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2) PREVALENCE OF CAMPYLOBACTER JEJUNI IN POULTRY BREEDER FLOCKS

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Abstract The aim of this work is to present the preliminary results of a study about breeders carrying out in order to demonstrate the supposed correlation between *Campylobacter* isolated from breeders and the ones isolated from broilers, supporting the theory of the vertical transmission of the germ. It was examined three different breeder flocks of Bojano in Molise region.
A total of 360 cloacal swabs and 80 enviromental swabs was collected. Of the 3 flocks studied, 6,9% tested were positive for *Campylobacter spp*. The most-prevalent isolated species is *C. jejuni* (8,2%). Only 3 of the 360 cloacal swabs samples examined were associated with *C. coli*. The environmental swabs resulted negative. This results confirms again that poultry is a reservoir of this germ.

Indagine sulla Prevalenza di *Campylobacter jejuni* in Gruppi di Riproduttori Avicoli

Riassunto Il presente lavoro si propone di illustrare i risultati preliminari, sui riproduttori, di uno studio più ampio volto a stabilire, mediante studi di biologia molecolare, una eventuale correlazione tra i *Campylobacter* isolati dai riproduttori e quelli isolati dai broiler in modo da definire l'eventuale trasmissione verticale del germe. Sono stati esaminati 3 gruppi di riproduttori ubicati a Bajano in Molise. Dei tre gruppi valutati, il 6,9% risultava positivo a *Campylobacter spp*. *C. jejuni* era la principale specie isolata (8,2%). Solo 3 dei 360 tamponi cloacali esaminati era associata a *C. coli*. I tamponi ambientali risultavano negativi. Tali risultati confermano ancora una volta il ruolo del pollame come reservoir di questo microrganismo.

Introduction

Campylobacter jejuni is the leading cause of bacterial foodborne illnesses in human medicine (Newell and Fearnley, 2003). The vast majority of human campylobacteriosis cases primarily result from consumption of undercooked poultry or other foods cross-contaminated with raw poultry meat during food preparation. However, other risk factors besides poultry such as contact with house pets, or consumption of raw milk, untreated water, and undercooked beef or pork have also been linked to human infections (Corry and Atabay, 2001). As poultry is considered a major reservoir for human campylobacteriosis, reduction or elimination of poultry contamination with *C. jejuni* would greatly reduce the risk of *Campylobacter* for public health. Although numerous farm-based studies have been conducted in the past decades, the sources of flock infection, modes of transmission, and the host and environmental factors affecting the spread of *Campylobacter* on poultry farms are still poorly understood (Sahin et al. 2002). Potential sources of flock infection include used litter, untreated drinking water, other farm animals, domestic pets, wildlife species, house flies, insects, farm equipment and workers, and transport vehicles (Newell and Fearnley, 2003). However, none of these suspected sources has been conclusively identified as the formal source of infection for broilers farms. Despite these observations, vertical transmission of *C. jejuni* is still questionable because live *Campylobacter* have not detected in the eggs of commercial breeders, young hatchlings or hatcheries under natural conditions. Therefore the exact role of vertical transmission in introducing *Campylobacter* to broiler flocks remains unclear (Sahin et al., 2003).

An estimated 2.1-2.5 million cases of human campylobacteriosis, characterized by watery and/or bloody diarrhoea, occur annually in the United States, exceeding the cases of salmonellosis (Friedman et al. 2000). The reported incidence of *Campylobacter* infection in Europe is estimated to be 1000-2300 cases per 100.000 (Padungton and Kaneene, 2003).

The aim of this work is to present the preliminary results of a study about breeders carrying out in order to demonstrate the supposed correlation between *Campylobacter* isolated from breeders and the ones isolated from broilers, supporting the theory of the vertical transmission of the germ.

Materials and Methods

This study was conducted during the period October 2003/July 2004 in the Arena breeders-farm of Bojano in Molise region.

Samples collection

It was examined three different breeder flocks respectively named A, B and C.

Each flock was visited five times. The first visit occurred during cleaning and disinfection procedures before placing the chicks. The second visit took place at one day of age, the third visit at 4 weeks of age, the fourth at 20 weeks of age and the last visit took place at 30 weeks. During every visit 30 cloacal swabs samples and 10 environmental samples (wall, water, litter, feed) was collected.

Isolation and identification procedure

The samples were added to Campylobacter Selective Enrichment broth (Oxoid) and incubated at 42° .C for 24 h under microaerophilic conditions. Then, each sample was streaked onto Campylobacter blood free selective agar base - Modified CCDA Preston (Oxoid) plates. Plates were incubated at 42° .C under microaerophilic conditions for 48 h. Therefore, the isolates was streaked onto blood-agar plates and incubated at 42°C for 24 h. Isolates were identified using a commercial identification method (API Campy, bioMérieux).

Multiplex PCR

A multiplex PCR assay was carried out to all isolates in accordance with the Cloak and Fratamico procedure (Cloak and Fratamico, 2002). The primers employed in this assay are shown in table 1.

Table 1. PCR primers for *C.jejuni* and *C.coli* employed in the multiplex PCR

Species targeted	Product size (bp)	Primer name (target gene)	Sequence (5' – 3')
C. coli/C. jejuni	400	cadF2B (*cad*F) cadR1B	TTGAAGGTAATTTAGATATG CTAATACCTAAAGTTGAAAC
C. coli	894	COL 1 (*ceu*E) COL 2	ATGAAAAAATATTTAGTTTTTGCA ATTTTATTATTTGTAGCAGCG
C. jejuni	160	C-1 (?) C-2	CAAATAAAGTTAGAGGTAGAATGT GGATAAGCACTAGCTAGCTGAT

Results and Discussion

Of the 3 flocks studied, 6,9% tested were positive for *Campylobacter spp*. (table 2). The most-prevalent isolated species is *C. jejuni* (8,2%). Only 3 of the 360 cloacal swabs samples examined were associated with *C. coli*. The environmental swabs resulted negative.

Table 2. Percentage of positivity from cloacal swabs

Number of Cloacal swabs	30	30	30	30
Age of breeder flocks	*1 day of age*	*4 weeks of age*	*20 weeks of ages*	*30 weeks of age*
Flock A	0%	0%	50%	40%
Flock B	0%	0%	3,3%	3,3%
Flock C	0%	0%	10%	3,3%

The results of this study show a low prevalence of *Campylobacter* in the breeder flocks examined. This result confirms again that poultry is a reservoir of this germ.

Conclusions

The literature suggests that standard biosecurity procedures are inadequate for the maintenance of flock negativity (Newell and Fearnley, 2003). This is a consequence of high exposure, low dose, and rapid bird-to-bird transmission rates. Nevertheless, stringent biosecurity may either delay positivity or reduce the number of flocks that become positive. However, it is generally considered that adeguate biosecurity procedures are difficult to substain in the farm environment (Pattison, 2001). For example, routine procedures such as the effective use of hygiene barriers, hand washing, and boot disinfection may be readily undertaken under normal conditions, but during emergencies, such as fan failure, such procedures may be ignored. Well-designed and well-located farms, the development of appropriate standard operating procedures to minimize risk factors, staff education, and incentives to maintain biosecurity at the highest level would all contribute to the reduction of flock positivity.

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