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Oterh Dissemination actitivites

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Campyylobacter challenge for EU poultry producers

Expected submission date:

Actual submission date:

Grant Agreement number	605835
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Project title	Control of Campylobacter infection in broiler flocks through two-steps strategy: nutrition and vaccination
Project website	<a href="http://www.campybro.eu">www.campybro.eu</a>
Project coordinator organization name	IMASDE AGROALIMENTARIA, S.L.
Project coordinator name & email address	Dr. Pedro Medel <a href="mailto:pmedel@e-imasde.com">pmedel@e-imasde.com</a>

Dissemination Level		
PU	Public	X
PP	Restricted to other program participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

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## 1. SUMMARY

A presentation of the *Campylobacter* problem for EU poultry producers was made in Madrid on 11/02/2014. The public were the CEO of the 16 bigger poultry producer in Spain, and Ángel Martín. The presentation included the *Campylobacter* description, and how the project deal with it. Also, the first results were presented, including the results that will be published at EPC congress in Norway in the summer of 2014. The presentation was made by Dr. Pedro Medel.

Figure 1. Ángel Martín with part of the companies representants.



Figure 2. Pedro Medel with the rest of the companies representants.



Control of Campylobacter infection in broiler flocks through  
two-steps strategy: nutrition and vaccination  
-CAMPYBRO-  
FP7-SME-2013-605835

## *Campylobacter*: un reto para la avicultura de carne europea



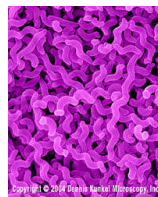
Madrid, 11/02/2014



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## *¿Quién es Campylobacter?*

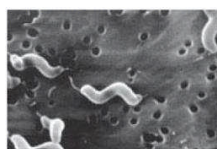
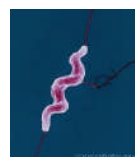
- ❑ **Género *Campylobacter* spp.**
  - ❑ **Bacilos gram negativos**
  - ❑ **Curvados o con forma espiral**
  - ❑ **Móviles mediante un flajelo uni ó bipolar**
  - ❑ **No esporulados**
  - ❑ **Microaerofílicos**
    - ❑ **Óptimo 5-10% O<sub>2</sub> y 1-10% CO<sub>2</sub>.**



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## ¿Quién es *Campylobacter*?

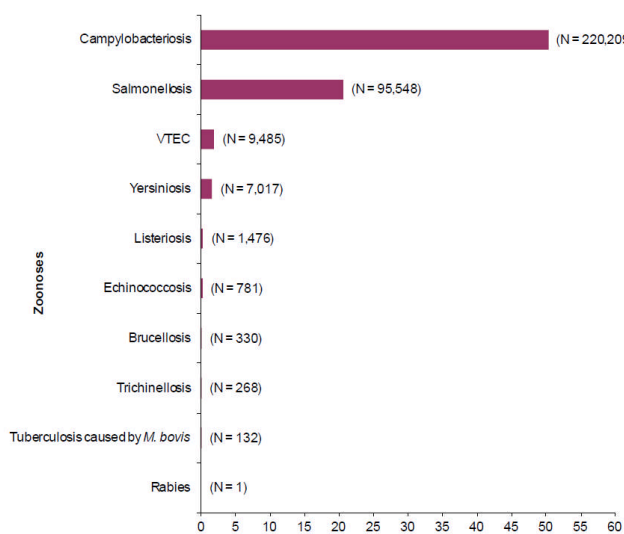
- ❑ 23 especies
- ❑ Termófilos (32-47°C, Óptimo: 42°C)
  - ❑ 25°C no crecen
  - ❑ Causantes gastroenteritis humanas
  - ❑ *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis* y *C. helveticus*
- ❑ Alta sensibilidad
  - ❑ Oxígeno
  - ❑ Dsecación o baja actividad de agua
  - ❑ Congelación
  - ❑ Luz ultravioleta, desinfectantes
  - ❑ Calor: 55°C, 1min
  - ❑ NaCl 2%
  - ❑ pH<4,7



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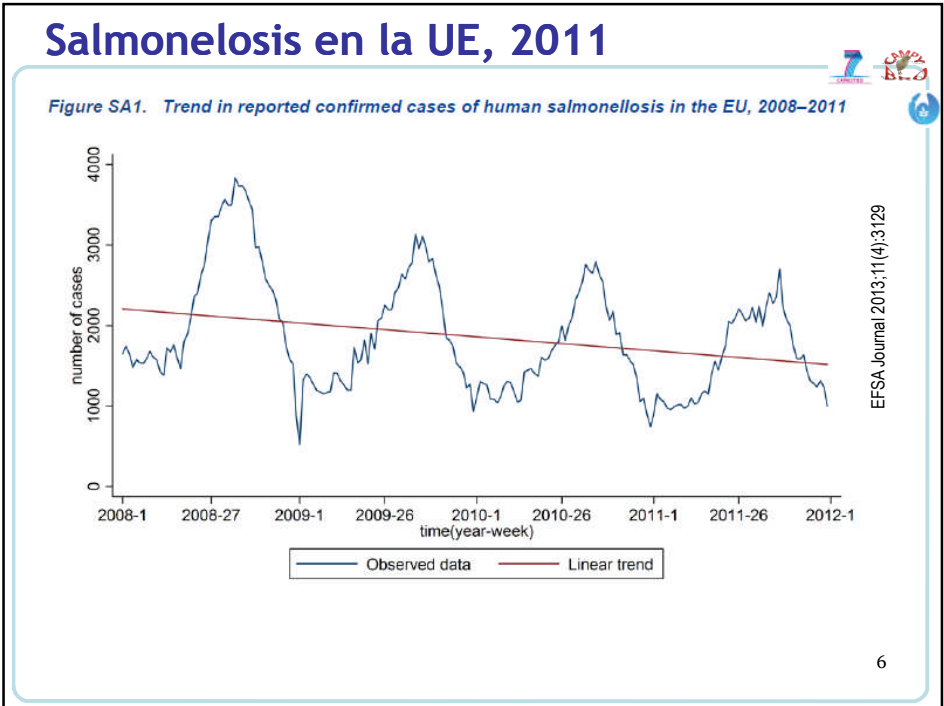
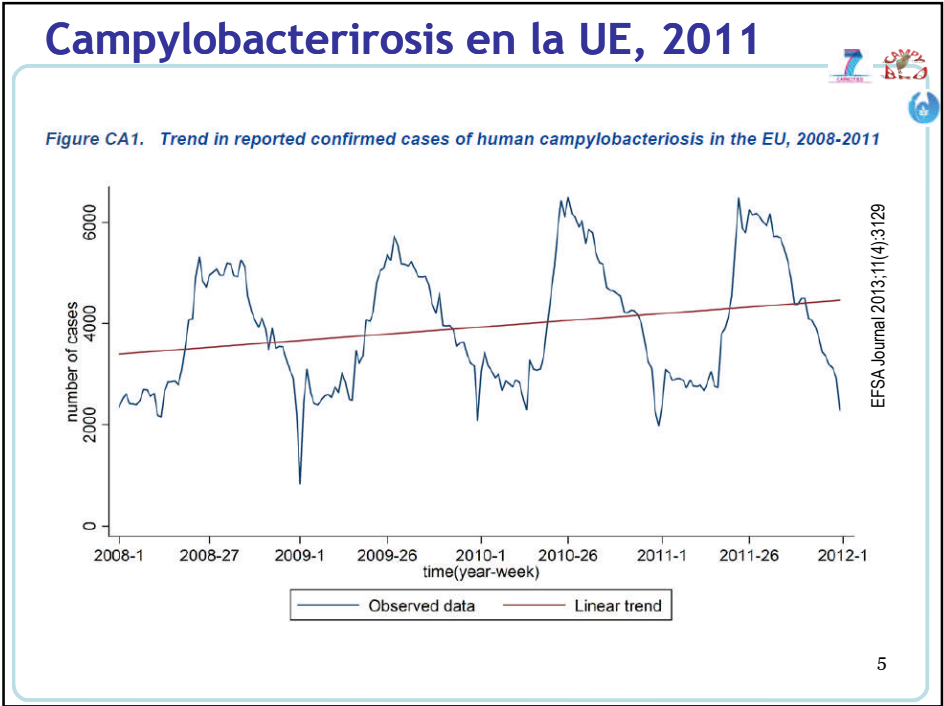
## Campylobacteriosis en la UE, 2011

Figure SU1. Reported notification rates of zoonoses in confirmed human cases in the EU, 2011



EFSA Journal 2013;11(4):3129

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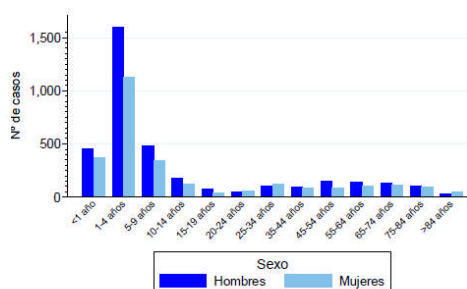




## Campylobacteriosis en España, 2011

Figura 4. Vigilancia de *Campylobacter*, 2011

Casos por grupos de edad y sexo



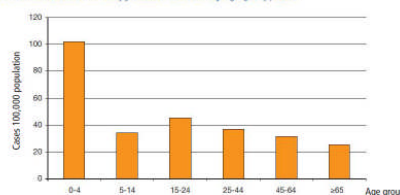
Fuente: Sistema de Información Microbiológica (SIM)



RESULTADOS DE LA VIGILANCIA  
EPIDEMIOLÓGICA DE LAS  
ENFERMEDADES TRANSMISIBLES.  
INFORME ANUAL. AÑO 2011



Figure CA3. Incidence of *Campylobacter* infection by age group, 2006



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## Campylobacteriosis y carne de pollo, EU 2011

### Resumen

*Campylobacteriosis* continued to be the most commonly reported zoonosis in humans in the EU since 2005. In 2011, the number of notified cases of thermotolerant *Campylobacter* in the EU increased by 2.2 % compared with 2010. The EU notification rate of confirmed cases of human campylobacteriosis has shown a statistically significant increasing trend in the last four years (2008–2011). The reasons for this increasing

Considering the high number of campylobacteriosis cases, the severity in terms of fatalities reported was low (0.04 %). The proportion of hospitalised cases was, on the other hand, larger than expected taking into

Broiler meat is considered to be a major source of human campylobacteriosis, as a result of undercooking and cross-contamination of RTE foods, as well as through direct hand-to-mouth transfer during food preparation. The EFSA's Panel on Biological Hazards (BIOHAZ) concluded in its scientific opinion<sup>26</sup> that handling, preparation and consumption of broiler meat may account for 20 % to 30 % of human campylobacteriosis cases in the EU, while 50 % to 80 % may be attributed to the chicken reservoir as a whole. *Campylobacter* strains from the broiler reservoir may also be transmitted to humans via routes other than food (e.g. via the environment or by direct contact). The principal reservoirs of *Campylobacter* spp. are the alimentary tracts of wild and domesticated birds and mammals. There are multiple pathways of human

In 2011, fresh broiler and other poultry meat were again the foodstuffs in which *Campylobacter* was most frequently reported. Overall, about one third of the samples were reported as positive, although there were

reported food-borne outbreak data from 2011. Approximately half (17 out of 37) of the *Campylobacter* outbreaks, in which information on the implicated food vehicle was provided, were linked to broiler meat. In

□ ≈ 9.000.000 casos/año (UE-27)

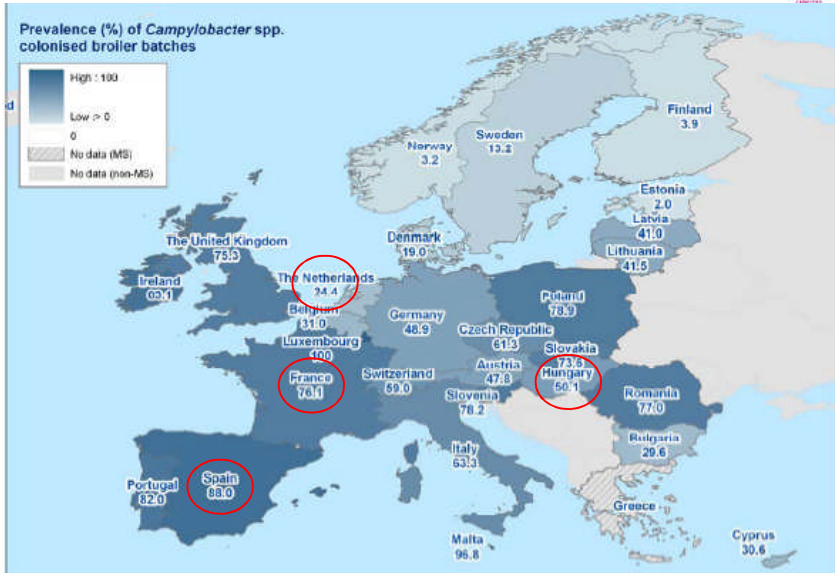
□ Coste estimado: 2.400 M€/año

EFSA Journal 2013;11(4):3129

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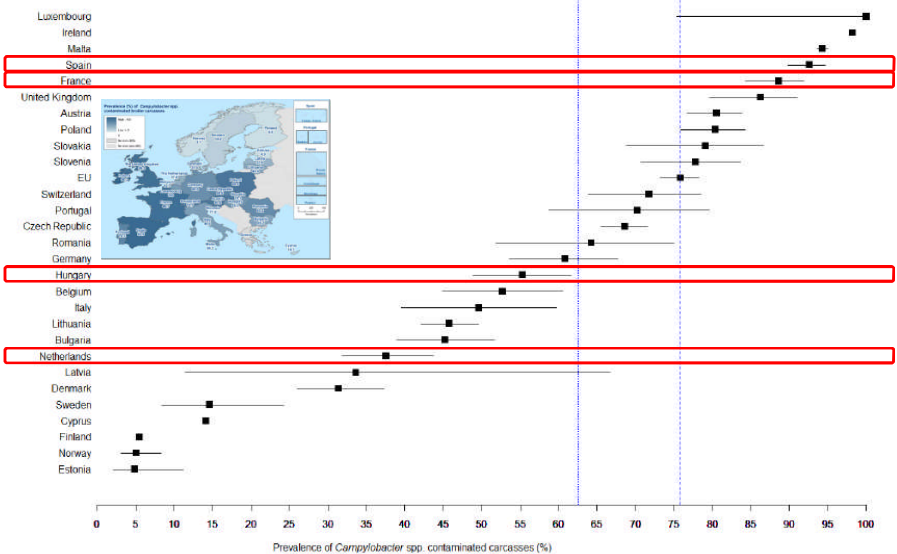
Estudio prevalencia, manadas



EFSA Journal 2011; 9(4):2105

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Estudio de prevalencia, canales



EFSA Journal 2010; 8(03):1503

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## **Campylobacter vs campylobacteriosis**



### **¿Cómo puedo reducir el riesgo para los consumidores?**

**La reducción de la contaminación de la carne de pollo y el consiguiente riesgo para la salud humana se puede lograr mediante la reducción de la concentración de *Campylobacter* en los broilers, y otras medidas en el matadero, sala de despiece y envasado**

Reduction achieved with interventions at primary production is expected to vary considerably between MSSs. 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

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## **Estrategias de control**

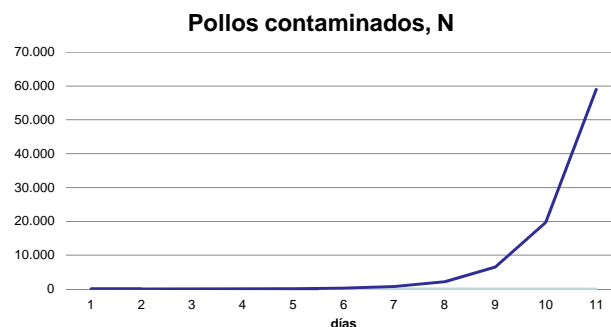


- ☐ Estrategias presacrificio
  - ☐ Estrategias post sacrificio
  - ☐ Etiquetado
  - ☐ Información y educación
- 
- ☐ **Premisa: sin transmisión vertical**

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## Dinámica de infección

- ❑ Baja dosis infectiva (5-50 UFC/pollo)
- ❑ Transmisión eficaz MUY EFECTIVA (100%)
- ❑  $10^8$ CFU/g en heces
  - ❑  $10^8$ CFU/g\*25g\*25.000pollos= $6 \cdot 10^{13}$  UFC/d
  - ❑ Supuesto: cada pollo infecta a 3/d



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## Estrategias presacrificio

- ❑ Reducción de la edad de sacrificio
  - ❑ Una reducción de la edad de sacrificio disminuye significativamente la probabilidad de altos conteos. Posible aplicación en manadas de alta prevalencia, y en meses de verano
- ❑ Programa limpieza y desinfección y vacío sanitario
  - ❑ La transmisión horizontal es muy eficaz, por lo que se debe evitar cualquier fuente de contaminación
- ❑ Cebo de sexos separados
  - ❑ Mayor homogeneidad de canales, mejor ajuste de equipos de sacrificio en matadero.

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## Estrategias presacrificio

- ❑ **Aclarado (Thining)**
  - ❑ Técnica de extracción de parte de la población de una nave, para ajustar la densidad y comercializar pollo asador
  - ❑ Ruptura de bioseguridad
  - ❑ Claro factor de riesgo para infección de *Campylobacter*
  - ❑ Si se hace
    - ❑ Evitar más de una
    - ❑ Lo más cercana al sacrificio
    - ❑ Bioseguridad en el proceso: ropa y botas de operarios específica de cada nave, camiones, desinfección de utensilios entre naves (incluso dentro de misma explotación)
    - ❑ Formación de operarios

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

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## Consortium



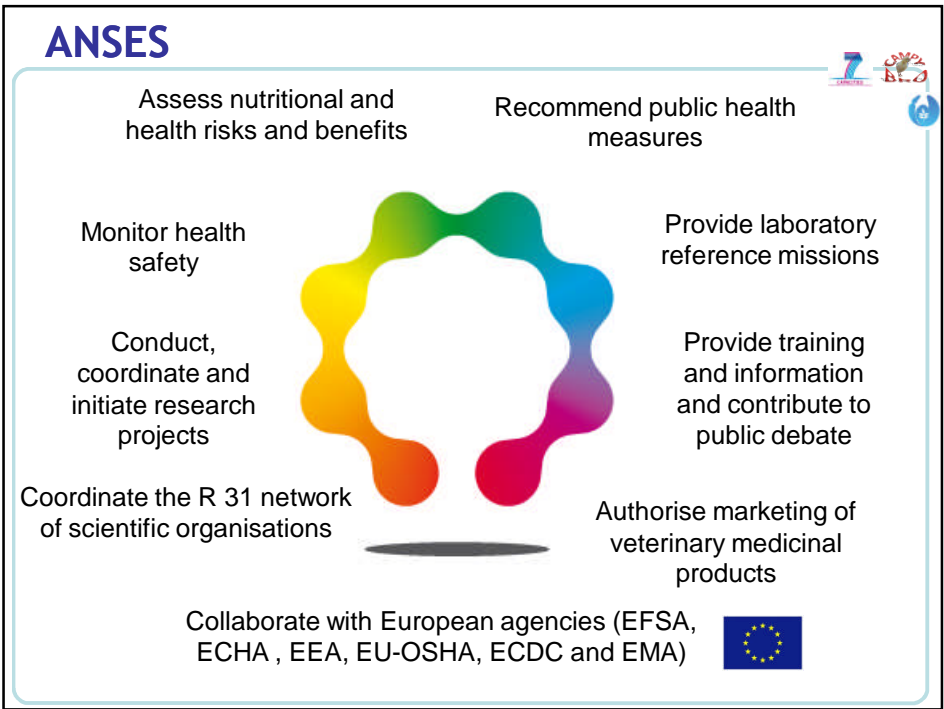
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## Imasde Agroalimentaria, S.L.


- ❑ Research center.
- ❑ Coordinator



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# Paquetes de trabajo

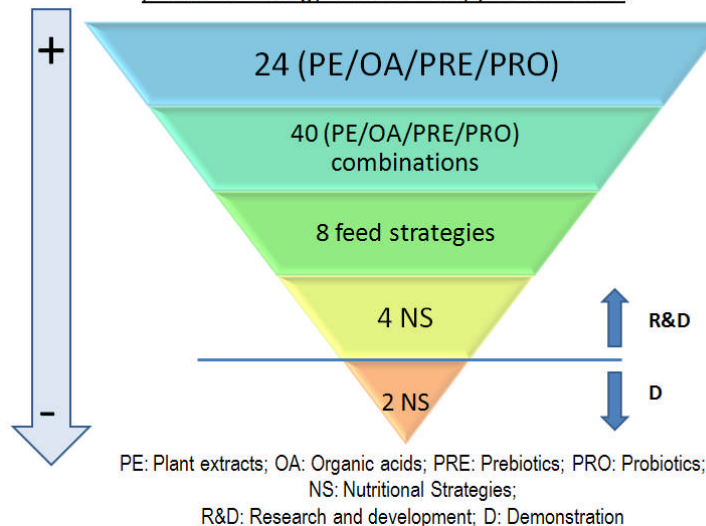


CAMPYBRO	WP	Year 1												Year 2												Year 3												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	
WP 1. Efficacy of several compounds against Campylobacter in broilers orally infected looking for synergies	WP1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
T1.1. In vivo effectiveness of products based on plant extracts, organic acids, prebiotics, and probiotics against Campylobacter.	T1.1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T1.2. In vivo effectiveness of mixtures of products: Synergistic effect	T1.2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T1.3. In vivo effectiveness of product mixtures based on plant extracts, organic acids, prebiotics, and probiotics against Campylobacter.	T1.3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
WP 2. Feed presentation strategies against Campylobacter.	WP2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T2.1. Effect of feed composition, particle size and feed presentation on the prevalence of Campylobacter in broilers orally infected	T2.1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T2.2. Effect of whole grain feeding on the prevalence of Campylobacter in broilers orally infected.	T2.2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
WP 3. Interactions between products and feed presentation against Campylobacter: Synergies.	WP3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T3.1. Interactions between product mixtures and feeding strategies against Campylobacter looking for synergies	T3.1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T3.2. Studies in the effect of the duration of treatment on the final infection: design of functional diets	T3.2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T3.3. Study on the correlation between in vitro and in vivo results. Cost-Benefit analyses.	T3.3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
WP 4. Application of different nutritional strategies against Campylobacter in experimental farm and field trials.	WP4	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T4.1. Effect of different strategies against Campylobacter on performance parameters and level of infection of broilers chickens in experimental farms	T4.1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T4.2. Effect of different strategies against Campylobacter on performance parameters and level of infection of broilers chickens in commercial farms.	T4.2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T4.3. Effect of different strategies against Campylobacter on performance parameters and level of infection of turkeys in commercial farms.	T4.3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
WP 5. Development of a novel vaccine against Campylobacter based on reverse vaccinology	WP5	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T5.1. Exhaustive identification of new potential vaccine antigens against Campylobacter using the reverse vaccinology strategy.	T5.1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T5.2. Development of an in vitro test to visualize the recognition of Campylobacter antigens by antibodies.	T5.2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T5.3. Determination of an efficient sub-unit vaccination protocol	T5.3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T5.4. Selection of the Campylobacter proteins that will be evaluated for their protective capacity	T5.4	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T5.5. Assessment of the protective potentials against Campylobacter induced by the selected vaccine candidates.	T5.5	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
WP 6. Evaluation of the developed nutritional strategies in different geographical situations.	WP6	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T6.1. Evaluation of developed nutritional strategies in South, Central, and East European conditions	T6.1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
WP 7. Project Management	WP7	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T7.1. Contractual, legal, Administrative and financial management and overseeing of ethical and gender issues	T7.1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T7.2. Monitoring and coordination of technical activities of the project, and planning, organizing and reporting of Project Coordinating Committee and General Assembly	T7.2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T7.3. Relationship with the European Commission	T7.3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
WP 8. Dissemination, training and exploitation	WP8	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T8.1. Dissemination of project results	T8.1	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T8.2. Training to achieve project results implementation	T8.2	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
T8.3. Exploitation of project results	T8.3	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	
MILESTONES																																						

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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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Control of *Campylobacter* infection in broiler flocks through  
two-steps strategy: nutrition and vaccination  
-CAMPYBRO-  
FP7-SME-2013-605835

*Effect of feeding medium chain fatty acids  
as monoglycerides or acids on  
*Campylobacter* counts in broilers*



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## Material and methods

- ✓ Experimental design
  - ✓ 3 treatments
    - ✓ Control diet
    - ✓ 0,6% Medium chain fatty acids (AB, 60% caproic and caprylic acids, 35% silica)
    - ✓ 0,8% Monoglycerides of caproic and caprylic acids (MGB, 48% C8-C10 monoglycerides, 35% silica)
  - ✓ All diets in mash form
  - ✓ Timing
    - ✓ Common diet 1-21d
    - ✓ Treatments form 21 to 42d

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## Material and methods

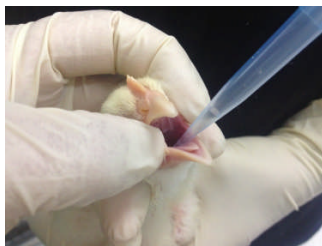
- ✓ Experimental animals
  - ✓ Day-of-hatch chicks (n=90)
  - ✓ Cobb, 50% male 50% female
- ✓ Experimental facilities
  - ✓ ABL2
  - ✓ 10 cages of 3 chickens per treatment
  - ✓ Feed and water *ad libitum*



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## Material and methods

- ✓ *Campylobacter* challenge
  - ✓ 14d
  - ✓ Orally gavaged
  - ✓ 100  $\mu$ l of a solution containing  $1 \times 10^5$  cfu/ml
  - ✓ *C. jejuni*
  - ✓ Two strains (ST-21 and ST-45)



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## Material and methods

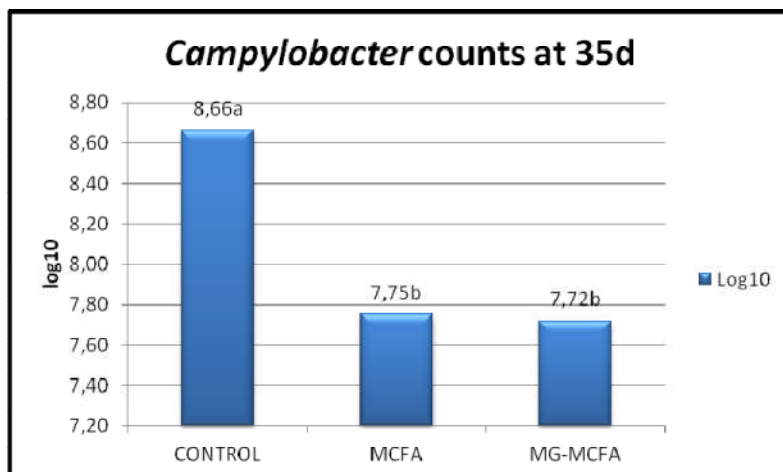
- ✓ *Campylobacter* infection
  - ✓ 21d
  - ✓ 5 chicks/treatment
  - ✓ Chicks were sacrificed by cervical dislocation, and GIT immediately removed
  - ✓ Two ceca were extracted, and identified
  - ✓ Ceca was maintained at 3°C 24h
- ✓ *Campylobacter* controls
  - ✓ 35d, 10 birds/treatment
  - ✓ 42d, 10 birds/treatment



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## Results

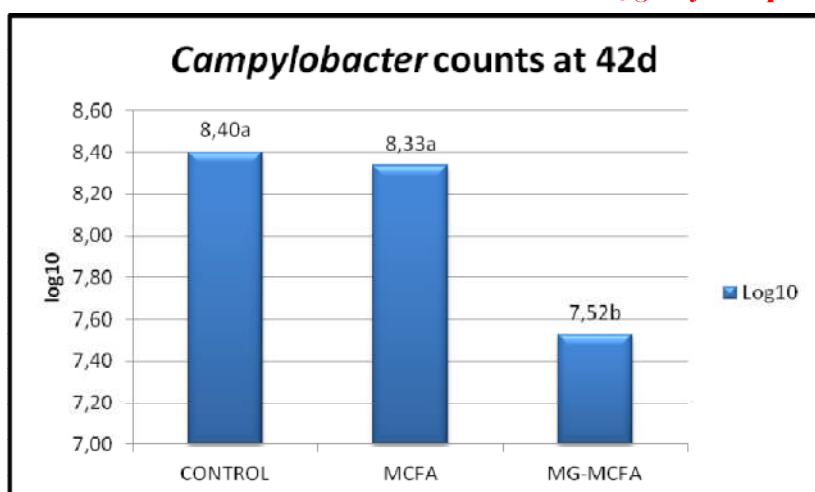
✓ *Campylobacter* counts: 35d



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## Results

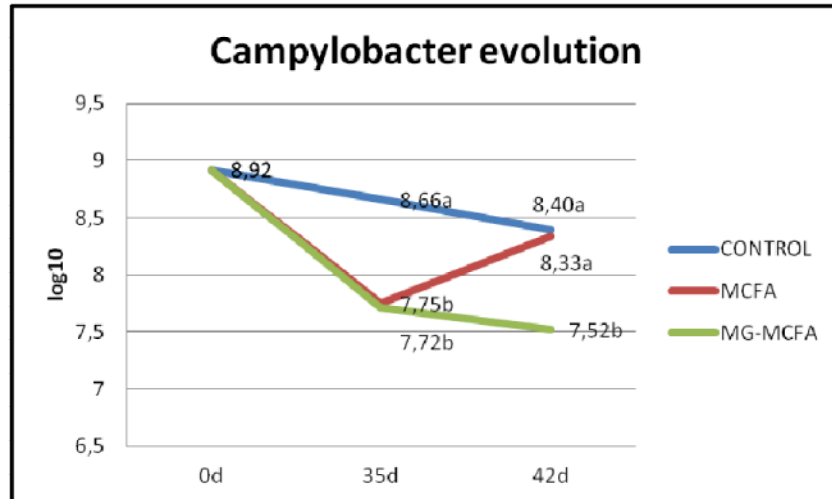
✓ *Campylobacter* counts: 42d **9.000MCFU/pollo**  
**225BillonesCFU/granja 25kpollos**



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## Results

### ✓ *Campylobacter* counts evolution



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## Conclusions

- ✓ Both the supplementation of a blend of caproic and caprylic as acids or monoglycerides in the feed from 21d, significantly decrease *Campylobacter* counts in the ceca of infected birds at 35d by about 1<sub>log</sub>
- ✓ However, only monoglycerides of C8+C10 significantly decreased *Campylobacter* counts in market-aged chickens (42d).
- ✓ The decrease is still low, and the combinations with other additives or feed forms, or extend the application period in order to amplify the reduction level to 3<sub>log</sub>

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**Effect of feeding caproic and caprylic as acids or monoglycerides on *Campylobacter* counts in broilers**  
 C. Millán<sup>1</sup>, O. Casabuena<sup>1</sup>, S. Porras<sup>1</sup>, J. Sánchez<sup>1</sup>, M.I. Gracia<sup>1</sup>  
<sup>1</sup>Imasde Agroalimentaria, S.L., Madrid, Spain

An experiment was conducted within the FP7 project CAMPYBRO in order to evaluate the effect of a blend of medium chain fatty acids (AB, 60% caproic and caprylic acids, 35% silica) and a blend monoglycerides of caproic and caprylic acids (MGB, 48% C8-C10 monoglycerides, 35% silica) on *Campylobacter* counts in broilers. There were three treatments applied from 21 to 42 days of age, T1: Positive controls (*Campylobacter*, no additives), T2: BA at 0.6% and T3: BMG at 0.8%. Day-of-hatch chicks (n=84) were offered a common starter diet until 21 days of age. Chicks were orally gavaged with 100 µl of a solution containing 1 x 10<sup>5</sup> cfu/ml of two *C. jejuni* strains (ST-21 and ST-45) on day 14. On day 21, cecal contents of 15 birds were collected and *Campylobacter* counts determined in order to assure the *Campylobacter* infection. On days 35 and 42, ceca were collected from 10 birds per treatment and *Campylobacter* counts determined. At 35 days of age, BA and BMG supplementation consistently decreased the *Campylobacter* counts compared with T1 (by 10.5 and 10.8% respectively; P < 0.05). At 42 days of age, only the BMG decreased the colonization of *Campylobacter* compared with positive controls (7.52 vs 8.40 log<sub>10</sub>(cfu/g); P < 0.05). The results suggest that the supplementation of a blend of caproic and caprylic acids in the feed, especially as monoglycerides, can effectively decrease *Campylobacter* in market-aged chickens.

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Control of *Campylobacter* infection in broiler flocks through  
 two-steps strategy: nutrition and vaccination  
 -CAMPYBRO-  
 FP7-SME-2013-605835

*Effect of feeding a garlic-based extract or  
 a probiotic in *Campylobacter* counts in  
 broilers*

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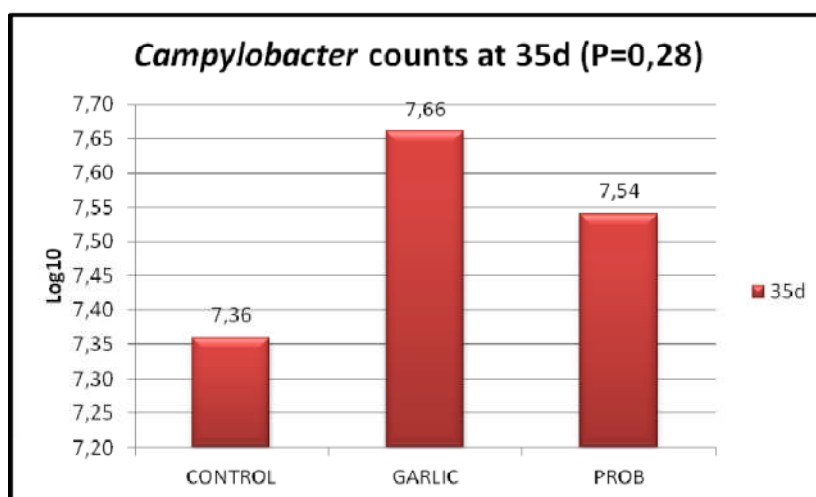
## Material and methods

- ✓ Experimental design
  - ✓ 3 treatments
    - ✓ Control diet
    - ✓ 800ppm of a herbal extract based on garlic
    - ✓ 500ppm of a probiotic based in *Bacillus subtilis* (DSM 17299):  $1,6 \times 10^9$ /g;  $8 \times 10^6$ UFC/g
  - ✓ All diets in mash form
  - ✓ Timing
    - ✓ Common diet 1-21d
    - ✓ Treatments form 21 to 42d
  - ✓ Rest of procedures as the previous trial

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## Results

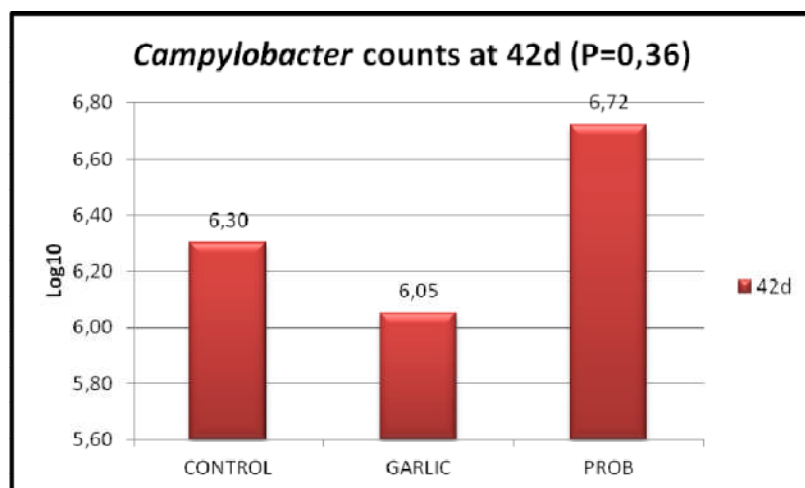
- ✓ *Campylobacter* counts: 35d



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## Results

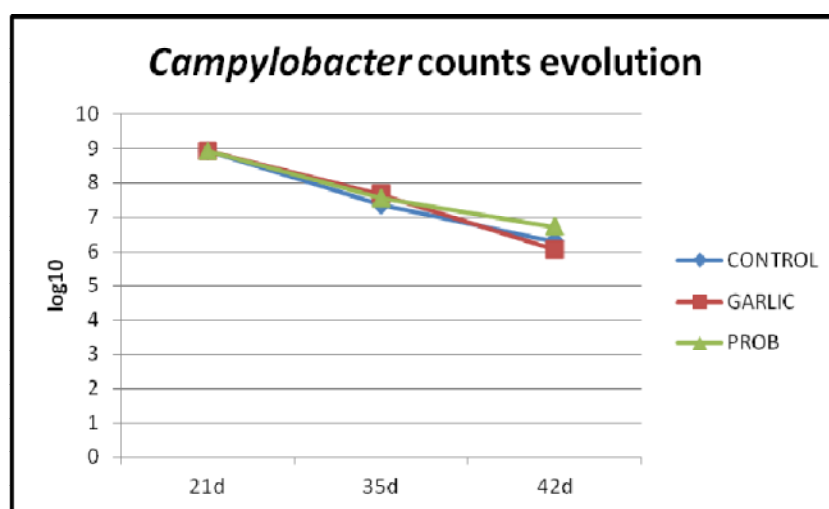
### ✓ *Campylobacter* counts: 42d



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## Results

### ✓ *Campylobacter* counts evolution



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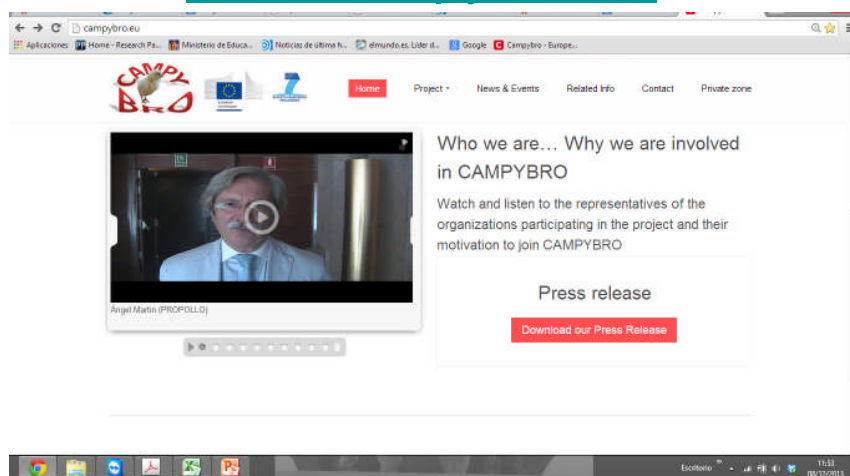
## Conclusions

- ✓ Both the supplementation of a plant extract based on garlic or a probiotic in the feed from 21d, do not affect *Campylobacter* counts in the ceca of infected birds at 35d or 42d
- ✓ Therefore, once the flock is infected is very difficult to decrease the contamination through this additives. However, its use from the beginning could release different results.

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## Consortium

[www.campybro.eu](http://www.campybro.eu)



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