Campylobacter fetus subsp. jejuni in a Turkey Processing Plant

NANCY W. LUECHTEFELD* AND WEN-LAN LOU WANG

Microbiology Laboratory, Denver Veterans Administration Medical Center, Denver, Colorado 80220, and Department of Pathology, University of Colorado School of Medicine, Denver, Colorado 80262

Cecal cultures taken over a 1-year period from 600 turkeys at a poultry processing plant were all positive for Campylobacter fetus subsp. jejuni. Swabs of the cloaca and of fresh feces were likewise all positive. Of 33 freshly dressed turkey carcasses, 94% were positive before chilling in tanks of chlorinated ice and water; 34% of 83 carcasses were still positive after overnight soaking in the tanks. Increasing the chlorine content from 50 to 340 ppm (50 to 340 μg/ml) did not cause a decrease in the number of positive carcasses. C. fetus subsp. jejuni was isolated from wastewater gutters as well as from chutes and conveyor belts in the packaging room. Water samples from the five water treatment lagoons for the plant were all positive for C. fetus subsp. jejuni while the plant was in operation, but 4 days after the plant closed for the winter, all water samples were negative.

Campylobacter fetus subsp. jejuni has been isolated from stools of 3 to 14% of unselected patients with diarrhea in several industrialized countries and is now recognized as a common cause of enteritis in humans (1, 5, 6, 22). The organism has been isolated from the intestinal tracts of numerous wild and domestic mammals and birds (3, 7, 12, 19, 20). Poultry have been implicated as a source of Campylobacter infections in humans (5, 10, 11, 14–16, 18), and C. fetus subsp. jejuni has been isolated from the intestines, carcasses, and processed meat of chickens (5, 9, 14–18). We conducted the present study to determine the patterns of Campylobacter contamination in a turkey processing plant and the effectiveness of processing procedures in decreasing the rate of contamination in the final marketable product.

MATERIALS AND METHODS

Plant description. The turkey processing plant studied is a vertically integrated, federally inspected operation. Separate rooms are used for the various stages of processing, including killing, scalding and plucking, eviscerating, chilling, and packaging. Turkeys from eight farms are loaded into large crates (40 to 55 birds per crate) and then trucked to the processing plant; the total time from crating to slaughter is approximately 3 to 7 h. Each carcass is examined by a federal inspector, and any bird contaminated by improper evisceration is discarded. Freshwater is constantly flowing from faucets at each station for workers to rinse their hands and equipment before beginning work on each bird; this rinse water also picks up solid waste material during evisceration and then flows out of the building to the water treatment area. Before packaging, turkey carcasses are chilled overnight in chlorinated ice water; the chlorine content ranges from 20 to 50 ppm (20 to 50 μg/ml). Plant processing equipment is constructed of stainless steel and is easily cleaned. The floors are made of concrete and are washed down several times each day during processing. The plant capacity is 900 birds per h, and about 30,000 turkeys are processed each week. The wastewater (approximately 150,000 gallons [ca. 567,750 liters] per day) is treated by sedimentation and by aerobic digestion in a series of five open lagoons covering 12 acres of fenced land. Evaporation is rapid enough from this surface area that there is no outflow of water from the lagoons.

Sampling and culture techniques. Cecal samples were taken from 600 freshly killed turkeys from 24 different flocks over a period of 10 months (July through December 1979 and March through June 1980). The plant is closed in January and February. The turkeys came from eight different farms and included both hens and toms, ranging in age from 18 to 22 weeks. Cecal specimens were collected by cutting one cecum from each bird with sterile scissors; cecal material was then expressed into sterile plastic tubes and transported immediately to the laboratory for culturing. The transport medium for all other specimens was Campy-thio (Pasco Laboratories, Inc., Wheatridge, Colo.) consisting of thioglycolate broth, 0.16% agar, and the following antimicrobial agents (per liter): vancomycin, 10 μg; polymyxin B, 2,500 U; trimethoprim, 5.0 mg; amphotericin B, 2.0 μg; and cephalothin, 15 mg (1). Cloacal swabs from 30 freshly slaughtered birds and 30 swabs of fresh feces from turkey carcasses on the delivery trucks were taken. Specimens from moist surfaces, such as gutters and chutes, were taken by swabbing with dry swabs. Turkey carcasses were cultured by dipping sterile swabs into Campy-thio and then swabbing the surface of the carcass for approximately 30 s. Packages of edible viscera (hearts, livers, and gizzards) were opened, and the contents were swabbed in a similar manner. Each swab was immediately placed in 0.5 ml of Campy-thio and transported to the laboratory within 2 h. All cecal specimens and swabs were inoculated onto Campy-BAP (Pasco), which consisted of tryptose agar base for brucella (Difco Laboratories, Detroit, Mich.), 5% sheep blood, and the same concentrations of the anti-
microbial agents found in Campylobacters (1). All plates were streaked for isolation (four-quadrant technique) and incubated for 48 h at 42°C in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% nitrogen. C. fetus subsp. jejuni was identified by techniques described previously (1).

Ten dressed toms were tagged and cultured both before and after chilling to correlate the effects of chlorine on individual birds. The chlorine content of the ice and water used for chilling was tested on multiple occasions by the Colorado State Health Department with an amperometric titrator (Fisher Scientific Co., Pittsburgh, Pa.) (4). On 1 day, the chlorine content of a chill tank was increased to 340 ppm; 20 processed turkey carcasses were chilled in this tank overnight as usual and then cultured on the next day.

Multiple water samples were taken from each lagoon, both during processing and again in January, as the plant shut down for 2 months. Also, samples of chlorinated water from the chill tanks were taken. Each 30-ml water sample was filtered through a 0.22-

\mu m membrane filter (Millipore Corp., Bedford, Mass.) which was then cultured by pressing it several times onto a plate of Campy-BAP. This material from the filter was then streaked in three directions for maximum separation of colonies and incubated as described above. On day 4 after the plant shut down for winter, five sediment samples (approximately 0.3 g each) from the lagoons were directly plated onto Campy-BAP and incubated as described above.

\section*{RESULTS}

Table 1 shows the isolation rates of C. fetus subsp. jejuni from turkey samples and from environmental samples at the processing plant. Overnight chilling of carcasses in chlorinated water reduced the rate of isolation from 94 to 34%. Also, positive swabs taken from carcasses before chilling grew a median of 2+ colonies (first quadrant confluent or almost so; ≥3 colonies in the second quadrant, but <3 colonies in the third quadrant) per plate, whereas positive swabs from chilled carcasses grew a median of only 1 colony per plate. Five 30-ml samples of water from chill tanks were negative for C. fetus subsp. jejuni.

Of the 10 toms tagged and cultured before and after chilling, all were positive before chilling, and 4 remained positive after chilling.

On one occasion, 9 of 20 turkeys (45%) were still culture positive after soaking overnight in ice water with a chlorine content of 340 ppm.

All water samples from the five water treatment lagoons were positive for C. fetus subsp. jejuni while the plant was operating. Each 30-ml water sample produced numerous colonies of the organism, but accurate counting was not possible due to spreading of the Campylobacter organisms and to masking by other organisms growing on the plate. Campylobacter organisms were not isolated from any of 10 water samples or 5 sediment samples by the 4th day after the plant closed in January.

\section*{DISCUSSION}

The extremely high isolation rate (100%) of C. fetus subsp. jejuni from turkey cecal specimens is comparable to rates reported for chickens (68 to 87%) (5, 9, 15, 17). Despite the different techniques used to quantitate organisms in cecal samples, our previously reported mean of 2.7×10^8 colony-forming units per g of turkey feces N. W. Luechtefeld, W.-L. L. Wang, M. J. Blaser, and L. B. Reller, submitted for publication) is quite similar to the mean of 4.4×10^8 colony-forming units per g of chicken feces obtained by Grant et al. (9). These rates of isolation of Campylobacter are much higher than rates reported for Salmonella in poultry (both chickens and turkeys), whether one compares initial sampling of intestinal contents before processing or swabs of the carcasses taken after processing (8, 13).

Why such a high carrier rate of C. fetus subsp. jejuni is seen in turkeys and chickens is not known. The organism propensity of the organism for wild (12, 19) and domestic birds in general may be related to the high body temperature of birds (42°C), which corresponds to the optimal temperature for growth of C. fetus subsp. jejuni in laboratories.

The survival of even small numbers of Campylobacter on turkey carcasses soaked in chlorinated water seems to conflict with data from this laboratory in which 0.625 ppm of chlorine completely killed a suspension of 10^7 colony-forming units of C. fetus subsp. jejuni per ml in 4 h (W.-L. L. Wang unpublished data). Since protein rapidly inactivates chlorine (23), perhaps the protein on the surface of processed turkey carcasses protects some of the organisms from the high concentrations of chlorine in the chill water. Chlorination of the chill water is not required by the U.S. Department of Agriculture, and many plants do not use chlorine treatment at all. Poultry processed at such plants could
have a higher level of contamination of the marketable product with \textit{C. fetus} subsp. \textit{jejuni} than did the plant studied here. Simmons and Gibbs reported that 11 of 12 turkeys were positive for \textit{C. fetus} subsp. \textit{jejuni} after processing and chilling (92%), but the concentration of chlorine in the chill water and the number of organisms isolated from each carcass were not reported (17). At this time, it is not known what the minimal infective dose of the organism is for humans; one human volunteer became ill after ingesting a suspension of 10\(^6\) organisms in a glass of milk (22), but smaller doses of the organism have not been tested.

Although the effluent from the plant was heavily contaminated with \textit{C. fetus} subsp. \textit{jejuni}, we were unable to detect the organism in the water treatment lagoons within 4 days after the plant shut down for winter. The lagoons at that time (January 1980) were completely frozen over, and the ice was broken to obtain the specimens. Perhaps the cold temperature was detrimental to the growth or survival of the organism in the lagoon water. Previous studies showed that pure cultures of \textit{C. fetus} subsp. \textit{jejuni} survived for 5 to 33 days in autoclaved mountain stream water at 4°C (2); on the other hand, the organism did not survive in turkey cecal samples frozen at -20°C (Luechtefeld et al., submitted for publication). An alternative explanation is that perhaps many of the organisms were concentrated in the sediment at the bottom of the lagoons. Five sediment samples were negative, but this small sampling may not have been adequate to detect the organism.

Further studies are needed regarding the virulence for humans of \textit{C. fetus} subsp. \textit{jejuni} from turkeys and chickens, the minimal cooking time and temperature required to kill the organism in poultry, and techniques to further decrease contamination levels in processed poultry.

**ACKNOWLEDGMENTS**

We thank Martin J. Blaser for reviewing the manuscript and the Water Quality Laboratory of the Colorado State Health Department for testing the chlorine content of water samples.

**LITERATURE CITED**


