

CAMPYLOBACTER JEJUNI

RELATIONSHIP OF MESSENGER RNA REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION SIGNAL TO *CAMPYLOBACTER* SPP. VIABILITY

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Chicken colonization by cells that have positive mRNA signal but that are noncultivable would provide a correlation in cell viability and persistence of mRNA. To examine whether this scenario exists, levels of four strains of *Campylobacter* spp., previously isolated from poultry feces, were stored for 6 to 7 wk in phosphate-buffered saline at 4°C and resulted in loss of cultivability. Cold-stored, noncultivable and heat-inactivated (60°C for 10 min) *Campylobacter* spp. produced inconsistent amplified products from RT-PCR assay, depending on the target transcripts and strains used, although all fresh cultures showed mRNA signals. For the most part, signals of mRNA species from viable but noncultivable (VBNC) and heat-killed *Campylobacter* spp. AH-1, AH-2, and CH-3 persisted. RT-PCR amplification of transcripts originating from the *tkt* and *cmp* genes and a 256-bp amplicon of haem-copper oxidase provided consistent signals, whereas transcripts from the M gene did not. Presumed VBNC and heat-inactivated *Campylobacter* spp., which produced positive mRNA signal but was not cultivable by conventional culture-based methods, did not establish colonization in the intestine of chicks 7 days after challenge. These results lead us to question the correlation between mRNA durability with cell viability as well as the significance of the VBNC cells in environmental transmission of *Campylobacter* spp.

DNA ISOLATION FROM NONCULTIVABLE *CAMPYLOBACTER JEJUNI* ISOLATES

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Campylobacter jejuni isolates, associated with poultry production, were subjected to 5 treatments following storage in Wang's transport medium: prolonged storage at room temperature; prolonged incubation at 42°C; multiple rounds of freezing and thawing; boiling; or contamination with *Pseudomonas aeruginosa*. Two nucleotide amplicons from the *flaA* gene (~ 400 nucleotides) and 16S rDNA (~ 800 nucleotides) were readily obtained from the noncultivable stored cells and may be used for isolate typing schemes including *flaA* short variable region (*flaA* SVR) sequencing, multilocus sequence typing (MLST) and *flaA* PCR-RFLP.

CONTAMINATION OF INTERNAL ORGANS BY *CAMPYLOBACTER JEJUNI* FOLLOWING ORAL OR INTRACLOACAL INOCULATIONS OF BROILER CHICKS

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Campylobacter jejuni was administered to day-old chicks (n = 30) through oral and intracloacal inoculations. After inoculation, broilers were aseptically opened and internal organs (thymus, spleen, liver/gallbladder, bursa of Fabricius, and ceca) individually analyzed for *C. jejuni*. Overall, *C. jejuni* was isolated after oral inoculation from 13%, 17%, and 28% of the 1-h, 1-day, and 1-wk samples, respectively. Following the intracloacal route of inoculation, *C. jejuni* was recovered from 32%, 8%, and 16% of the 1-h, 1-day, and 1-wk samples, respectively. *C. jejuni* was isolated from 10%, 8%, 19%, 25% and 40% of the thymus, spleen, liver/gallbladder, bursa of Fabricius, and ceca samples, respectively. The rapid movement of *Campylobacter* to internal organs following both oral and intracloacal inoculation may be significant, particularly if it persists in these organs as reservoirs throughout the 65-wk life cycle of breeding birds.

**CONTAMINATION OF COMMERCIAL BROILER BREEDER ROOSTERS BY
CAMPYLOBACTER, *SALMONELLA*, AND *CLOSTRIDIUM PERFRINGENS***

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The present study was conducted to determine if several foodborne pathogens (*Campylobacter*, *Salmonella*, and *Clostridium perfringens*) could be isolated from the ductus deferens, testes, and ceca of 45- to 65-wk old commercial broiler breeder roosters. Aseptic necropsy was performed on 15 roosters (five roosters from 3 separate commercial breeder farms) to remove the ductus deferens, testes, and ceca without surface contamination from blood and other tissues. None of the foodborne pathogens were isolated from the testes of the roosters. In the ductus deferens, *C. perfringens* was isolated from 1 of the 15 roosters, whereas no *Campylobacter* or *Salmonella* was isolated from this tissue. *Campylobacter* was cultured from the ceca of all 15 roosters, *C. perfringens* was isolated from 14 of 15 roosters, and *Salmonella* from 2 of 15 roosters. These data suggest that the contamination of semen by these foodborne pathogens is via fecal or cecal contamination as the semen passes through the cloaca and not from bacterial colonization of the testes and ductus deferens.

CHARACTERIZATION OF BACTERIOCINS ISOLATED FROM *BACILLUS CIRCULANS* AND *PAENIBACILLUS POLYMYXA* STRAINS AND INHIBITORY EFFECTIVENESS TOWARD *CAMPYLOBACTER JEJUNI*

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Three hundred sixty-five *Bacillus* and *Paenibacillus* isolates from poultry production environments were screened for anti-*Campylobacter* activity. Crude cell-free preparations, prepared from 4 promising isolates (*B. circulans* and 3 *P. polymyxa* strains), exhibited zones of *Campylobacter jejuni* growth inhibition in a spot test. Inhibition was consistent with the presence of a bacteriocin based on resumption of growth when the crude preparation was exposed to protease enzymes. The molecular mass of the antimicrobial peptides was estimated at ~ 3,500 Da based on sodium dodecyl sulfate polyacrylamide gel electrophoresis and matrix-assisted laser desorption and ionization time of flight analysis. The bacteriocins from *P. polymyxa* NRRL B-30507, NRRL B-30508, and NRRL B-30509 had pIs of 4.8, 4.8 and 7.2, whereas the bacteriocin from *B. circulans* NRRL B-30644 had a pI of 7.2. Amino acid sequences were consistent with those of class IIa bacteriocins. Following purification of the bacteriocin from *P. polymyxa* NRRL B-30509 and microencapsulation in polyvinylpyrrolidone, the bacteriocin (0.25 g) was incorporated into 1 kg of chicken feed. The feed was administered to chickens for 3 days that had been orally challenged with *C. jejuni* 1-day after hatch followed by ad libitum access to standard broiler starter feed and water for 7 days. Significant reductions in colonization by *C. jejuni* were observed with untreated 10-day old chickens exhibiting 7.2 log₁₀ CFU/g of feces and treated chickens exhibited no detectable numbers of *C. jejuni* in their feces.