
  - ❑ UK (2015): batches  $> 3 \log_{10}$  cfu/g lower 10%
  - ❑ EC?

Broiler (cecum)	Broiler (fecal)	Broiler (carcass after chilling)
$10^n$	$10^{n-1}$	$10^{n-3}$
$10^7$	$10^6$	$10^4$
$10^6$	$10^5$	$10^3$



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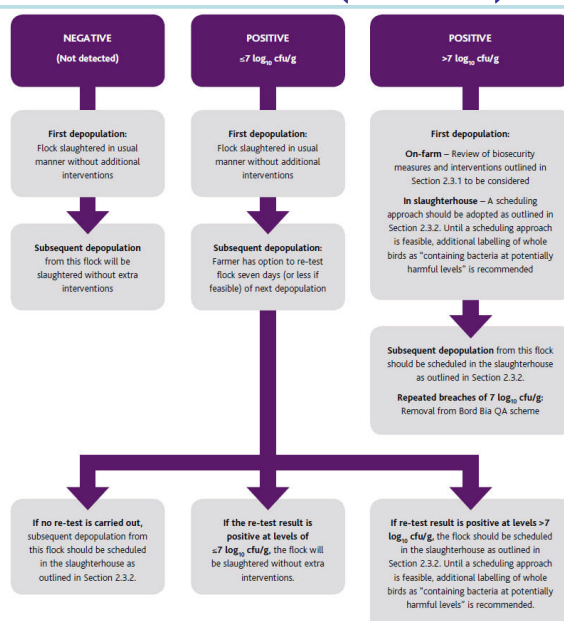
## Pre-harvest sampling



- ❑ A limit of  $\leq 6-7 \log_{10}$  cfu/g in pooled caecal samples is recommended (depending the limit in the carcass to be achieved)
- ❑ Sampling should be carried out on-farm seven days (or less if feasible) before slaughter.
- ❑ Testing of each house should be performed by randomly selecting 10 birds from various locations within the house which will be harvested and dispatched to the laboratory on the same day.
- ❑ The caecal contents of the 10 birds will be pooled for analysis in the laboratory.
- ❑ Enumeration of campylobacter in the pooled samples will be carried out by a laboratory with accreditation

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## Pre-harvest criteria (Ireland)



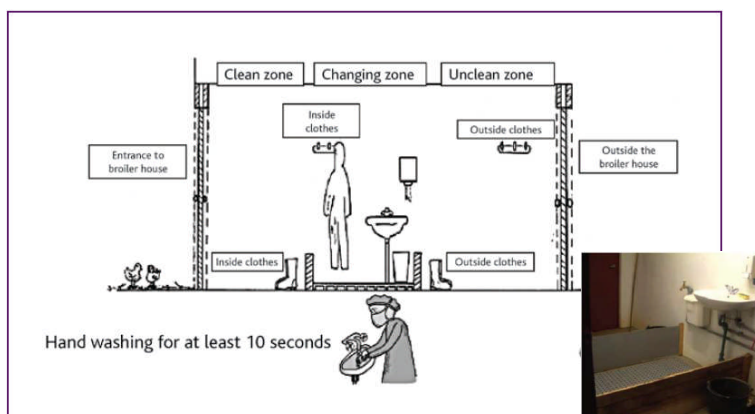
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## Pre-harvest (non nutritional) measures



- ❑ **Personnel access and hygiene:**
  - ❑ **A system of boot changes and hygiene measures in the anterior room**
  - ❑ **Personnel access and hygiene during non-routine events**



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## Pre-harvest (non nutritional) measures



- ❑ **Surrounds of broiler house:**
  - ❑ **A concrete apron (maintained in good condition) is recommended in front of the doors to the broiler house**
  - ❑ **It is recommended that pebbles/gravel are placed along the sides of the houses to permit rainage, prevent dust circulation and rodents**



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## Pre-harvest (non nutritional) measures

- ❑ **Fly control**
  - ❑ Wherever possible, fitting of fly screens is recommended, particularly in high prevalence flocks.
  - ❑ Alternative methods of fly and insect control may be appropriate where fly screening is not possible
- ❑ **Biosecurity during preparation and stocking of house**
  - ❑ It is recommended that the truck ramp is thoroughly cleaned and then disinfected and that the ramp is placed very close to the entrance of the house to ensure that the modules carrying the chicks are not a source of contamination

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## Pre-harvest (non nutritional) measures

- ❑ **Reduction of slaughter age**
  - ❑ A policy of slaughtering flocks at a younger age particularly during the summer months, may lead to a reduction for flocks with persistently high concentrations of *Campylobacter* spp.
- ❑ **Disposal and storage of waste**
  - ❑ Adequate storage and disposal of broiler farm waste is important to eliminate the potential of waste being a source of contamination for subsequent flocks.
- ❑ **Sexing of birds**
  - ❑ Greater homogeneity of birds at time of slaughter

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## Pre-harvest (non nutritional) measures

- ❑ **Partial depopulation (Thining)**
  - ❑ No more than one partial depopulation prior to the final depopulation
  - ❑ Period between the partial depopulation and the final depopulation should not exceed five days
  - ❑ Effective cleaning and disinfection of forklift wheels and forks prior to entry into broiler house
  - ❑ Improved crate, module and truck washing and disinfection in the slaughter plant
  - ❑ Thinning teams must put on clean protective clothing and clean, disinfected boots prior to entry into the broiler house.
  - ❑ There should be dedicated footwear supplied for use by the thinning teams for each house on-farm
  - ❑ Education of thinning personnel to enforce the importance of biosecurity

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## Post-harvest sampling (flocks)

- ❑ Should take place at the time of evisceration in a way to minimize external contamination from caeca content. Intact caeca
- ❑ At random through the batch (avoiding the first part of the batch)
- ❑ 10 intact caeca collected may be placed in a single sterile bag/pack for transport= a pooled sample

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## Post-harvest sampling (carcasses)



- ❑ Should be confined to broilers from those farms whose pre-depopulation sampling results are positive and have levels that are  $\leq 6-7 \log_{10} \text{ cfu/g}$ .
- ❑ Should take place after chilling and prior to further processing
- ❑ Should be carried out when approximately half of the flock have been slaughtered.
- ❑ Fifteen carcasses should be sampled. A piece of approximately 10g of neck skin shall be obtained from each carcass. The neck skin samples from three carcasses shall be pooled before examination in order to form  $5 \times 25\text{g}$  final sampling units.
- ❑ Samples should be dispatched to a suitable laboratory for isolation and enumeration of *Campylobacter* spp

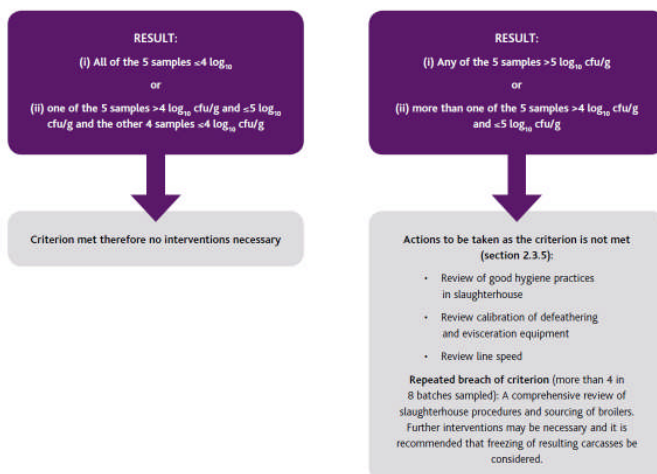
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## Post-harvest criteria (Ireland)



Post-harvest sampling should be conducted once a week on a carcass from a flock whose pre-harvest result is positive and  $\leq 7 \log_{10} \text{ cfu/g}$ .

Sampling plan and limits:  $n=5$  samples;  $c=1$  sample between  $m$  and  $M$ ;  $m=4 \log_{10} \text{ cfu/g}$ ; and  $M=5 \log_{10} \text{ cfu/g}$  (for details see section 2.2.3 and Appendix 2)



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## UK



**THE JOINT GOVERNMENT AND INDUSTRY TARGET  
TO REDUCE *CAMPYLOBACTER* IN UK PRODUCED CHICKENS BY 2015  
DECEMBER 2010**

**TARGET TO REDUCE *CAMPYLOBACTER* IN UK PRODUCED CHICKENS**

**The Target**

6. The target will be to reduce *Campylobacter* contamination on whole chickens in UK slaughterhouses and will be based on *Campylobacter* counts (enumeration) as this is

The target will be monitored using a banding approach, where samples are grouped into 3 bands according to whether the *Campylobacter* counts in chicken are above or below a set level (i.e. <100 cfu/g, 100-1,000 cfu/g, and >1,000 cfu/g). The target is limited to 3 bands for simplicity and to allow sensible interpretation when monitoring progress against the baseline. The target focuses on decreasing the proportion of birds in the most contaminated group i.e. >1,000 cfu/g. A number of factors affect the likelihood of

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## UK



**THE JOINT GOVERNMENT AND INDUSTRY TARGET  
TO REDUCE *CAMPYLOBACTER* IN UK PRODUCED CHICKENS BY 2015  
DECEMBER 2010**

The UK target for reduction of *Campylobacter* is a reduction in the percentage of chickens produced in UK poultry slaughterhouses that have the highest level of contamination, i.e. those with more than 1,000 cfu per gram, from a baseline of 27% in 2008 to 10% by 2015, measured post-chill. It is expected that the least contaminated chickens i.e. less than 100 cfu per gram, will get no worse or will improve upon the baseline of 42% by 2015. The baseline was determined in 2008 by the EU survey of *Campylobacter* in broiler batches and on *Campylobacter* and *Salmonella* on broiler carcasses<sup>7</sup>.

The target will be set in the slaughterhouse at the end of the slaughter process, post chill. The advantages, and disadvantages, of a slaughterhouse target were compared to

enhanced biosecurity to keep *Campylobacter* out of UK poultry farms. The new on-farm standards will be implemented throughout the UK by the Red Tractor Farm Assurance Poultry Standards – Broiler and Poussin, in April 2011. The new standards will be

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## UK



**THE JOINT GOVERNMENT AND INDUSTRY TARGET  
TO REDUCE CAMPYLOBACTER IN UK PRODUCED CHICKENS BY 2015**

**DECEMBER 2010**

**Slaughterhouse interventions**

	Campylobacter enumeration		
	<100 cfu/g	100-1,000 cfu/g	>1,000 cfu/g
Baseline	42%	31%	27%
2013 Expected progress	Expected improvement	Expected improvement	19%
Target reviewed 2013 2015 target reset as appropriate			
Model estimates (2015)	68%	22%	10%
Target 2015	Expected improvement	Expected improvement	10% Target

3  
3

## Post-harvest measures



- ❑ Freezing of chicken
- ❑ Adjustment and monitoring of slaughtering equipment
- ❑ Crust-freezing of chicken
  - ❑ The CO<sub>2</sub> crust-freezing technique has been shown to produce consistent reductions (approx. 0.5 log<sub>10</sub>)
  - ❑ It can be sold as fresh meat, provided the temperature of the meat remains greater than or equal to -2°C (Regulation (EC) No. 543/2008).
- ❑ Removal of skin from chicken
  - ❑ skinless products have counts several logs lower than the respective meat products with skin
- ❑ Logistical approach to slaughter
  - ❑ broilers from farms with higher counts of Campylobacter spp. in the caeca) will be slaughtered later in the day

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## Post-harvest measures



- ❑ **Hot water treatment of carcasses**
  - ❑ 1.66 log<sub>10</sub> cfu/cm<sup>2</sup> following hot water immersion treatment at 75°C for 30 seconds (Corry et al. 2007)
- ❑ **Steam treatment of carcasses**
  - ❑ steam can penetrate cavities, crevices and feather follicles
  - ❑ 1.3 log<sub>10</sub> cfu/g decrease in *Campylobacter* numbers with 90°C atmospheric steam for 12 seconds
  - ❑ BUT denatured appearance of the skin or meat surface
- ❑ **Combined steam and ultrasound**
  - ❑ ≥2.52 log<sub>10</sub> cfu/carcass
  - ❑ Denatured appearance of the skin or meat surface

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## Post-harvest measures



- ❑ **Diversion of carcasses to value added products**
  - ❑ **Marinating**
  - ❑ **Heat treatment of chicken**
    - ❑ D-value (decimal reduction time – the time required at a certain temperature to kill 90% of a particular organism) for *Campylobacter* spp. in cooked chicken at 55°C is 2.12-2.25 minutes and at 57°C 0.79-0.98 minutes (ICMSF, 1996)
- ❑ **Modified atmosphere packaging (MAP)**
  - ❑ Boysen et al. (2007) reported reductions in *Campylobacter* numbers of 2.0-2.6 log<sub>10</sub> cfu/g after eight days in 70%/30% O<sub>2</sub>/CO<sub>2</sub> mixture BUT no reduction with 70%N<sub>2</sub>/30%CO<sub>2</sub>.
  - ❑ Modified atmosphere of 80% O<sub>2</sub>/20% N<sub>2</sub> resulted in a reduction of approximately 1.2 log<sub>10</sub> cfu/g (Rajkovic et al., 2010)

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## Post-harvest measures



- ❑ **Module, Crate and Vehicle Washing**
  - ❑ Ensure that crates, modules and vehicles are effectively cleaned and disinfected.
  - ❑ Appropriate temperatures for cleaning (hot water) and disinfection and adequate concentrations of disinfectants should be used.
  - ❑ This cleaning should be validated through periodic microbiological testing

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## Post-harvest measures



- ❑ **Scalding**
  - ❑ 50-52°C (soft scald) to 56-68°C (hard scald).
  - ❑ Humphrey et al. (2007): death rate in scald tank water maintained at 52°C was 9 minutes (not achieved)
  - ❑ Counter-current scald tanks: water in the tank should move through the system flowing against incoming carcasses. This flow creates a 'dirty to clean' gradient
  - ❑ High water flow rates in the tanks
  - ❑ Multi-stage scald tanks
    - ❑ 1-4log<sub>10</sub> reduction

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## Post-harvest measures



- ❑ **Defeathering**
  - ❑ Cross-contamination of carcasses by the machinery may occur at this step
  - ❑ Contamination increases largely due to the escape of faecal material through the cloaca by the action of the picker fingers pressing on the abdomen
  - ❑ The feather follicles in the skin at this stage are open and may lead to movement of campylobacter cells inside the follicles
  - ❑ Recommendations to minimise cross-contamination
    - ❑ Correct alignment of machinery based on bird size
    - ❑ Adequate flow rates of water
    - ❑ Regular equipment sanitation and maintenance<sub>39</sub>

## Post-harvest measures



- ❑ **Evisceration**
  - ❑ Minimize the rupture of the exposed intestines and prevent the spread of faecal bacteria
  - ❑ Excessive pressure by the equipment on the abdominal cavity (Berrang et al., 2004) or an accidental cut may occur
  - ❑ Significant amounts of *Campylobacter* can be introduced during this process step (Takahashi et al., 2006) potentially resulting in extensive cross-contamination
  - ❑ Recommendations
    - ❑ Control alignment of equipment
    - ❑ Visual inspection of carcasses to identify problems with evisceration
    - ❑ Regular equipment sanitation and maintenance<sub>40</sub>



## Post-harvest measures



- ❑ **Chilling**
  - ❑ Carcasses are still warm and must be chilled as soon as possible to inhibit microbial growth
  - ❑ Chilled room until they are correctly chilled ( $<4^{\circ}\text{C}$ ).
  - ❑ Decrease in counts of campylobacter of 1  $\log_{10}$
  - ❑ **Recommendations**
    - ❑ Appropriate design and maintenance of equipment
    - ❑ Appropriate line speeds
    - ❑ Washing of carcasses prior to chilling is recommended to remove surface debris and contamination

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## WP OBJECTIVES AND PROJECT SCHEDULE



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## What is 7th FP?



- ❑ **7th Framework Programme for Research and Technological Development (RTD)**
- ❑ **Two main strategic objectives:**
  - ❑ to strengthen the scientific and technological base of European industry;
  - ❑ to encourage international competitiveness while promoting research that supports EU policies.
- ❑ **2007 – 2013**
- ❑ **Budget of over €55 billion**

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## FP7 Specific programmes



1. Cooperation → *Collaborative trans-national research activities*
2. Ideas → *Frontier, basic research.*
3. People → *Human Potential. Marie Curie actions and other initiatives*
4. **Capacities** → *Research infrastructures, Benefits of SME, **Benefits of SME Associations**, regions of knowledge, science and society, international cooperation,...*

Encourage and facilitate SME AG participation across FP7



## FP7 Capacities

### “Capacities” –

#### Research for the benefit of SMEs

##### Objectives

Strengthen the innovation capacities and competitiveness of SMEs, to develop new products and markets by **outsourcing of research**.

Clear exploitation potential with economic benefits for the SMEs (or for the SME members of the associations) involved.

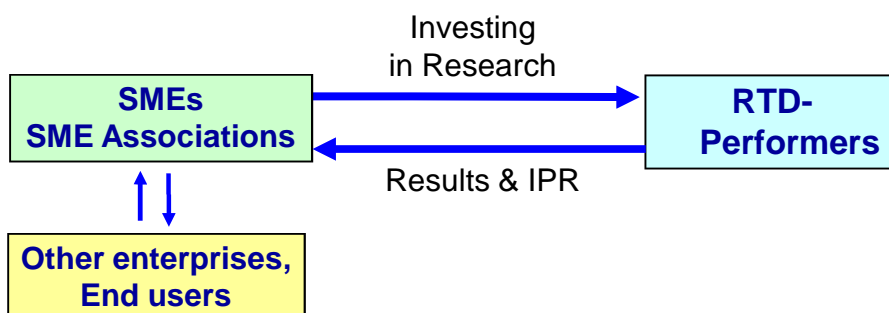
→ bottom-up approach, no thematic focus

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## FP7 Capacities

### “Capacities” –

#### Research for the benefit of SMEs



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## Consortium



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## Imasde Agroalimentaria, S.L.

- ❑ Private research center with R&D projects in agroindustries as our main activity.
- ❑ Coordinator



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## ANSES - Missions



## Emerging interest for Governments and R&D institutions

### Review

#### Bacteriocins to control *Campylobacter* spp. in poultry—A review

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**ABSTRACT** The unacceptably high frequency of *Campylobacter jejuni* transmission from poultry to humans encourages scientists to consider and create alternative intervention strategies to control the pathogen in poultry production. Extremely high numbers of *Campylobacter* (often  $>10^8$  cfu/g of poultry intestinal material) potentiate high numbers of the organism on the processed broiler carcasses with increasing consequent human health risk. Many scientists believe interventions during poultry production portend the greatest opportunity for reducing risk of disease. Over the past 10 yr, we have focused our studies on nonantibiotic bacteriocin application to intervene during animal production and this is the subject of the current review. The application of therapeutic bacteriocin treatments to reduce poultry colonization diminishes *Campylobacter* from  $>10^8$  cfu/g of cecal materials to nondetectable or very low levels in broiler birds. Further, the review provides scientists with a useful starting point for the further development of industry-applicable interventions leading to reduced transmission of this agent in human disease.

**Key words:** *Campylobacter*; bacteriocin; colonization; broiler; intervention

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### ORIGINAL ARTICLE

#### Reduced spread of *Campylobacter jejuni* in broiler chickens by stimulating the bird's natural barriers

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## Emerging interest for Governments and R&D institutions



### Evaluating the efficacy of an avian-specific probiotic to reduce the colonization of *Campylobacter jejuni* in broiler chickens

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**ABSTRACT** Campylobacteriosis is the most frequent zoonotic disease in humans worldwide, and the contaminated poultry meat by *Campylobacter jejuni* can be considered one of the important sources of enteric infections in humans. The use of probiotics, which can help to improve the natural defense of animals against pathogenic bacteria, is an alternative and effective approach to antibiotic administration for livestock to reduce bacterial contamination. In vitro experiments showed that *Enterococcus faecium*, *Poliovaccinia aciditica*, *Lactobacillus salivarius*, and *Lactobacillus reuteri* isolated from healthy chicken gut inhibited the growth of *C. jejuni*. To demonstrate this effect in vivo, 1-d-old broiler chicks received 2 mg/bird per day of a multispecies probiotic product via the drinking water. Controls

received no probiotic treatment, and all chicks were infected with *C. jejuni* orally. Results showed that the cecal colonization by *C. jejuni* was significantly reduced by probiotic treatment at both 8 and 15 d postchallenge. To confirm this effect, in a second in vivo experiment, 1-d-old broiler chicks received the same dose of the same probiotic via the drinking water and controls received no probiotic, and all chicks were infected with *C. jejuni* orally. Similarly, probiotic treatment reduced ( $P = 0.001$ ) cecal colonization by *C. jejuni* at both 8 and 15 d postchallenge. **The results of our in vivo experiments conclude that probiotic administration reduced the colonization of *C. jejuni* in broiler chickens.**

**Key words:** probiotic, *Campylobacter jejuni*, colonization, food-borne illness, broiler

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**Table 1.** The effect of administration of a multispecies probiotic product (PoultryStar soil, BIOMIN GmbH, Herzogenburg, Austria) on the cecal colonization of *Campylobacter jejuni* in broiler chickens in 2 in vivo experiments<sup>a</sup>

Item	Treatment		SEM	P-value
	Control (n = 10)	PoultryStar soil (2 mg/bird/day) (n = 10)		
First experiment				
<i>C. jejuni</i> (log cfu/g) (8 d postchallenge)	6.77 <sup>a</sup>	3.09 <sup>b</sup>	0.51	0.001
<i>C. jejuni</i> (log cfu/g) (15 d postchallenge)	8.09 <sup>a</sup>	2.59 <sup>b</sup>	0.23	0.001
Second experiment				
<i>C. jejuni</i> (log cfu/g) (8 d postchallenge)	7.81 <sup>a</sup>	<2.00 <sup>b</sup>	0.52	0.001
<i>C. jejuni</i> (log cfu/g) (15 d postchallenge)	7.80 <sup>a</sup>	<2.00 <sup>b</sup>	0.51	0.001

<sup>a,b</sup>Means within the same row with different superscripts are significantly different (Mann-Whitney test was performed for the first experiment,  $n = 10$ /treatment and Kruskal-Wallis test followed by Mann-Whitney test for the second experiment,  $n = 10$ /treatment).

<sup>c</sup>Data presented as means of logarithms of 10 cecal samples per group (log cfu/g).

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### A *Bifidobacterium*-based synbiotic product to reduce the transmission of *C. jejuni* along the poultry food chain

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Poultry food chain

#### ABSTRACT

With the ban of dietary antimicrobial agents, the use of probiotics, prebiotics and synbiotics has attracted a great deal of attention in order to improve intestinal health and control food-borne pathogens, which is an important concern for the production of safe meat and meat products. Recently, *Campylobacter jejuni* has emerged as a leading bacterial cause of food-borne gastroenteritis in humans, and epidemiological evidences indicate poultry and poultry products as the main source of human infection. This work aimed at the development of a synbiotic mixture capable of modulating the gut microflora of broiler chickens to obtain an increase of the beneficial bacteria (i.e. *Bifidobacterium*, *Lactobacilli*) and a competitive reduction of *C. jejuni*. The prebiotic compound used in the mixture was chosen after an in vivo trial: a fructooligosaccharide and a galactooligosaccharide were separately administered to broilers mixed with normal feed at a concentration of 0.5% and 3%, respectively. Quantitative PCR on DNA extracted from fecal samples revealed a significant ( $p < 0.05$ ) increase of *Bifidobacterium* spp. in broilers treated with the galactooligosaccharide, coupled to a decrease ( $p < 0.05$ ) of *Campylobacter* spp. The galactooligosaccharide was then combined with a probiotic *Bifidobacterium* strain (*B. longum* subsp. *longum* PC133), possessing in vitro antimicrobial activity against *C. jejuni*. The strain was microencapsulated in a lipid matrix to ensure viability into the feed and resistance to stomach transit. Finally, the synbiotic mixture was administered to broiler chickens for 14 days mixed with normal feed in order to have an intake of  $10^9$  CFU of PC133/day. *Bifidobacterium* spp., *Lactobacillus* spp., *Campylobacter* spp., *B. longum* subsp. *longum* and *C. jejuni* were quantified in fecal samples. PC133 was recovered in feces of all animals. *C. jejuni* concentration in poultry feces was significantly ( $p < 0.05$ ) reduced in chickens administered with the synbiotic mixture. This study allowed to highlight the positive effect of the synbiotic approach for *C. jejuni* reduction in broiler chickens, which is of fundamental importance for the safety of poultry meat consumers.

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## Emerging interest for Governments and R&D institutions



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### Characterization of probiotic strains: An application as feed additives in poultry against *Campylobacter jejuni*

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#### ARTICLE INFO

Keywords:  
Probiotics  
Feed additives  
Poultry  
- *jejuni*  
Food safety

#### ABSTRACT

*Campylobacteriosis* is at present the most frequent zoonosis in humans and the main source is poultry meat contaminated by *Campylobacter jejuni*. An alternative and effective approach to antibiotic administration to livestock to reduce bacterial contamination is the use of probiotics, which can help to improve the natural defence of animals against pathogenic bacteria. In this study 55 lactic acid bacteria and bifidobacteria were screened for desirable properties for their application as probiotics against *Campylobacter* in poultry. All bacteria were examined for their antimicrobial activity against three *C. jejuni* strains. Strains exhibiting the highest anti-*Campylobacter* activity were examined for their survival in the gastro intestinal tract (low pH and presence of bile salts) and food/feed processing conditions (high temperature, high NaCl concentration and starvation) and basic safety aspects such as antibiotic susceptibility and hemolytic activity were studied. On the basis of these activities, two strains, namely *Lactobacillus plantarum* PCS 20 and *Bifidobacterium longum* PCB 133, were chosen for an *in vivo* trial in poultry. They were separately administered to healthy chickens in order to evaluate their capability of colonizing the GI tract of poultry and to estimate their effect on *C. jejuni* population. The results evidenced that *L. plantarum* PCS 20 was not present in poultry feces at detectable concentration, whereas *B. longum* PCB 133 significantly increased after two weeks of daily administration and its amount was still high after a wash-out period of 6 days. In the same period, *C. jejuni* concentration in poultry feces was significantly reduced in chickens administered with *B. longum* PCB 133. Therefore, *B. longum* PCB 133, possessing interesting probiotic properties and a marked anti-*Campylobacter* activity both *in vitro* and *in vivo*, is an excellent candidate for being employed as additives to feed for poultry for the reduction of food-borne campylobacteriosis in humans.

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### Bactericidal Activities of Plant Essential Oils and Some of Their Isolated Constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*

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#### ABSTRACT

An improved method of sample preparation was used in a microplate assay to evaluate the bactericidal activity levels of 96 essential oils and 23 oil compounds against *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella enterica* obtained from food and clinical sources. Bactericidal activity (BA50) was defined as the percentage of the sample in the assay mixture that resulted in a 50% decrease in CFU relative to a buffer control. Twenty-seven oils and 12 compounds were active against all four species of bacteria. The oils that were most active against *C. jejuni* (with BA50 values ranging from 0.003 to 0.009) were marigold, ginger root, jasmine, patchouli, gardenia, cedarwood, carrot seed, celery seed, mugwort, spikenard, and orange bitter oils; those that were most active against *E. coli* (with BA50 values ranging from 0.046 to 0.14) were oregano, thyme, cinnamon, palmarosa, bay leaf, clove bud, lemon grass, and allspice oils; those that were most active against *L. monocytogenes* (with BA50 values ranging from 0.057 to 0.092) were gardenia, cedarwood, bay leaf, clove bud, oregano, cinnamon, allspice, thyme, and patchouli oils; and those that were most active against *S. enterica* (with BA50 values ranging from 0.045 to 0.14) were thyme, oregano, cinnamon, clove bud, allspice, bay leaf, palmarosa, and marjoram oils. The oil compounds that were most active against *C. jejuni* (with BA50 values ranging from 0.003 to 0.034) were cinnamaldehyde, estragole, carvacrol, benzaldehyde, citral, thymol, eugenol, perillaldehyde, carvone R, and geranyl acetate; those that were most active against *E. coli* (with BA50 values ranging from 0.057 to 0.28) were carvacrol, cinnamaldehyde, thymol, eugenol, salicylaldehyde, geraniol, isoeugenol, citral, perillaldehyde, and estragole; those that were most active against *L. monocytogenes* (with BA50 values ranging from 0.019 to 0.43) were cinnamaldehyde, eugenol, thymol, carvacrol, citral, geraniol, perillaldehyde, carvone S, estragole, and salicylaldehyde; and those that were most active against *S. enterica* (with BA50 values ranging from 0.034 to 0.21) were thymol, cinnamaldehyde, carvacrol, eugenol, salicylaldehyde, geraniol, isoeugenol, terpineol, perillaldehyde, and estragole. The possible significance of these results with regard to food microbiology is discussed.

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## Emerging interest for Governments and R&D institutions



### Is allicin able to reduce *Campylobacter jejuni* colonization in broilers when added to drinking water?

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**ABSTRACT** Reducing *Campylobacter* shedding on the farm could result in a reduction of the number of human campylobacteriosis cases. In this study, we first investigated if allicin, allyl disulfide, and garlic oil extract were able to either prevent *C. jejuni* growth or kill *C. jejuni* in vitro. Allyl disulfide and garlic oil extract reduced *C. jejuni* numbers in vitro below a detectable level at a concentration of 50 mg/kg (no lower concentrations were tested), whereas allicin reduced *C. jejuni* numbers below a detectable level at a concentration as low as 7.5 mg/kg. In further experiments we screened for the anti-*C. jejuni* activity of allicin in a fermentation system closely mimicking the broiler cecal environment using cecal microbiota and mucus isolated from *C. jejuni*-free broilers. During these fermentation experiments, allicin reduced *C. jejuni* numbers below a detectable level after 24 h at a concentration of 50 mg/kg. In contrast, 25 mg/kg of allicin killed *C. jejuni* in the first 28

h of incubation, but anti-*C. jejuni* activity was lost after 48 h of incubation, probably due to the presence of mucin in the growth medium. This had been confirmed in fermentation experiments in the presence of broiler cecal mucus. Based on these results, we performed an in vivo experiment to assess the prevention or reduction of cecal *C. jejuni* colonization in broiler chickens when allicin was added to drinking water. We demonstrated that allicin in drinking water did not have a statistically significant effect on cecal *C. jejuni* colonization in broilers. It was assumed, based on in vitro experiments, that the activity of allicin was thwarted by the presence of mucin-containing mucus. **Despite promising in vitro results, allicin was not capable of statistically influencing *C. jejuni* colonization in a broiler flock, although a trend toward lower cecal *C. jejuni* numbers in allicin-treated broilers was observed.**

**Key words:** *Campylobacter jejuni*, allicin, in vivo, broiler, drinking water

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## Emerging interest for Governments and R&D institutions



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### The cinnamon-oil ingredient trans-cinnamaldehyde fails to target *Campylobacter jejuni* strain KC-40 in the broiler chicken cecum despite marked in vitro activity.

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#### Abstract

*Campylobacter jejuni* is the most common bacterial cause of diarrheal disease in humans worldwide, with poultry products being a major source. Therefore, strategies to decrease *Campylobacter* colonization during primary production might aid in reducing the number of human campylobacteriosis cases. Several plant-derived compounds have been reported to possess anti-*Campylobacter* properties in vitro, so they could be promising candidates to reduce *Campylobacter* colonization in broiler chickens. To test this hypothesis, selected plant-derived antimicrobials (caffeic, gallic, protocatechuic, and vanillic acids, epigallocatechin gallate, trans-cinnamaldehyde, and thymol) were screened for anti-*Campylobacter* activity by determining MICs and setting up time-kill curves for *C. jejuni* strain KC-40. These experiments revealed marked antibacterial activity, especially for the cinnamon-oil ingredient trans-cinnamaldehyde (CIN). This compound was tested in a broiler chick seeder model; it was added to the feed in coated form at an effective concentration of 0.3% from day-of-hatch for the entire 22-day duration of the experiment. At 14 days of age, one-third of the birds were inoculated with *C. jejuni* strain KC-40 and served as seeders. CIN was not able to reduce cecal *Campylobacter* colonization in this model, which was confirmed in a cecal loop experiment. Despite CIN concentrations much higher than the MIC, *C. jejuni* numbers were not reduced compared with those in nontreated ceca at 2 and 24 h after injection. **In conclusion, this study shows a marked discrepancy between in vitro and in vivo activity of CIN against *C. jejuni* strain KC-40.**

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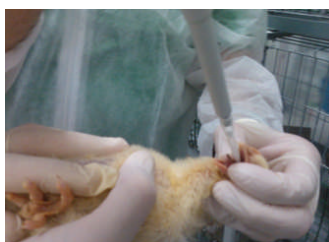


## Work Packages



### **WP 1. Efficacy of several compounds against *Campylobacter* in broilers orally infected looking for synergies.**

- T1.1. In vivo effectiveness of products based on plant extracts, organic acids, prebiotics, and probiotics against *Campylobacter*.
- T1.2 In vitro effectiveness of mixtures of products: Synergistic effect
- T1.3. In vivo effectiveness of product mixtures based on plant extracts, organic acids, prebiotics, and probiotics against *Campylobacter*.

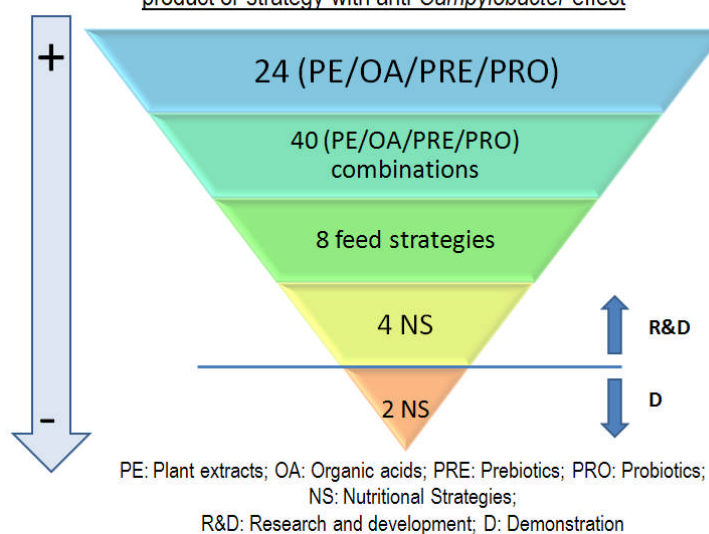


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## Work Packages



Figure 1.4.1. Selective pressure procedure to detect to best product or strategy with anti *Campylobacter* effect



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## Work Packages



### **WP2. Feed presentation strategies against *Campylobacter*.**

- ❑ T2.1. Effect of feed composition, particle size and feed presentation on the prevalence of *Campylobacter* in broilers orally infected
- ❑ T2.2 Effect of whole grain feeding on the prevalence of *Campylobacter* in broilers orally infected.

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## Work Packages



### **WP 3. Interactions between products and feed presentation against *Campylobacter*. Synergies.**

- ❑ T3.2. Interactions between product mixtures and feeding strategies against *Campylobacter* looking for synergies
- ❑ T3.2 Studies in the effect of the duration of treatment on the final infection: design of functional diets
- ❑ T3.3. Study on the correlation between in vitro and in vivo results. Cost-Benefit analyses.

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## Work Packages



### **WP 4. Application of different nutritional strategies against *Campylobacter* in experimental farm and field trials.**

- ❑ T4.1. Effect of different strategies against *Campylobacter* on performance parameters and level of infection of broilers chickens in experimental farm.
- ❑ T4.2. Effect of different strategies against *Campylobacter* on performance parameters and level of infection of broilers chickens in commercial farms.
- ❑ T4.3. Effect of different strategies against *Campylobacter* on performance parameters and level of infection of turkeys in commercial farms.

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## Work Packages



### **WP 5. Development of a novel vaccine against *Campylobacter* based on reverse vaccinology**

- ❑ T5.1. Exhaustive identification of new potential vaccine antigens against *Campylobacter* using the reverse vaccinology strategy.
- ❑ T5.2. Development of an in vitro test to visualize the recognition of *Campylobacter* antigens by antibodies.
- ❑ T5.3. Determination of an efficient sub-unit vaccination protocol
- ❑ T5.4. Selection of the *Campylobacter* proteins that will be evaluated for their protective capacity
- ❑ T5.5. Assessment of the protective potentials against *Campylobacter* induced by the selected vaccine candidates.

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## Work Packages



### **WP 6. Evaluation of the developed nutritional strategies in different geographical situations.**

- T6.1. Evaluation of developed nutritional strategies in South, Central, and East European conditions

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## Work Packages



### **WP 7. Project Management.**

- T7.1. Contractual, legal, Administrative and financial management and overseeing of ethical and gender issues
- T7.2. Monitoring and coordination of technical activities of the project, and planning, organizing and reporting of Project Coordinating Committee and General Assembly
- T7.3. Relationship with the European Commission

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## Work Packages



### WP 8. Dissemination, training and exploitation.

- ☐ T8.1. Dissemination of project results
- ☐ T8.2. Training to achieve project results implementation
- ☐ T8.3. Exploitation of project results