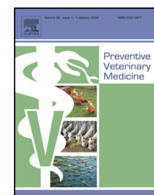
Preventive Veterinary Medicine 114 (2014) 106–113

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed



Prevalence and risk factors of *Campylobacter* infection in broiler flocks from southern Spain

Alicia Torralbo [a], Carmen Borge [a], Alberto Allepuz [b,c], Ignacio García-Bocanegra [a], Samuel K. Sheppard [d], Anselmo Perea [a], Alfonso Carbonero [a,*]

[a] *Department of Animal Health, Campus de Excelencia Internacional Agroalimentario ceiA3, University of Cordoba, Cordoba, Spain*
[b] *Centre de Recerca en Sanitat Animal (CReSA), UAB-IRTA, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
[c] *Departament de Sanitat i Anatomia Animals, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain*
[d] *College of Medicine, Swansea University, Swansea SA2 8PP, United Kingdom*

ARTICLE INFO

Article history:
Received 19 March 2013
Received in revised form 7 January 2014
Accepted 19 January 2014

Keywords:
Broilers
Campylobacter
Prevalence
Risk factors
Spain

ABSTRACT

An extensive epidemiological study was performed to determine the prevalence and risk factors of *Campylobacter* infection in broiler farms in Andalusia (southern Spain). A total of 2221 cloacal swabs and 747 environmental swabs from 291 broiler flocks were screened between April 2010 and May 2012. The prevalence of *Campylobacter* in individual animals was 38.1%, and the flock prevalence was 62.9%. Flocks were predominantly infected by *C. jejuni* and *C. coli* but were also infected by untyped *Campylobacter* spp., and mixed-species infection could be found. Risk factors for *Campylobacter* infection were assessed from direct interview of the farmers. The number of positive samples by flock was modelled assuming a binomial distribution. Analysis indicated five factors associated with increased intra-flock prevalence: presence of dogs or cats on the farm, older age of the broiler flock, the application of thinning of flocks, the presence of windows with canvas blinds, and the presence of rodents in the poultry house. Two factors were associated with decreased intra-flock prevalence: the treatment of drinking water and having an entrance room for access into the poultry house. This is the first study performed on broilers farms from Spain reporting the risk factors of *Campylobacter* infection and is the largest study on the prevalence of *Campylobacter* infection.

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1. Introduction

Campylobacteriosis has been the most frequently reported zoonotic disease in humans in the EU since 2005 (EFSA Journal, 2012), and the economic and public health burden of *Campylobacter* is significant (Tam et al., 2003).

Campylobacter is frequently observed in the digestive tract of poultry (Ansari-Lari et al., 2011) as well as in other livestock species, such as cattle and pigs (Keller et al., 2007). As a result, undercooked poultry meat is the most important source of campylobacteriosis for humans (Sheppard et al., 2009). In addition, cross contamination from raw chicken meat through knives, cutting board or hands has been reported as a major risk factor (Luber et al., 2006).

The two most common species in the *Campylobacter* genus, *C. jejuni* and *C. coli*, are able to infect poultry. *C. jejuni*

* Corresponding author. Tel.: +34 957 21 87 25; fax: +34 957 21 87 25.
E-mail addresses: aliciaveterinaria231985@hotmail.com (A. Torralbo), sa2borom@uco.es (C. Borge), Alberto.Allepuz@cresa.uab.cat (A. Allepuz), nacho.garcia@uco.es (I. García-Bocanegra), s.k.sheppard@swansea.ac.uk (S.K. Sheppard), sa1perej@uco.es (A. Perea), espargen@hotmail.com, sa1camaa@uco.es (A. Carbonero).

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doi:10.1016/j.prevetmed.2014.01.019

generally more frequently infects domestic chickens than does *C. coli* (Keller et al., 2007; Sasaki et al., 2011), although Ansari-Lari et al. (2011) report the opposite.

The epidemiology of *Campylobacter* from broiler farms is not fully understood (Ridley et al., 2011), although it is well known that horizontal transmission is largely responsible for the colonisation of broilers (Newell et al., 2011). At present, it has not been possible to demonstrate vertical transmission from hens to their chicks (Callicott et al., 2006). Broilers are rarely colonised before fourteen days of age (Bull et al., 2006).

Previous epidemiological studies have identified risk factors associated with the prevalence of *Campylobacter* in chicken farms, such as the higher age of broilers at slaughter (Ansari-Lari et al., 2011), drinking water distribution (Näther et al., 2009), the presence of other animals in the vicinity of the farm (Hansson et al., 2010) and heavy rainfall some weeks before the slaughter (Jonsson et al., 2012). In contrast, good hygiene practices by farmers (Hansson et al., 2010) or seldom or never thinning (Hansson et al., 2010) have been shown to be protective factors against *Campylobacter* infection.

The prevalence of *Campylobacter* among broiler flocks seems to differ according to studies and locations, ranging from 34.2% in Great Britain (Ellis-Iversen et al., 2009) to 63.9% in Italy (Di Giannatale et al., 2010). The prevalence of broiler flocks colonised with *Campylobacter* was 88.0% in Spain (EFSA Journal, 2010). This prevalence is higher than the average EU *Campylobacter* flock prevalence (71.2%) (EFSA Journal, 2010). However, information on epidemiology of *Campylobacter* spp. in broiler flocks in Spain remains very limited.

The aims of this study were to determine the prevalence, sources of infection, level of contamination of the house environment and risk factors associated with *Campylobacter* infection on broiler farms in Andalusia (southern Spain).

2. Materials and methods

2.1. Study design and sampling

The present study was performed in Andalusia (southern Spain; 36° N–38°60′ N, 1°75′ W–7°25′ W) between 2010 and 2012. In the last census in Andalusia, there were 14,105 broiler flocks (Ministry of Agriculture and Fisheries, Government of Andalusia), representing one fifth of all broilers reared in Spain (Livestock Farms Registry of Spain, 2008). The lines used were primarily Ross and Cobb.

The sampling unit was the flock. The flock is defined as a group of broilers entering a house at one day old who are bred in the house until they reach the weight of sacrifice, approximately 45–50 days, when they are taken as a group for slaughter. A stratified random sampling by provinces (number of sampled flocks was proportional to the total population of each province) was performed. To calculate sample size (n), we considered a population size of 14,105 (number of flocks), an expected flock prevalence of 75% (Powell et al., 2012) ($p = 0.75$; $q = 1 - p = 0.25$), a 95% confidence level ($Z_\alpha = 1.96$) and an acceptable error of 5% ($L = 0.05$). Using the formula (Thrusfield, 2005): $n = (Z_\alpha^2 \times p \times q)/L^2$ this resulted in a minimum sample size of 289 flocks.

A total of 291 flocks from 134 different broiler farms located in all eight provinces of Andalusia were finally recruited (Fig. 1 and Table 1). To determine the number of samples needed in each flock to detect *Campylobacter* infection, we considered a flock size (N) of 5000 (flock with minimal number of animals in previous data), a minimum expected prevalence of 37% (lower than the within-flock prevalence described by Gregory et al. (1997), Heuer et al. (2001) and Shreeve et al. (2002)) and a confidence level (a) of 95%. Using the formula (Thrusfield, 2005): $n = [1 - (1 - a)^{1/D}] \times [N - (D - 1)/2]$ this resulted in a minimum sample size of 7 animals per flock (D is the number of infected animals: 37% of 5000 = 1850).

We collected 7–8 cloacal swabs from the 291 flocks included in the study. These samples were taken at different points within the house to get a better representation of the infection distribution. In addition, 2–7 environmental swabs were collected in almost all of the flocks (281/291) at the same time the cloacal samples were taken. The types of environmental samples were decided according to the possibility of contamination. Thus, samples from feeders, drinkers, the litter and installations were collected from many of the flocks, but other types of samples were not so frequently collected because the existence of tools or other samples (e.g., dead birds or combustible material) inside the houses was infrequent.

A total of 2221 cloacal swabs from broilers and 747 environmental swabs were obtained (Table 2). Cloacal samples and environmental samples were collected using sterile swabs placed in tubes containing a transport medium (Amies, Eurotubo®, Rubí, Spain). The samples were kept refrigerated until arrival at the laboratory and then processed within 24 h.

In addition, we designed a questionnaire based upon previous studies of risk factors for *Campylobacter* infection in broiler farms. The questionnaire included variables related to the location, handling, facilities, biosecurity, measures and control health-programmes (Tables S1 and S2). The questionnaire was completed by a direct interview with each farmer.

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2014.01.019.

2.2. Isolation and identification of Campylobacter

Cloacal and environmental swabs were cultured in *Campylobacter* Blood-Free Selective Agar Base (Oxoid® CM0739, Basingstoke, UK) with CCDA Selective Supplement (Oxoid® SR0155, Basingstoke, UK). After 24–48 h of incubation at 42 °C in a CO_2-enriched atmosphere achieved by AnaeroGen sachets (Oxoid®, Basingstoke, UK), colonies that exhibited morphology compatible with *Campylobacter* were cultured in blood agar and incubated under the same conditions as described above. These selected strains were confirmed by examination of morphology and Gram staining, motility under dark field microscopy and oxidase and catalase testing. Strains phenotypically classified as *Campylobacter* were stored at −80 °C in a semi-solid medium

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