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***Campylobacter* sp in organs and meconium of day-old broiler chicks derived from naturally infected breeder hens**

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Campylobacter sp, chickens, vertical transmission, epidemiology.

ABSTRACT

Campylobacter sp is an important agent that causes foodborne infection, particularly in food of poultry origin. Therefore, the efficient control of the transmission routes in chicken farms is of outmost importance to prevent it from spreading. In chicken farms, the main transmission route of this microorganism is horizontal, as the vertical route continues to be the object of inconclusive researches. The objective of this study was to verify the presence of *Campylobacter* sp in breeder hens, meconium, and other organs of day-old chicks derived from these breeders in order to obtain information on vertical transmission. Microbiological analyses were performed, using cloacal swabs from 279 breeder hens. Positive breeders were segregated, and the presence of *Campylobacter* sp. was verified in their progeny by analyzing 117 meconium samples; 36 heart, liver and spleen samples (pool of 3 day-old chicks per sample), and 34 intestine samples (pool of 3 chickens in each sample). The analysis of the 279 breeder hens showed that 39 (13.97%) were positive for *Campylobacter* sp, using the cloacal swab method. The meconium and the organs of day-old chicks were not positive. The physiological characteristics of breeder hens, of eggs, and of *Campylobacter* sp favor the entrance of bacteria and their survival inside the eggs, and therefore, this probably is the contamination route of day-old chicks. However, chick meconium and organs were negative in the present experiment, indicating that the vertical way of transmission is a rare event.

INTRODUCTION

The role of the genus *Campylobacter* as an emergent microorganism related to food contamination, particularly of avian origin, has been emphasized in different parts of the world (Aquino, 1995). It is the main cause of gastroenteritis in humans in the United States of America and other developed countries (Friedman *et al.*, 1998). It was demonstrated that the intestinal tract of domestic poultry is an important reservoir of *Campylobacter jejuni* (Hiett, 2002). Infections in birds are not often associated with clinical disease, despite the large numbers of bacteria shed in feces.

Due of the concern of the association of *Campylobacter* sp with foods of avian origin, and the knowledge of its transmission route, the efficient control of chicken farms is necessary. Refrégier-Petton *et al.* (2001) reported several studies conducted to determine the infection pathways in birds. Environmental factors, such as high temperature, deficient air circulation inside the shed, poor water quality, infected workers' boots, and the presence of small insects are critical points for flock contamination.

The vertical transmission route of *Campylobacter* sp is still discussed by many authors, but no definitive conclusion was reached. Therefore,



further studies are necessary to understand it. The difficult isolation of the agent in eggs and day-old chicks using the traditional culture method (Baker, 1987; Rabie, 1992; Zaki And Reda, 1995; Sahin, 1993 and Young, 1999), and the existence of viable and non-culturable forms of the microorganism (Moreno *et al.*, 2001), reinforce the need for further investigations.

The aim of this study was to verify the presence of *Campylobacter* sp in breeder flocks, meconium samples, and organs of their progeny on the first day of life, in order to investigate indications of vertical transmission.

MATERIALS AND METHODS

Campylobacter sp in breeder hen flocks

Samples were collected in a broiler breeder farm, and in a hatchery, both located in the state of Minas Gerais, Brazil. Individual cloacal samples were collected using sterile swabs from 279 55-week-old broiler breeders. Samples were placed in Clary Blair transportation broth (Oxoid®), and submitted to the laboratory for microbiological analyses.

Positive breeders were identified by placing a metal band on their wings, and were then housed in a separate pen inside the shed.

Campylobacter sp in newly-hatched chicks

A total number of 202 eggs laid by breeders identified as positive by cloacal swabs, and kept in a separate pen inside the shed, were aseptically collected for 7 consecutive weeks. Eggs were sent to the hatchery, and, after being stored for 1 to 3 days, were placed in an incubator. After 21 days, out of all chicks that hatched, 117 had their meconium and organs collected.

Meconium was collected from each chick by a massaging its abdominal region. The sample was then put into a small sterile plastic bag containing 10mL Bolton broth (Oxoid®), supplemented with 20mg/L sodium cephaloperazone, 20mg/L vancomycin, 20g/L trimethoprim, and 50mg/L of cycloheximide (Oxoid®).

After meconium collection, chicks were anesthetized with ether, and euthanasia was performed by neck dislocation. Heart, spleen, and liver were aseptically collected, and placed into a sterile small plastic bag containing 50mL Bolton broth, supplemented with the same antibiotic mixture as described above. Each sample consisted of a pool of three chicks, and, therefore, a total number of 36

samples was collected. The 34 intestine samples (each sample was a pool from 3 different animals) were each placed in a small sterile bag with 50mL Bolton broth.

All samples were placed in ice boxes, and immediately sent to the laboratory.

Laboratory Analyses

Cloacal swabs from the breeder hens were enriched in tubes containing 10mL Bolton broth supplemented with the above described antibiotic mixture, and incubated for a period of 24 hours, at a temperature of 37°C, in microaerobic atmosphere. The same procedures were followed with the chick samples.

After enrichment, aliquots of each culture were seeded on *Brucella* agar (Oxoid®), supplemented with the same antibiotic mixture, hemolyzed sheep blood, and FBP supplement (0.4g/L sodium pyruvate, 0.4g/L ferrous sulfate, and 0.4g/L sodium metabisulfite – Oxoid®). Plates were incubated at 37°C for 48 hours in anaerobic jar placed in microaerobic atmosphere.

Characteristic colony-forming units that appeared on the plates were confirmed as *Campylobacter* sp by Gram staining, and further examined under phase-contrast microscopy for typical movement and morphology. The curved, S-shaped morphology or spiral rods measuring 0.2 to 0.8 µm width and 0.5 to 5.0 µm length, were classified as *Campylobacter* sp.

RESULTS

Campylobacter sp was detected in 39 of the 279 cloacal swabs taken from breeder hens (13.97% positive). No presence of *Campylobacter* sp was verified in the meconium or organ samples of day-old chicks.

DISCUSSION

The prevalence of *Campylobacter* sp in cloacal swabs taken from breeder hens was lower than the results obtained and described by Kazwala *et al.* (1990), who observed a prevalence of 80%. Buhr (2002) found 90 to 100% of the breeder hen flocks were positive. The lower isolation rate found in the present study, as compared with the results of other authors, is probably due to the excellent management and health conditions of the broiler breeder farm, where samples were taken. This is reinforced by Fernandez *et al.* (1993), who found a 66.7% positive rate in free-range chickens, 29.4% in broilers, and 7.3% in SPF (Specific Pathogen Free) chickens. The authors concluded that the prevalence of *Campylobacter* sp declined as management and health conditions improved.



Organs from day-old chicks were negative for *Campylobacter* sp. These results are consistent with those found by Jacobs-Reistman (1995), Evans (2000), Newell and Wagenaar (2000), and Shreeve (2000). The mentioned authors assert that day-old chicks were contaminated only when they were 2 or 3 weeks of age. In principle, their resistance to *Campylobacter* sp could result from the immunity transmitted by the mother, but there are few scientific studies on this factor. Sahin *et al.* (2003) studied two groups of SPF chickens: one group was contaminated with *Campylobacter jejuni*, and the other was not. They observed that chicks from contaminated flocks presented specific antibodies against *C. jejuni*, and that these antibodies derived from their mothers. Chicks from contaminated flocks that continued to receive an additional dose of *C. jejuni* presented lower contamination rates as compared to chicks with no maternal antibodies.

The experimental challenge of day-old chicks by Shanker *et al.* (1990), however, did not support the theory of maternally-derived immunity, as *Campylobacter* was isolated from feces samples of these chicks at two days of age.

None of the meconium samples were positive. There are no reports in scientific literature on the presence of *Campylobacter* sp in these specimens, only of *Salmonella* sp, when vertical transmission was investigated (Rocha *et al.*, 2003; Soncini and Back, 2001).

Campylobacter width (0.2 to 0.8 µm) and length (0.5 to 5.0 µm) (Vandame, 1992), the size of the egg shell pores (11 to 12 µm), the bird's temperature (42°C) (Sesti, 2000), and the ability of this bacterium to be naturally present in parts of the reproductive tract of commercial chickens (Buhr *et al.*, 2002) suggest that vertical transmission of *Campylobacter* sp to the progeny of contaminated breeder flocks is possible. However, probably some factors present inside the egg or the chicks do not allow the agent to survive, which indicates that vertical transmission is probably a rare event in poultry flocks.

CONCLUSIONS

Breeder flocks need to be recognized as a reservoir of *Campylobacter* sp. However, it may be more appropriate to consider them as a potential risk factor in horizontal transmission routes in poultry production, rather than a risk factor for vertical transmission via the egg.

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