Prevalencia de Campylobacter en pollos. Estudios epidemiológicos y de transmisión

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Resumen

El objetivo de este estudio fue conocer la prevalencia de *Campylobacter spp.* en las granjas de pollos de Cataluña (España) a la edad de matadero, alrededor de los 45 días de edad. El estudio se realizó entre los años 1998 y 2010. Se analizaron un total del 1.306 granjas, tomando muestras de contenido cecal de 5 pollos por manada.

La positividad a *Campylobacter spp.* osciló entre el 62,5% y el 100%, variando según los años. La positividad media de todos los años fue del 84,80%. La prevalencia media de *Campylobacter jejuni* fue del 34,83%.

También durante el año 2010 se realizó un estudio preliminar sobre transmisión vertical y horizontal de *Campylobacter.* No se constató la presencia de transmisión vertical.

En Mayo de 2011 se inició un nuevo estudio más amplio, a lo largo de la cadena de producción, sacrificio y distribución de carne de pollo.

Palabras clave: Campylobacter, granjas de pollos, prevalencia, Cataluña.

Introducción

Las infecciones zoonóticas que con más frecuencia se observan en la Unión Europea están, mayoritariamente, causadas por agentes zoonóticos bacterianos, agentes que pueden ser transmitidos por animales de granja asintomáticos. Encabezando de esta lista encontramos el *Campylobacter* y la *Salmonella*.

Los Campylobacter spp, terrmotolerantes, y, principalmente *Campylobacter jejuni*, son uno de los más importantes agentes zoonóticos en la Unión Europea y en el mundo. Entre otras fuentes, como el agua, animales salvajes y de granja, etc., se ha descrito el consumo y la manipulación de la carne de pollo como una de las vías de infección. Las tres especies que con más frecuencia se aislan en pollos son *C. jejuni,* seguido de *C. coli* y ocasionalmente *C. lari.*

El CESAC es el Centro de Sanidad Avícola de Aragón y Cataluña, situado en el Noreste de España. La misión del CESAC es el control de las enfermedades aviares oficialmente reguladas por la Unión Europea y el control de las enfermedades aviares de interés económico para la industria avícola. También la organización de cursos de formación técnica para veterinarios, técnicos y granjeros. Entre otras tareas, se encarga de los programas de control de los agentes zoonóticos en las regiones de Cataluña y Aragón (España) desde el año 1989.

Durante un periodo de 13 años, siguiendo el mandato de la Directiva de la Unión Europea 92/117/CEE del 17 de Diciembre de 1992, que establece medidas para la protección contra las zoonosis y sus agentes, el CESAC ha estado controlando manadas de pollos, antes de sacrificio, en mataderos de Cataluña, para conocer la prevalencia de *Campylobacter spp.* en su industria de producción avícola.

Material y métodos

Estudios de prevalencia:

Para este estudio de prevalencia se cogieron muestras, por los propios técnicos del laboratorio, del contenido cecal de 5 pollos de un total de 1.306 manadas de pollos diferentes y de diferentes mataderos del pais. El muestreo se hizo a la edad de sacrificio, alrededor de los 45 días de edad. El mismo día de muestreo las muestras se llevaron al laboratorio, para su análisis inmediato, sin retraso, a fin de no perder viabilidad de *Campylobacter*.

Método de detección de *Campylobacter* spp: La técnica empleada fue el aislamiento e identificación de *Campylobacter* spp. El contenido cecal se diluyó 1:10 (volumen húmedo) en una solucion salina tamponada de fosfatos (PBS), (BR0014G, OXOID, Hampshire, UK) y la mezcla se homogeneizó. Posteriormente, la mezcla homogeneizada, se sembró directamente en agar selectivo sin sangre para *Campylobacter* (CBFA) (CM0739, OXOID, Hampshire, UK). Las placas se incubaron de forma microaerófila a 41,5±1ºC durante 44±4h. Despues de la incubación se confirmaron las colonias típicas de *Campylobacter.*

Estudios de transmisión:

82 pollitos de 1 día, procedentes de una sala de incubación comercial, se colocaron en las instalaciones experimetales del CESAC.
Para el estudio preliminar de transmisión vertical, 10 de los 82 pollitos de 1 día se analizaron para la presencia de *Campylobacter*.
Para el estudio preliminar de transmisión horizontal, los otros 72 pollitos se desafiaron con *Campylobacter jejuni* introduciendo en el grupo pollitos infectados inoculados oralmente.

Cepa del inóculo de *Campylobacter*: Para infectar los pollitos se inocularon con una cepa de campo de *Campylobacter jejuni* procedente de una manada de pollos comercial. La cepa se almacenó congelada a -80ºC y se reconstituyó en agar selectivo sin sangre para *Campylobacter* (CBFA) (CM0739, OXOID, Hampshire, UK). Las placas se incubaron de forma microaerófila a 41,5±1ºC durante 44±4h. Usando los estandares de McFarland, se preparó una suspensión bacteriana en PBS (OXOID, BR0014G) y se hicieron diluciones seriadas para conseguir la población de desafío deseada. Se preparó un inóculo conteniendo aproximadamente 10^6 unidades formadoras de colonia (ufc) por mililitro, y se administró oralmente a las aves 0,1ml del inóculo con una micropipeta. La dosis de desafio fue 10^5 ufc por ave inoculada.

Método cualitativo de detección de *Campylobacter* spp: La técnica empleada fue el aislamiento e identificación de *Campylobacter* spp. El contenido cecal se diluyó 1:10 (volumen húmedo) en una solució salina tamponada de fosfatos (PBS), (BR0014G, OXOID, Hampshire, UK). A continuación se homogeneizó la mezcla. Posteriormente la mezcla homogeneizada se sembró directamente en agar selectivo sin sangre para *Campylobacter* (CBFA) (CM0739, OXOID, Hampshire, UK). Las placas se incubaron de

forma microaerófila a 41,5±1ºC durante 44±4h. Despues de la incubación se confirmaron las colonias típicas de *Campylobacter.*

Método cuantitativo de detección de *Campylobacter* spp: Cada ciego se diluyó 1:10 (volumen húmedo) en una solució salina tamponada de fosfatos (PBS), (BR0014G, OXOID, Hampshire, UK) y se homogeneizó la mezcla. A continuación se realizaron diluciones en base a diez de la suspensión original y cada dilución se sembro directamente en agar selectivo sin sangre para *Campylobacter* (CBFA) (CM0739, OXOID, Hampshire, UK). Las placas se incubaron de forma microaerófila a 41,5±1ºC durante 44±4h. Despues de la incubación de contaron y confirmaron las colonias típicas *Campylobacter spp.*

Instrumentación: Todos los instrumentos utilizados se especificaron, calibraron y/o verificaron de acuerdo con el Procedimiento Normalizado de Trabajo del Departamento de Microbiología del CESAC.
Soluciones y reactivos: Todas las soluciones estaban estandarizadas por escrito y controladas antes de su uso de acuerdo con el Procedimiento Normalizado de Trabajo del Departamento de Microbiología del CESAC.
Procedimientos**:** Todas las tarea realizadas estaban definidas en el correspondiente Procedimiento Normalizado de Trabajo del Departamento de Microbiología del CESAC.

Resultados

Estudios de prevalencia:

La mayoría de las manadas analizadas fueron positivas a *Campylobacter spp*, oscilando del 62,5% al 100%, dependiendo de los años. La positividad media de todos los años fue del 84,80%.

La prevalencia media de *Campylobacter jejuni* fue del 34,83%. Solo en el año 1998 la prevalencia de *Campylobacter jejuni* fue superior al 50% (57,14%). No parece que haya ninguna tendencia en tipo de aislado, aunque parece que *Campylobacter jejuni* a ido decreciendo a lo largo de los años.

Ninguna de la mejoras en bioseguridad o cualquier otra medida implementada para el control de *Salmonella*, según se regula en la Directiva 2003/99/CE del Parlamento Europeo y del Consejo, del 17 de Noviembre de 2003, sobre el control de zoonosis y agentes zoonóticos, ha tenido eficacia alguna para el control o reducción de la prevalencia de *Campylobacter* en nuestro pais.

Ver Gráfico 1 y Tabla 1.

Estudios de transmisión:

Transmisión vertical: El 100% de los pollitos de día fueron negativos a *Campylobacter spp*. En este estudio se vio que los pollitos de 1 día recién nacidos no contenían *Campylobacter* de forma natural en el ciego.
Transmisión horizontal: 7 días despues de ser alojados junto con las aves inoculadas, el 100% de las 72 aves se volvieron positivas a *Campylobacter jejuni*. De esta forma se demostró la rápidez y facilidad con que se colonizan las manadas de pollos vía transmisión horizontal.

Discusión

Estudios de prevalencia:

Si se quiere reducir la pevalencia de de *Campylobacter* de las manadas de pollos a fin de reducir su incidencia y prevalencia en humanos se necesita mucha más investigación, ya que los resultados de este estudio epidemiológico indican que la bioseguridad no sirve para reducir *Campylobacter* en las manadas de pollos. Cabe añadir que las vías de infección de *Campylobacter* en las manadas de pollos se desconocen todavía.

Se deberían realizar también posteriores estudios para determinar si la tendencia a la disminución de *Campylobacter jejun*i observada en este estudio es correcta, y si tiene alguna relación con el incremento de las medidas de bioseguridad implementadas a partir de los programas de control de *Salmonella*.

Estudios de transmisión:

Considerando que el aislamiento e identificación de *Campylobacter* no es una cosa fácil, y que se podría pasar por alto la presencia de pequeñas cantidades de *Campylobacter* en el ciego de los pollitos de 1 día, se deberían realizar más estudios sobre la posibilidad de transmisión vertical y sobre la posibilidad de contaminación de los pollitos de 1 día en la sala de incubación.

Aunque la transmisión horizontal parece ser la vía más probable, la transmisión vertical o la contaminación al nacimiento no deberían descartarse.

Conclusiones

Nos falta mucho por aprender acerca de *Campylobacter*. Hay muy poca bibliografía sobre las características básicas de la infección en pollos, y su comportamineto a lo largo del proceso de producción de la carne de pollo, de la granja a la mesa. Solo poodemos llegar a la conclusión que *Campylobacter* es extremadamente ubicuo y está presente en la mayoría de las manadas de pollos a la edad de sacrificio. Y esto lleva a la contaminación de la carne fresca de pollo en el matadero.

El CESAC, en colaboración con la Agencia Catalana de Seguridad Alimentaria, ha iniciado recientemente, en Mayo 2011, un nuevo estudio en Cataluña. En este nuevo estudio, participan 6 empresas, con toma de muestras desde las granjas de reproductoras, salas de incubación, granjas de pollos, transporte, matadero y distribución.

Los objetivos de este estudio son:
1.- Estudiar la epidemiología de *Campylobacter* a lo largo de todas las fases de la cadena de producción y distribución de la carne de pollo.
2.- Estudiar la prevalencia de *Campylobacter* en las diferentes fases de la cadena de producción y distribución de la carne de pollo.
3.- Estudiar la contaminación cruzada de *Campylobacter* en los mataderos.
4.- Estudiar vía biología molecular (Gel Electroforesis de Campo Pulsado - PFGE) la variabilidad de los aislados de *Campylobacter*.

Gráfico1.- Resultados de prevalencia de *Campylobacter* 1998 – 2010

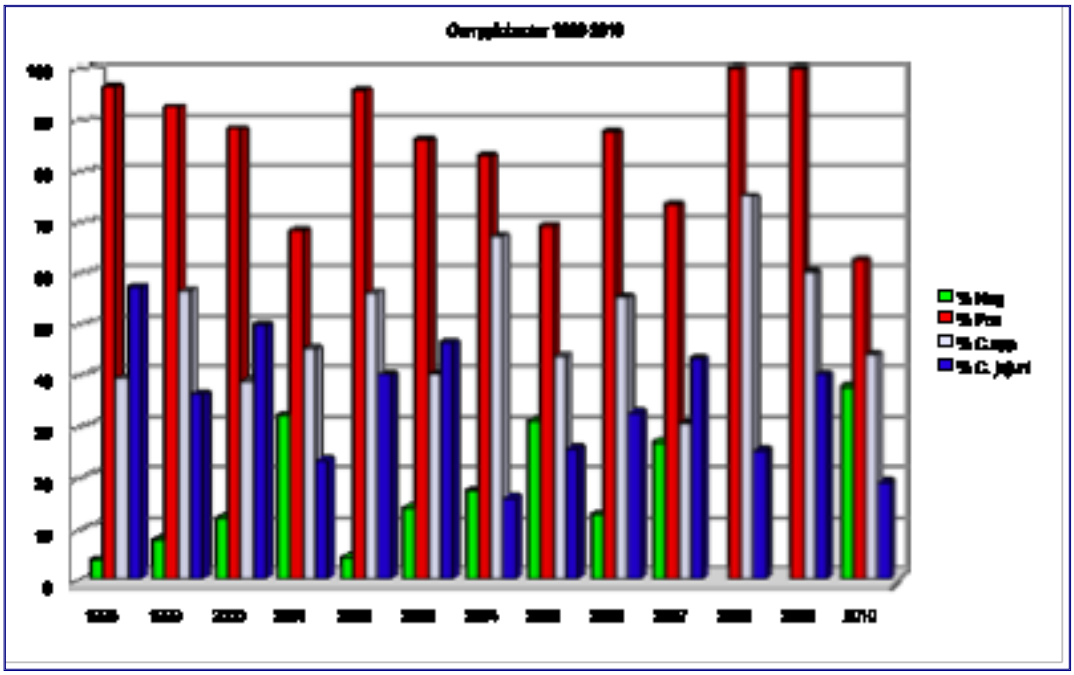

Tabla1.- Resultados de prevalencia de *Campylobacter* 1998 – 2010

Año	Manadas	% Neg	% Pos	% C.spp	% C.jejuni
1998	28	3,57	96,43	39,29	57,14
1999	119	7,56	92,44	56,3	36,13
2000	101	11,9	88,12	38,6	49,5
2001	91	31,9	68,13	45,1	23,21
2002	143	4,2	95,8	55,9	39,9
2003	173	13,9	86,13	39,9	46,2
2004	164	17,1	82,93	67,1	15,9
2005	282	30,9	69,15	43,6	25,5
2006	80	12,5	87,5	55	32,5
2007	86	26,7	73,26	30,2	43
2008	8	0	100	75	25

Campylobacter prevalence in broilers. Epidemiological and transmission studies

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Summary

The goal of this study was to know the prevalence of *Campylobacter spp* in broiler farms in Catalonia (Spain) at slaughter age, about 45 days of age. The study was carried out between 1998 and 2010 and a total of 1.306 flocks were analyzed sampling the ceca content of 5 chickens per flock.

Positiveness to *Campylobacter spp.* ranged from 62,5% to 100% depending on the years. Average positiveness of all the years was 84,80%. Average prevalence to *Campylobacter jejuni* was 34,83%.

Also in 2010 a preliminary study on vertical and horizontal transmission was carried out. No apparent vertical transmission was found.

A new more comprehensive study all along the broiler production, processing and distribution chain has been started in May 2011.

Key words: Campylobacter, broiler farms, prevalence, Catalonia.

Introduction

The zoonotic infections more commonly observed in humans in the European Union are, mostly, caused by bacterial zoonotic agents that can be transmitted by asymptomatic farm animals. Heading this list we find *Campylobacter* and *Salmonella*.
Thermotolerant *Campylobacter spp,* and mainly *Campylobacter jejuni*, are one of the most important zoonotic agents in the European Union and in the world. Amongst other sources, like water, wild and farm animals, pets, etc, consumption and manipulation of raw chicken has been described as one of its causes. The three species most commonly isolated in poultry are C. jejuni followed by C. coli and rarely C. lari.

The CESAC is the Official Poultry Health Institute of Catalonia, in the north east of Spain. The missions of the CESAC are the control of the poultry diseases officially regulated by the European Union and the control of the diseases of economical interest for the poultry industry. Also the organization of technical meetings for veterinarians and farmers. Amongst other tasks, carries out routine testing for zoonotic agents in the regions of Catalonia and Aragón (Spain) since 1989.

Over a period of 13 years, following the mandate of the EU regulation 92/117/CEE from December 17th 1992, that establishes measures for the protection against zoonosis and its agents, the CESAC has been checking broiler flocks at slaughterhouses in Catalonia to know the prevalence of *Campylobacter spp.* in its poultry production industry.

Material and methods

Prevalence studies:

For this prevalence study samples were collected, by the own technicians of the laboratory, from the ceca content of 5 chickens from a total of 1.306 different flocks in different slaughterhouses in the country. Sampling was at slaughter age, about 45 days of age. The same day of sampling, samples were brought to the laboratory, to be analyzed immediately, without delay, in order to not lose *Campylobacter* livability.

Detection method of *Campylobacter* spp: The technique used was the isolation and identification of *Campylobacter* spp. The ceca content was diluted 1:10 (wt:vol) in Phosphate Buffered Saline Solution (PBS), (BR0014G, OXOID, Hampshire, UK) and the mixture was homogenized. Then the homogenized was direct-plated on *Campylobacter* Blood-Free Agar (CBFA) (CM0739, OXOID, Hampshire, UK). The plates were incubated microaerophilically at 41,5±1ºC for 44±4h, after incubation, typical *Campylobacter* spp colonies were confirmed.

Transmission studies:

82 day old broiler chicks, coming from a commercial hatchery, were placed at CESAC experimental facilities.
For the preliminary vertical infection study, 10 out of 82 day old broiler chicks were tested for *Campylobacter*.
For the preliminary horizontal infection study, 72 day old chicks were challenged with *Campylobacter jejuni* by introducing seeder birds orally inoculated.

Campylobacter innoculum strain: A field strain of *Campylobacter jejuni* from a broiler chicken flock was used to infect the seeder birds. The strain was stored frozen at -80ºC and it was reconstituted in *Campylobacter* Blood-Free Agar (CBFA) (CM0739, OXOID, Hampshire, UK), plates were incubated microaerophilically at 41,5±1ºC for 44±4h. Using the McFarland standards, a bacterial suspension in PBS (OXOID, BR0014G) was prepared and serial dilutions were made to achieve the target challenge population. An inoculum containing approximately 10^6 colony forming units (cfu) per milliliter was prepared and seeder birds were given 0,1ml of the inoculum orally with a micro-pipette. The challenge dose was 10^5 cfu per seeder bird.

Detection method of *Campylobacter* spp: The technique used was the isolation and identification of *Campylobacter* spp. The ceca content was diluted 1:10 (wt:vol) in Phosphate Buffered Saline Solution (PBS), (BR0014G, OXOID, Hampshire, UK) and the mixture was homogenized. Then the homogenized was direct-plated on *Campylobacter* Blood-Free Agar (CBFA) (CM0739, OXOID, Hampshire, UK). The plates were incubated microaerophilically at 41,5±1ºC for 44±4h, after incubation, typical *Campylobacter* spp colonies were confirmed.

Quantitative method of *Campylobacter* spp: Each ceca was diluted 1:10 (wt:vol) in Phosphate Buffered Saline Solution (PBS), (BR0014G, OXOID, Hampshire, UK) and the mixture was homogenized. Then ten-fold dilutions were made from the stock suspension, and each dilution was direct-plated on *Campylobacter* Blood-Free Agar (CBFA) (CM0739, OXOID, Hampshire, UK). The plates were incubated microaerophilically at 41,5±1ºC for 44±4h, after incubation, typical Campylobacter spp colonies were counted and confirmed.

Instruments: All the used instruments, were specified, calibrated and/or verified according to the Normalized Work Protocol by CESAC's Bacteriology Department.

Solutions and reagents: All used solutions were written up and perfectly checked before theirs use according to the Normalized Work Protocol by CESAC's Bacteriology Department.
Procedures**:** All the carried tasks were perfectly defined at their correspondent Normalized Work Protocol by CESAC's Bacteriology Department.

Results

Prevalence studies:

Most of the flocks tested were positive to *Campylobacter spp*, ranging from 62,5% to 100%, depending on the years. Average positivity of all the years was 84,80%.

Average prevalence of *Campylobacter jejuni was* 34,83%. Only one year, 1998, *Campylobacter jejuni* was above 50% (57,14%). There seems not to be a tendency on the type of isolate, though it seems that *Campylobacter jejuni* is decreasing along the years.

None of the improvements in biosecurity or other measures implemented for the control of *Salmonella*, as regulated by the Directive 2003/99/CE of the European Parliament and of the Council, of 17 November 2003, on the monitoring of zoonoses and zoonotic agents, has been of any efficacy for the control or reduction of the prevalence of *Campylobacter* in our country.

See Chart 1 and Table 1.

Transmission studies:

Vertical transmission: The 100% day old chicks tested were negative for *Campylobacter spp*. It was demonstrated in this study that day-of-hatch birds did not naturally contain cecal *Campylobacter*.
Horizontal transmission: 7 days after being housed in a pen with inoculated seeder birds, 100% of the 72 birds became *Campylobacter jejuni* positive. At this point, it was demonstrated how rapidly broiler flocks can become colonized because the horizontal transmission.

Discussion

Prevalence studies:

If *Campylobacter* is to be reduced from poultry flocks in order to reduce the prevalence and its incidence to humans more research is needed, as data from this epidemiological study indicates that biosecurity is not useful to reduce *Campylobacter* in poultry flocks. Moreover the paths of infection of *Campylobacter* to poultry flocks are still unknown.

Further studies should also be carried out to determine if the decreasing *Campylobacter jejun*i tendency observed in this study is right, and if it has any relation with the increased biosecurity measures implemented with the *Salmonella* control programs.

Transmission studies:

Considering that growing and culturing *Campylobacter* is not an easy thing, and the presence of *Campylobacter* in the ceca of day all chicks can be dismissed, more studies on vertical, transmission, or at hatch contamination, should be carried out.

Though horizontal transmission seems to be the most suitable way, vertical transmission, or at hatch contamination, shouldn't be disregarded and more research should be conducted.

Conclusions

We are lacking a lot to know about *Campylobacter*. There is very few literature about the basic characteristics of the infection in broilers, and its behavior through all the process of production of poultry meat, from the farm to the table. We can only conclude that *Campylobacter* is extremely ubiquitous and it is present in most broiler flocks at slaughter age. And this leads to the contamination of raw poultry meat at slaughterhouses.

The CESAC has recently started a new study new study in Catalonia in May 2011. The study involves 6 companies, taking samples from parent stock farms, hatcheries, broiler farms, transport, slaughterhouses and retail.

The goals of the study are:
1.- Look for the epidemiology of *Campylobacter* through all the stages of the production and distribution of poultry meat.
2.- Look for the prevalence of the different *Campylobacter* at the different stages of the production and distribution of poultry meat.
3.- Look for the cross contamination of *Campylobacter* at slaughterhouses.
4.- Check with molecular biology (pulsed field) differences between *Campylobacter* isolates

Chart 1.- Results of the Campylobacter prevalence study 1998 – 2010

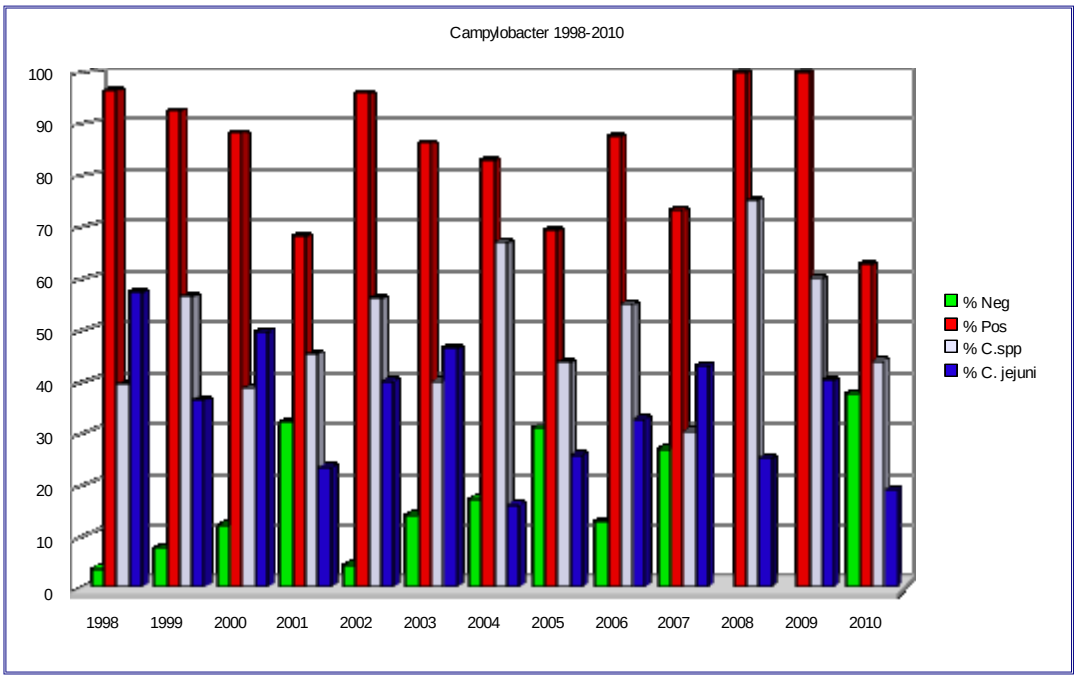

Table1.- Results of the Campylobacter prevalence study 1998 – 2010

Year	Flocks	% Neg	% Pos	% C.spp	% C.jejuni
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2006	80	12,5	87,5	55	32,5
2007	86	26,7	73,26	30,2	43
2008	8	0	100	75	25
2009	15	0	100	60	40

2010	16	37,5	62,5	43,8	18,8

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2009	15	0	100	60	40
2010	16	37,5	62,5	43,8	18,8

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