

Research Note—

Incidence of *Campylobacters* in the Intestine of Avian Species in Alabama

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SUMMARY. Avian species necropsied at the C. S. Roberts Veterinary Diagnostic Laboratory, Auburn, Alabama, from December 1993 until May 1994 were examined for the incidence of intestinal campylobacters. Ninety-one intestinal swabs, representing 66 separate cases and 17 different avian species, were collected and placed into Cary-Blair transport medium. Selective enrichment and culture media were used for initial isolation of *Campylobacter* spp. Presumptive colonies were identified as *Campylobacter* spp. by phase-contrast microscopy and Gram stain, and they were confirmed by serological latex agglutination. *Campylobacter* spp. were isolated in 18 (19.7%) of the 66 cases. From the remainder of the cases, 13 (15%) yielded presumptive colonies on Campy-Cefex agar; however, they were not confirmed serologically as *Campylobacter* spp. Use of Cary-Blair transport medium held in refrigeration for up to 24 days did not hinder the determination of campylobacters in intestinal samples. A variety of avian species, including chicken, emu, hawk, ostrich, and parrot, harbored commensal campylobacters and therefore should be considered potential reservoirs.

RESUMEN. *Nota de Investigación*—Incidencia de *Campylobacter* en el intestino de especies aviarias en Alabama.

Se determinó la incidencia de *Campylobacter* en el intestino de especies aviarias recibidas para necropsia en el Laboratorio de Diagnóstico Veterinario C. S. Roberts en Auburn, Alabama, desde Diciembre de 1993 hasta Mayo de 1994. A partir de 66 casos se tomaron 91 hisopos intestinales de 17 especies aviarias diferentes y se almacenaron en medio de transporte Cary-Blair. Para el aislamiento inicial de *Campylobacter* spp., se utilizaron medios de cultivo de enriquecimiento selectivo. Las colonias sospechosas se identificaron como *Campylobacter* spp. por microscopía de contraste de fases y por la coloración de Gram, y fueron confirmadas por aglutinación serológica en látex. Se aisló *Campylobacter* spp. en 18 (19.7%) de los 66 casos. De los casos restantes, 13 (15%) produjeron colonias sospechosas en agar Campy-Cefex, sin embargo, no se confirmaron serológicamente como *Campylobacter* spp. El uso de medio de transporte Cary-Blair mantenido en refrigeración hasta por 24 días, no alteró la determinación de *Campylobacter* en las muestras intestinales. Una variedad de especies aviarias, incluyendo el pollo, emú, halcón, avestruz y loro son portadoras de *Campylobacter* comensal y por lo tanto pueden ser consideradas como reservorios potenciales.

Key words: *Campylobacter*, incidence, avian species

Campylobacter is one of the most important foodborne pathogens transmitted to humans by mishandled poultry (2,5,20). In England and Wales, chicken is the second most important

food item associated with human campylobacteriosis (9,22). In the United States, human campylobacter infection rates exceed the combined infection rates of *Salmonella* and *Shigella* (11). In addition to being transmitted via the foodborne route, campylobacters can be transmitted to humans through the oral route during contact with pets, domestic and wild animals

(including birds), and infected persons, or by consumption of contaminated water (29).

Campylobacter jejuni establishes as a commensal in the gastrointestinal tract of wild and domestic ruminants, swine, cats, dogs, fowl, and rodents (4,5,24,32,34). Motility, by flagellum, and chemotaxis toward L-fucose (an exposed sugar component of mucin) play important roles in intestinal colonization (6,10,19,33). Once established in the mucin layer of cecal crypts, *Campylobacter* multiplies and creates an asymptomatic carrier condition in the host.

Other species of *Campylobacter* have also been isolated from the intestinal tract of chickens, turkeys, and other avian species. Two of them, *C. coli* and *C. lari*, are closely related to *C. jejuni* and therefore are difficult to distinguish from *C. jejuni* (1,2,7,17,28).

In modern poultry production, *C. jejuni* transmission between birds may result from oral or cloacal contamination (29). Chickens remain infected with *Campylobacter* for extended periods. In southern England, chickens in a broiler farm carried *C. jejuni* for at least 18 months after infection was detected (22). Likewise, a high rate of intestinal carriage occurs in mature breeder hens and pre-slaughter broilers and turkeys. Horizontal transmission is the primary route of infection in domestic food animals, particularly poultry, because vertical transmission is not a natural route of *C. jejuni* infection (25,27).

Although abundant research has incriminated poultry as an important reservoir, little information is available on the role of other avian species in the epidemiology of the disease. Surveys have shown that pigeons in both the United States and Japan are infected with *C. jejuni* (12,14,28). This organism has been also isolated from the intestinal tracts of Galliformes such as pheasants and quail and from Anseriformes (16,27,28). Omnivorous species and scavengers are more frequent carriers than granivores (3,13,18,21,27).

Given the fact that *Campylobacter* is spread exclusively by horizontal routes in poultry, the role that other avian species play in disseminating the pathogen into commercial poultry environments is very important to the understanding of the epidemiology of human campylobacteriosis.

Because of their sensitivity to desiccation and other environmental stresses, appropriate

methods for transport and storage of specimens are necessary for the survival of campylobacters (28). The use of a transport medium plus refrigeration is often required whenever immediate culturing of samples is not possible. *Campylobacter* spp. have been recovered from feces stored for 3 weeks at 4 C, but no viable organisms could be recovered from samples stored at 25 C for a week (8).

The objective of this study was to determine the incidence of intestinal carriage of *Campylobacter* spp. in different avian species. Also, the efficacy of transport, enrichment, and culture media for the analysis of *Campylobacter* spp. from intestinal swabs was evaluated.

MATERIALS AND METHODS

Samples from 91 individual birds were collected at the C. S. Roberts Veterinary Diagnostic Laboratory at Auburn, Alabama, from December 1993 to May 1994. These samples represented 66 different cases and 17 different avian species.

The sampling procedure consisted of collecting swabs of cecal content and mucosa (or rectal/colonic when the species did not have ceca), which were then immersed in 10 ml of Cary-Blair transport medium (Oxoid Ltd., Basingstoke, Hampshire, England) in screw-capped glass tubes (32). These samples were transported to and further processed at the Food Microbiology Laboratory, Department of Poultry Science, Auburn University.

Some samples were stored in transport medium at 4 C for up to 24 days before being processed. Samples were enriched using Hunt and Radle enrichment medium (30). Flasks were flushed with CO₂ to reduce oxygen tension (9). After enrichment, samples were subcultured onto Campy-Cefex selective agar (31).

Plates were placed under microaerophilic conditions by the use of anaerobic jars and Campy-Pak® gas-generating packets (BBL Microbiology Systems, Cockeysville, Maryland) and incubated at 42 C for 48 hr. *Campylobacter* spp. were identified to the level of genus on the basis of colony morphology, cell form, Gram stain, and motility under phase-contrast microscopy. Presumptive colonies were confirmed as *Campylobacter* spp. using a serological latex agglutination kit (Meritec-Campy; Meridian Diagnostics, Inc., Cincinnati, Ohio).

RESULTS AND DISCUSSION

Eighteen of the 66 cases (19.7%), consisting of 27 samples, yielded confirmed *Campylobacter* spp. These cases represented five different

Table 1. Prevalence of *Campylobacter* spp. in avian species.

Species	Total number cases/samples	Positive ^a cases/samples	Presumptive ^b cases/samples	Storage time ^c (days)
Blackbird	1/5	-/-	1/1	
Chicken	18/30	10/19	-/-	11
Cockatlel	4/4	-/-	2/2	
Dove	1/4	-/-	-/-	
Duck	1/4	-/-	1/1	
Emu	16/16	4/4	5/5	8
Goose	1/1	-/-	-/-	
Hawk	1/1	1/1	-/-	6
House finch	1/1	-/-	-/-	
Lovebird	1/1	-/-	-/-	
Owl	1/1	-/-	-/-	
Ostrich	7/9	2/3	2/2	24
Parrot	9/9	1/1	-/-	10
Pigeon	1/1	-/-	1/1	
Quall	1/2	-/-	1/2	
Rhea	1/1	-/-	-/-	
Swan	1/1	-/-	-/-	
Total	66/91	18/27	13/14	

^aConfirmed serologically.

^bCases not confirmed at *Campylobacter* spp. with serological tests.

^cNumber of days specimens were held at 4 C in Cary-Blair medium.

avian species (Table 1): chickens, emus, ostriches, a parrot, and a hawk. Fifty percent of the chicken cases were confirmed positive for *Campylobacter* spp. with the latex agglutination kit; consequently, these isolates can be considered as *C. jejuni*, *C. coli*, or *C. lari*.

Jones *et al.* (11) isolated *C. jejuni* from 20% of cloacal swabs taken from broilers entering the processing plant and before slaughter. Other studies indicated that 20 to 100% of chickens carry *Campylobacter* spp. in their intestinal tracts (3,24). Certainly, *Campylobacter* has adapted to the chicken intestinal tract, making this avian species a principal reservoir (1,4,7,11,27,28).

Free-living birds are also frequent carriers of *C. jejuni* and represent a possible source of infection for commercial poultry. Recently, *Campylobacter* spp. were reported isolated from liver lesions of a rhea chick and from the yolk sacs of ostrich chicks with yolk sacculitis (23). In the present study, campylobacters were confirmed in 25% of emu and 28% of ostrich cases (Table 1). These results plus the fact that two ostrich and five emu cases yielded *Campylobacter*-like isolates suggest that these avian species are potential livestock carriers of campylobacters.

In 13 (15%) cases (Table 1), cultural isolates had characteristics consistent with *Campylobacter* spp. but failed to give positive agglutination results. Typically, these specimens yielded combinations of two or three bacterial types, and in many instances the isolation of pure *Campylobacter*-like colonies was difficult. Moreno *et al.* (18) described similar findings when isolating campylobacters from pets and rodents. Thus, it is likely that these suspected cultures were species of campylobacters other than *C. jejuni*, *C. coli*, or *C. lari* and therefore did not agglutinate in the serological tests. In all of these cases, the isolates grew rapidly in Campy-Cefex agar under microaerophilia, were gram-negative curved rods with corkscrew motility, were similar to campylobacters in size, and produced small, whitish, *Campylobacter*-like colonies.

The blackbirds, doves, and pigeon were submitted dead to the laboratory, because pesticide intoxication was suspected. Laboratory tests failed to reveal the presence of any pesticide; unfortunately, avicide analysis could not be done because insufficient stomach content was left. In another diagnostic procedure using these specimens (data not shown), inoculation of filtered intestinal content into embryonated

chicken eggs caused embryonic death. Bacterial contamination of the eggs was evident, and the bacteria were morphologically consistent with *Campylobacter* spp. However, corresponding intestinal samples did not yield *Campylobacter* spp. (Table 1).

Samples that were kept in Cary-Blair medium under refrigeration for up to 24 days yielded confirmed *Campylobacter*, suggesting no deleterious effect on survival (Table 1). Luechtefeld *et al.* (15) isolated viable campylobacters from specimens in Cary-Blair medium with 0.16% agar when stored at 4 C for up to 18 days; they concluded that this medium was the best of six transport media for the preservation and survival of a small number of campylobacters in turkey fecal specimens held at either 4 or 25 C. Our results confirm that the use of Cary-Blair medium with 0.56% agar and refrigeration was highly suitable for handling intestinal samples that were not processed immediately.

Although originally developed for the isolation of campylobacters from food samples, Campy-Cefex selective agar performed adequately in this study. The combination of Hunt and Radle enrichment broth, Campy-Cefex selective agar, and incubation conditions was a highly selective isolation procedure, which proved to be appropriate for handling specimens. This procedure is labor intensive and requires trained personnel. Stern and Line (30) found this combination to be optimal for *Campylobacter* isolation from processed chicken carcasses without the use of serological confirmation. Yet culture confirmation seemed necessary when processing fecal samples.

None of the positive cases presented lesions consistent with *Campylobacter* spp. infection, indicating that the isolated campylobacters were commensals. These findings corroborate the accepted tenet that *Campylobacter* spp. establish commensal relations with free-living birds (5,27) without causing any clinical manifestations. However, Ruiz-Palacios *et al.* (26) reportedly induced diarrhea in 3-day-old chicks with as few as 90 cells of a human isolate of *C. jejuni*; in other experimentation, however, intestinal colonization achieved by oral and cloacal inoculation failed to produce disease in 2-to-15-day-old chickens (29). Infective dose, virulence of the strain, and age of the bird affect the pathogenesis of *Campylobacter* spp. in chickens (28). Nevertheless, data here confirm

that a wide variety of avian species can commensally harbor and potentially shed campylobacter into food animal environments and should therefore be considered potential reservoirs for this human pathogen.

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