Evaluation of antimicrobial susceptibility patterns in *Campylobacter* spp isolated from dairy cattle and farms managed organically and conventionally in the midwestern and northeastern United States

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Objective—To describe antimicrobial susceptibility patterns in *Campylobacter* spp isolated from dairy cattle and farms managed organically and conventionally in the midwestern and northeastern United States.

Design—Longitudinal study.

Sample Population—128 farms.

Procedure—Samples and data were collected every 2 months from August 2000 to October 2001. Fecal samples were collected from calves and cows. Milk samples were obtained from the bulk tank and milk line filters. Environmental samples were obtained from a water source, feed bunks of lactating cows, and cattle housing areas. *Campylobacter* identification and antimicrobial susceptibility testing were performed at a central laboratory by use of microbroth dilution with 2 customized antimicrobial susceptibility panels.

Results—460 and 1,570 *Campylobacter* isolates were obtained from organic and conventional dairy farms, respectively. Most isolates from both farm types were susceptible to most antimicrobial agents tested, and antimicrobial susceptibility of conventional dairy isolates was decreased, compared with organic dairy isolates. Low proportions of isolates resistant to ampicillin (< 10%) and moderate proportions resistant (30% to 60%) to kanamycin, sulfamethoxazole, and tetracycline were observed on both farm types. The proportion of isolates resistant to tetracycline was higher for conventional than organic farms.

Conclusions and Clinical Relevance—*Campylobacter* isolates from dairy cattle and farms managed organically and conventionally had similar patterns of antimicrobial resistance; the proportion of resistant isolates was higher for conventional than organic farms. (*J Am Vet Med Assoc* 2006;228:1074–1081)

Abbreviations

ATCC	American Type Culture Collection
BASB	*Brucella* agar with supplemental 5% defibrinated sheep blood
MIC	Minimum inhibitory concentration
MIC_{50}	MIC inhibiting growth of 50% of isolates
MIC_{90}	MIC inhibiting growth of 90% of isolates

Campylobacter spp are often identified as a cause of bacterial gastroenteritis in humans in the United States[1,2] and outnumber other infectious causes of foodborne illness, such as *Salmonella*, *Escherichia coli* O157:h7, and *Shigella*.[3] Each year, as many as 2 million cases of illness are estimated to be caused by *Campylobacter jejuni*.[4] Most cases of enteritis are mild, self-limiting episodes of vomiting, cramping, and diarrhea[5,6]; however, a more serious form of campylobacteriosis can develop in infants, geriatric patients, and immunocompromised individuals. In those individuals, hematochezia, dehydration, septicemia, and long-term sequela can develop,[7] including the demyelinating neurologic disorder Guillain-Barre syndrome or intermittent arthritis.[8,9] Guillain-Barre syndrome develops subsequent to approximately 1 in 1,000 cases of enteritis caused by *C jejuni* and is usually transient; however, some individuals with Guillain-Barre syndrome continue to have neurologic deficits throughout life.[8]

In humans, many *Campylobacter* infections are associated with direct or indirect exposure to animals.[10] Thermophilic *Campylobacter* spp can colonize the gastrointestinal tracts of mammals and birds without causing disease[11]; therefore, feces from apparently healthy animals may contaminate the environment with *Campylobacter* organisms. Research has been focused on *Campylobacter*-contaminated poultry in retail markets[12,13]; however, the

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dairy industry may also be a source of human exposure to *Campylobacter* organisms. Healthy adult cows and calves frequently shed this organism in their manure,[14-15,a] and a number of outbreaks of *Campylobacter* enteritis have been associated with consumption of raw milk,[16-19] dairy farm visits,[20] and water contamination.[21-23] Consequently, the role of dairy cattle in the transmission of *Campylobacter* to humans should be examined in greater depth.

Another public health concern associated with *Campylobacter* is that this organism has developed resistance to antimicrobial agents. *Campylobacter* isolates from humans are becoming increasingly resistant to numerous classes of antimicrobial drugs with time and with the introduction of new pharmaceutical drugs.[24] In developing countries, antimicrobial resistance may be associated with widespread availability of these drugs[7] because easy access to antimicrobial agents often results in self-medicating to compensate for poor sanitary conditions.[25,26] In developed countries, such as Denmark, domestically acquired *Campylobacter* spp isolated from humans in 2004 were resistant to nalidixic acid (31% of 107 isolates), ciprofloxacin (29%), tetracycline (24%), and erythromycin (5%).[27]

In countries with restricted availability of antimicrobial agents, including the United States, there is ongoing debate regarding the contribution of human medical, veterinary therapeutic, and animal husbandry practices to the decreased susceptibility of key bacteria to antimicrobials.[28-30] Increased fluoroquinolone resistance has been detected in *Campylobacter* and other bacteria once these antimicrobials were approved in some food animal species,[13,31] and there has been evidence of increased susceptibility in bacteria when certain antimicrobials are banned from use.[32] However, most studies supporting the decrease in susceptibility are based on ecologic (aggregative) analysis of data (ie, which drugs are approved for veterinary use in a particular country) without ascertaining actual exposure to the drugs being studied, and the focus of much research on *Campylobacter* resistance has been on drug classes such as fluoroquinolones and macrolides. Studies[33,34] of antimicrobial resistance to drugs used on dairy farms are limited; therefore, the role of dairy farm practices in the development of antimicrobial resistance in *Campylobacter* spp remains poorly defined.

To address the need for research on antimicrobial resistance and dairy cattle, the purpose of the study reported here was to describe antimicrobial susceptibility patterns of *Campylobacter* spp isolated from dairy cattle and farms managed organically and conventionally in the midwestern and northeastern United States.

Materials and Methods

The study reported here was part of a large longitudinal study investigating the prevalence and antimicrobial susceptibility patterns of *Campylobacter* spp and *Salmonella* spp isolated from dairy cattle from organic and conventionally managed farms in Michigan, Minnesota, New York, and Wisconsin. Results of the study associated with *Salmonella* spp have been reported.[35-38]

Herds—One hundred thirty-two dairy farms were chosen from Michigan, Minnesota, New York, and Wisconsin, from which 128 farms had *Campylobacter* isolates available for antimicrobial susceptibility testing. Herds were enrolled according to farm type (organic vs conventional) and by farm size (No. of lactating and nonlactating cows). To be included in the study, a farm had to meet the following criteria: have at least 30 milking cows in the herd, have at least 90% of cows in the herd of Holstein breed, raise their own calves for replacement cattle, and ship milk all year. Organic farms had to be certified as organic by a recognized organic certification agency and may not have used antimicrobials in cattle > 1 year old for at least 3 years. Lists of farms were obtained from the respective state departments of agriculture, and herd owners within approximately 100 miles of the respective universities were randomly chosen to receive a mailing describing the study. Owners of farms were asked to indicate interest in participation in the study by returning a postcard. The final list of farms was obtained by randomly choosing names of respondents that had indicated willingness to participate in the study. To evaluate potential herd management practices as risk factors, a predetermined number of farms was enrolled within the following herd size categories: 30 to 49, 50 to 99, 100 to 199, and ≥ 200 cows. Because of the limited availability of organic farms, owners of all known organic farms within approximately 150 miles of the respective universities were contacted to determine eligibility on the basis of selection criteria and their desire to participate in the study. Farm visits for the collection of cattle and environmental samples took place every other month during a 12-month period.

Collection of samples—Approximately 10 g of fecal material was collected from the rectum of cattle and placed into plastic bags.[b] A separate glove was used to collect each sample. The number of samples collected per herd and the number of samples collected from specific cattle groups were determined by herd size. The total number of fecal samples from herds with 30 to 49, 50 to 99, 100 to 199, and ≥ 200 cows was 30, 40, 50, and 55 samples, respectively. Cattle management groups included preweaned heifer calves, cows to be culled within 14 days, periparturient cows (due to calve within 14 days and cows within 14 days in lactation after calving), sick cows as determined by farm personnel or the herd veterinarian, and healthy lactating cows. No effort was made to collect samples from the same individual cattle at subsequent herd visits.

Farm environmental samples—One sample from each location was collected at each sampling visit by wiping areas to be tested with sterile gauze pads soaked in double-strength skim milk (skim milk powder[c] reconstituted with 50% of the volume of water normally used and sterilized via autoclaving), which were placed into plastic bags[b] for shipment. Sampling locations included areas in which cattle may be directly exposed to *Campylobacter*, including feed bunk of lactating cows; lagoon or manure pile; bird droppings in areas housing cows; and the walls, boards, or flooring of maternity pens and areas housing calves or sick cattle. In cows that were going to be culled, a sample was obtained by wiping the coat across the lower aspect of the flank and gluteal region with a swab soaked in double-strength skim milk. If a pen location was not used on a particular farm (eg, no sick pen), then no sample was collected for that location. Samples collected from pens used for more than 1 purpose, such as the sick cow pen and calving pen, were labeled according to the predominant use. A sample from a water source for cattle (eg, a water tank or a pooled swab specimen from 5 drinking cups), a bulk tank milk sample, and a milk line filter were also collected.

Shipment of samples—After collection, samples were shipped to a central laboratory at Michigan State University. Samples from Minnesota, New York, and Wisconsin were

shipped via overnight delivery in insulated foam boxes with ice packs. Samples were shipped the same day as collection whenever possible; however, some samples were stored at 2° to 4°C for 2 to 36 hours until the next shipping opportunity.

Isolation and identification of *Campylobacter* spp— Environmental swab specimens and milk filters were enriched in Bolton broth[d] containing 5% laked horse blood and selective antimicrobial agents (cefaperazone [20 mg/L], vancomycin [20 mg/L], trimethoprim [20 mg/L], and cycloheximide [50 mg/L]). Enriched samples were incubated at 42°C in 5% to 10% CO_2 for 48 hours. Fecal and milk samples were suspended in phosphate buffer saline solution. Fecal and milk samples suspended in phosphate buffered saline solution and enriched samples were streaked on selective *Campylobacter* plates with 5 antimicrobial agents (amphotericin B, cephalothin, trimethoprim, vancomycin, and polymyxin B) and 10% defibrinated sheep blood[e] and incubated at 42°C in 5% to 10% CO_2 for 48 hours. Typical colonies (small pinpoint gray colonies without hemolysis) were selected and streaked on sheep blood agar and incubated at 42°C in 5% to 10% CO_2 for 48 hours. Identification of *Campylobacter* spp was performed from isolated colonies by gram staining, oxidase testing, and motility testing. Hippurate hydrolysis was used to speciate *C jejuni* by use of *C jejuni* ATCC 33560 as a positive control and *Campylobacter coli* ATCC 33559 as a negative control.

In vitro antimicrobial susceptibility testing—In vitro antimicrobial susceptibility testing was performed by use of a microbroth dilution method. At the time this study was performed in 2000 to 2001, the NCCLS had not approved standardized recommendations for in vitro antimicrobial susceptibility testing for *Campylobacter* spp.[39] Because of the lack of a standard, in vitro antimicrobial susceptibility testing was performed by use of the microbroth dilution method, following the NCCLS guidelines available at that time for bacteria isolated from animals.[39,40] In 2003, the NCCLS approved agar dilutions as the standardized method for antimicrobial susceptibility testing for *Campylobacter* spp[39]; therefore, a study[41] was performed in our laboratory with a subset of those isolates to verify the performance of our microbroth dilution system with agar dilution. Briefly, results of that study indicated that there was no association in the classification of resistance by the testing methods used, and the quality control strain of *C jejuni* ATCC 33560 performed in a consistent manner for both agar dilution and microbroth dilution.[41]

Bacterial isolates from frozen stock were grown on BASB for 48 hours at 42°C in 5% to 10% CO_2. Individual colonies from each plate were subcultured on BASB in similar conditions. Bacteria were swabbed from the BASB and suspended in 5 mL of water, and the turbidity was adjusted to a 0.5 McFarland standard. This suspension was used to make a dilution in a 1:10 ratio in *Haemophilus* testing medium,[f] resulting in a final bacterial inoculum concentration of approximately 8×10^5 colony forming units/mL.

Commercially prepared microbroth dilution plates were used for antimicrobial susceptibility testing. For all plates, *C jejuni* ATCC 33560 and *C jejuni* ATCC 81176 were used as quality control strains. Each plate was inoculated by adding 100 µL of bacterial suspension by use of an autoinoculator,[g] covered with a gas-permeable seal, and incubated at 42°C in microaerophilic conditions for 48 hours. The MIC, the minimum antimicrobial dilution at which no bacterial growth developed, was read manually from each plate for each isolate. The MIC_{50} and MIC_{90} from a given herd were calculated from the microbroth dilution plate with the largest range of MIC values for each drug.

Two different microbroth dilution plates were used throughout the study. Initially, to determine whether patterns of resistance detected in isolates from dairy cattle were comparable to those detected in isolates from humans, a customized antimicrobial panel (CMV1USDA),[h] with a prepared range of concentrations for azithromycin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline, was purchased. After observing that isolates from dairy farms did not have resistance patterns similar to isolates from humans (decreased susceptibility to ciprofloxacin or azithromycin),[13,24,29] another customized

Table 1—Dilution ranges for antimicrobial agents used, by panel, and interpretative breakpoints for in vitro antimicrobial susceptibility testing for *Campylobacter* isolates obtained from dairy cattle and farms managed conventionally and organically in the midwestern and northeastern United States.

Antimicrobial	CMV1USDA[h] panel (µg/mL)	CMV2DMSU[h] panel (µg/mL)	Interpretative criteria for resistant strains (µg/mL)
Amoxicillin-clavulanic acid			
Amoxicillin	NA	2–64	≥ 32
Clavulanic acid	NA	1–32	≥ 16*
Ampicillin	NA	2–64	≥ 32*
Azithromycin	0.03–256	0.12–4	≥ 2†
Ceftiofur	NA	1–16	≥ 8*
Ceftriaxone	NA	4–128	≥ 64*
Cephalothin	NA	4–64	≥ 32*
Chloramphenicol	0.5–64	4–64	≥ 32*
Ciprofloxacin	0.03–64	0.5–16	≥ 4†
Clindamycin	0.06–256	NA	≥ 4†
Erythromycin	0.12–256	0.25–16	≥ 8†
Florfenicol	NA	2–32	≥ 16*
Gentamicin	0.12–256	2–32	≥ 16†
Kanamycin	NA	8–128	≥ 64*
Nalidixic acid	0.12–128	4–128	≥ 32†
Streptomycin	NA	16–128	≥ 64†
Sulfamethoxazole	NA	64–512	≥ 512*
Tetracycline	0.25–256	2–128	≥ 16†
Trimethoprim-sulfamethoxazole			
Trimethoprim	NA	1–8	≥ 4
Sulfamethoxazole	NA	16–512	≥ 64*

*Breakpoint from NCCLS.[41] †NCCLS (Clinical and Laboratory Standards Institute) General Enteric breakpoints used by the National Antimicrobial Resistance Monitoring System.[42]
NA = Not applicable.

antimicrobial panel (CMV2DMSU)[h] was developed to address drug exposures that are common to dairy cattle management and to permit comparison with patterns of resistance detected in *Salmonella* spp.[37,38] Antimicrobial panel CMV2DMSU included 17 drugs encompassing drug classes used on our study farms, such as β-lactams and cephalosporins,[36] and its use replaced the CMV1USDA panel. Breakpoints used to classify isolates as resistant or not resistant were those recommended by the National Antimicrobial Resistance Monitoring System for *Campylobacter* spp for azithromycin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline (**Table 1**).[41] For the CMV2DMSU panel, general enteric breakpoints were used to classify isolates as resistant for the additional antimicrobials. Whereas selective media with antimicrobial agents (cefaperazone, vancomycin, trimethoprim, and cycloheximide) were used to isolate *Campylobacter* spp, any isolates detected would have been expected to be resistant to these agents. Consequently, analysis of resistance to ceftiofur, cephalothin, and trimethoprim-sulfamethoxazole was not pursued.

Data analysis—To determine whether there was an association with the proportion of resistant isolates and farm type, descriptive breakpoints were used to classify isolates as resistant or susceptible for each antimicrobial agent. The proportion of resistant isolates by farm type (organic or conventional) was analyzed by use of χ^2 tests via a computer software program.[i]

Results

A total of 2,030 *Campylobacter* isolates were available for antimicrobial susceptibility testing. Organic

Table 2—Distribution of *Campylobacter* isolates obtained from dairy cattle and farms managed conventionally and organically in the midwestern and northeastern United States used for antimicrobial susceptibility testing.

***Campylobacter* isolates**	**Organic farm**	**Conventional farm**
Farm environmental isolates		
Feedbunk	0	0
Calf pen	2	1
Sick cow pen	0	1
Maternity pen	2	3
Water tank	1	3
Lagoon	1	3
Bulk tank milk	0	3
Milk filter	2	9
Bird droppings	0	4
Cull cow haircoat	0	6
Total	**8**	**33**
Cattle isolates		
Preweaned calves	132	427
Healthy lactating cows	238	683
Cull cows	3	32
Cows due to calve in 14 days	23	80
Cows that calved within 14 days	35	177
Sick cows	19	126
Total	**450**	**1,525**

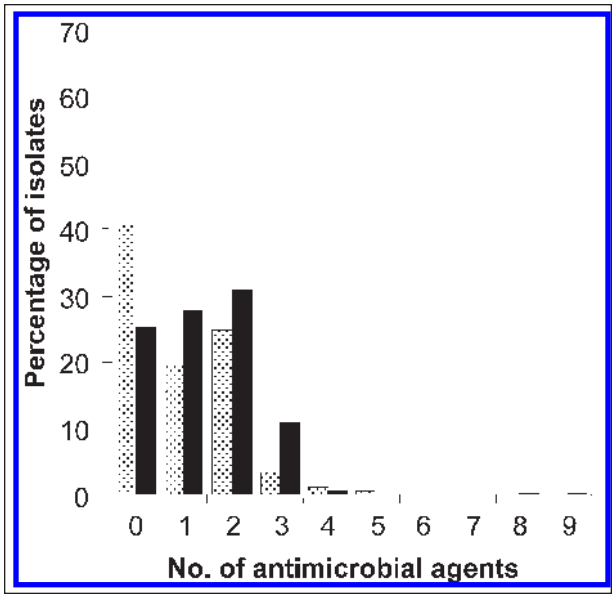

Figure 1—Percentage of *Campylobacter* isolates with multidrug resistance as determined by in vitro antimicrobial susceptibility testing (customized panel with 17 antimicrobials [CMV2DMSU]) of samples obtained from dairy cattle and farms managed conventionally (black bars; n = 696) or organically (stippled bars; 169) in the midwestern and northeastern United States.

Table 3—In vitro antimicrobial susceptibility of *Campylobacter* isolates from feces of dairy cattle on farms managed conventionally or organically in the midwestern and northeastern United States.

	Conventional farm isolates				**Organic farm isolates**			
Antimicrobial	**No. of cattle**	**MIC_{50}**	**MIC_{90}**	**Resistant (%)**	**No. of cattle**	**MIC_{50}**	**MIC_{90}**	**Resistant (%)**
Amoxicillin-clavulanic acid*	686	2	2	0.1	168	2	2	0
Ampicillin	686	4	8	8.6	168	8	16	7.1
Azithromycin†	1,525	0.12	0.12	1.3	450	0.06	0.12	1.1
Ceftriaxone*	686	16	16	1.4	168	16	32	2.3
Chloramphenicol†	1,525	2	4	1.1	450	2	4	0
Ciprofloxacin†	1,525	0.12	0.5	1.1	450	0.12	0.5	0.9
Clindamycin†	840	0.12	0.5	1.3	282	0.12	0.25	1.0
Erythromycin†	1,525	0.5	1.0	1.2	450	0.5	1.0	1.1
Florfenicol*	686	2	2	0.3	168	2	2	0
Gentamicin†	1,525	2	2	0.1	450	1	2	0
Kanamycin*	686	8	> 128	32.4	168	8	> 128	30.0
Nalidixic acid†	1,525	4	8	1.9	450	4	8	1.3
Streptomycin*	686	16	16	1.6	168	16	16	0.6
Sulfamethoxazole*	686	256	512	37.2	168	256	256	38.7
Tetracycline†	1,525	32	128	58.3	450	8	128	49.3

Sample numbers change because not all antimicrobials were present on all panels used to test for antimicrobial susceptibility.
*CMV2DMSU.[h] †CMV1USDA.[h]

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Table 4—Distribution of MICs for farm environmental *Campylobacter* isolates obtained from dairy farms managed organically and conventionally in the midwestern and northeastern United States.

Antimicrobial	MIC (µg/mL)	No. of isolates	
		Conventional	Organic
Amoxicillin-clavulanic acid* (MIC resistance breakpoint ≥ 32/16)	2	9	1
	4	1	0
	8	0	0
	16	0	0
Ampicillin* (MIC resistance breakpoint ≥ 32)	2	3	1
	4	1	0
	8	4	0
	16	1	0
	32	0	0
	64	1	0
Azithromycin*† (MIC resistance breakpoint ≥ 2)	0.03	10	3
	0.06	4	3
	0.12	15	2
	0.25	3	0
	0.5	0	0
	1	0	0
	2	0	0
	4	0	0
	8	1	0
	16	0	0
	32	0	0
	64	0	0
	128	0	0
	256	0	0
Ceftriaxone* (MIC resistance breakpoint ≥ 64)	4	1	0
	8	1	0
	16	6	1
	32	2	0
	64	0	0
	128	0	0
Chloramphenicol*† (MIC resistance breakpoint ≥ 32)	0.5	1	1
	1	8	3
	2	13	3
	4	10	1
	8	1	0
	16	0	0
	32	0	0
	64	0	0
Ciprofloxacin*† (MIC resistance breakpoint ≥ 4)	0.03	1	0
	0.06	6	3
	0.12	13	3
	0.25	1	1
	0.5	11	1
	1	0	0
	2	1	0
	4	0	0
	8	0	0
	16	0	0
	32	0	0
	64	0	0
Clindamycin† (MIC resistance breakpoint ≥ 4)	0.06	4	4
	0.12	3	2
	0.25	9	1
	0.5	6	1
	1	0	0
	2	0	0
	4	0	0
	8	0	0
	16	0	0
	32	0	0
	64	1	0
	128	0	0
	256	0	0
Erythromycin*† (MIC resistance breakpoint ≥ 8)	0.12	1	0
	0.25	10	0
	0.5	11	3
	1	8	3
	2	2	2
	4	0	0
	8	0	0
	16	0	0
	32	1	0
	64	0	0
	128	0	0
	256	0	0
Florfenicol* (MIC resistance breakpoint ≥16)	2	10	1
	4	0	0
	8	0	0
	16	0	0
	32	0	0
Gentamicin*† (MIC resistance breakpoint ≥16)	0.12	0	0
	0.25	0	1
	0.5	0	1
	1	18	3
	2	14	3
	4	0	0
	8	1	0
	16	0	0
	32	0	0
	64	0	0
	128	0	0
	256	0	0
Kanamycin* (MIC resistance breakpoint ≥ 64)	8	10	1
	16	0	0
	32	0	0
	64	0	0
	128	0	0
Nalidixic acid*† (MIC resistance	0.12	0	0
	0.25	0	0
	0.5	0	0
	1	0	0
	2	2	1
	4	25	3
	8	5	3
	16	0	0
	32	1	1
	64	0	0
	128	0	0
Streptomycin* (MIC resistance breakpoint ≥ 64)	16	10	1
	32	0	0
	64	0	0
	128	0	0
Sulfamethoxazole* (MIC resistance breakpoint ≥ 512)	64	1	0
	128	1	0
	256	3	0
	512	3	1
	> 512	2	0
Tetracycline*† (MIC resistance breakpoint ≥16)	0.25	12	5
	0.5	0	1
	1	0	0
	2	6	1
	4	0	0
	8	1	0
	16	3	0
	32	2	0
	64	2	1
	128	4	0
	256	3	0

*CMV2DMSU (10 conventional isolates and 1 organic isolate). †CMV1USDA (23 conventional isolates and 8 organic isolates).

farms yielded 450 fecal, 8 environmental, and 2 milk and milk filter *Campylobacter* isolates for antimicrobial susceptibility testing, whereas conventional farms yielded 1,525 fecal, 33 environmental, and 12 milk and milk filter isolates (**Table 2**). Greater than 97% of isolates were classified as *C jejuni,* and the rest were not further speciated.[a]

Across farm type, conventional farms had slightly higher proportions of resistant isolates to most antimicrobial agents than organic farms. For fecal isolates, there were slightly higher proportions of reduced antimicrobial susceptibility in conventional farm isolates (9.9% resistant overall and 6.4% resistant excluding tetracycline), compared with organic farm isolates (8.9% resistant overall and 6.0% resistant excluding tetracycline; **Table 3**). The only significant ($P = 0.007$) difference detected was in resistance to tetracycline, in which the proportion of resistant isolates was higher for conventional farms (58.3%) than organic farms (49.3%). Similarly, there were no significant differences in MIC_{50} and MIC_{90} dilutions between conventional and organic isolates, with the exception of tetracycline. Conventional farm isolates required 4 times the antimicrobial concentration of tetracycline (32 µg/mL) to inhibit growth of 50% of the isolates than required for organic farm isolates (8 µg/mL). Higher proportions of reduced antimicrobial susceptibility were detected for antimicrobial agents in the CMV2DMSU panel (mean percentage resistant, 11.4%) than in the CMV1USDA panel (mean percentage resistant, 7.6%), and a higher proportion of multidrug resistance was detected in isolates from conventional farms (47%), compared with isolates from organic farms (40%), when the CMV2DMSU panel was used (**Figure 1**).

Given the low numbers of isolates from farm environmental samples and milk and milk filters, MIC_{50} and MIC_{90} were not calculated. Resistance in isolates from farm environmental samples was low, with no resistant isolates detected for amoxicillin-clavulanic acid, ceftriaxone, chloramphenicol, ciprofloxacin, florfenicol, gentamicin, kanamycin, and streptomycin (**Table 4**). Resistance to sulfamethoxazole was detected in 50% of isolates from conventional farms. Decreased antimicrobial susceptibility to tetracycline was detected in 5 of 12 *Campylobacter* isolates from milk and milk filters from conventional farms.

Discussion

Although infections and outbreaks in humans caused by *Campylobacter* spp have been associated with or linked to dairy cattle sources,[20,21,43] little critical evaluation of the antimicrobial susceptibility of those isolates has been performed. Although multidrug-resistant *Salmonella* infections in humans have been traced to dairy farms through meat or milk consumption,[44] evaluation of this association would also seem prudent for *Campylobacter* spp. An additional concern is that the current consumer interest in organic and alternative food sources has resulted in some consumers bypassing such food safety measures as pasteurization.[45] In light of increased consumer interest in raw milk and minimally processed food products, it is noteworthy that decreased susceptibility was observed in some isolates from raw milk and milk filter samples to the 8 antimicrobials of interest in treating infections in humans. The practice of drinking raw milk has lead to infections in humans with *Campylobacter* and *Salmonella* spp.[43,44,46] Therefore, unprocessed dairy products may be capable of transmitting not only foodborne pathogens but also antimicrobial resistance determinants through the exchange of mobile genetic elements such as plasmids or integrons.

In addressing the primary purpose of evaluating the susceptibility of *Campylobacter* spp by farm type in the United States, results of our study indicated that *Campylobacter* isolates from both types of dairy farms are generally susceptible to most antimicrobials. This finding agrees with results of studies[47-50] on farming systems in countries in which antimicrobial use is regulated more than it is in the United States. With the exception of tetracycline, no significant association between decreases in antimicrobial resistance and organic farming practices was detected, which agrees with results of a study[51] indicating that no clear associations between on-farm antimicrobial use and susceptibility patterns in *Campylobacter* isolates to tetracycline, kanamycin, ciprofloxacin, erythromycin, or nalidixic acid were detected. Increased susceptibility to tetracycline in isolates from organic dairies was detected, which agrees with results of other studies[52,53] indicating that antimicrobial susceptibility among organic farming systems increased, compared with isolates from conventional farms.

In addition to differences in selection pressures from antimicrobial use, organic farms are often small and use different animal management practices, such as pasture grazing or exposure of cattle to free-living bird environments,[36,54] compared with large, conventionally managed dairies. Resistance of *Campylobacter* spp in free-living wild birds has also been detected, suggesting that wildlife may play a role in the ecology of antimicrobial resistance.[55] Consequently, various management practices must be considered when evaluating the ecology of antimicrobial resistance in a farm environment.

There were 2 drugs, kanamycin and tetracycline, for which resistance was common to both farm types. Antimicrobial susceptibility to tetracycline, which has a much wider spectrum of use in cattle than kanamycin, was significantly decreased on conventional farms, compared with organic farms. In other species, resistance to tetracycline has been found to be associated with use of this drug in broiler chicken flocks and in birds that had been exposed to a coccidiostat only.[56] Coccidostats are frequently used in dairy heifer rations on conventional farms[36]; however, this was not a common practice in organic herds used in our study. Reportedly, genetic determinants for kanamycin and tetracycline resistance (KanR and tetO, respectively) are carried on plasmids in *C jejuni,*[57] and it is possible that these mobile genetic elements are continually exchanged between other bacteria and *C jejuni*, despite a lack of selective pressure in the animal host from which it was isolated. Genetic markers for tetracycline resistance have been detected in farming environments,[58] making environmental contamination a viable source for an animal to acquire resistance factors regardless of whether an individual animal was treated with antimicrobial drugs.

Results of our study indicated that compared with conventional farms, *Campylobacter* isolates obtained from organic farms were not more susceptible to all classes of antimicrobials studied. However, differences observed in tetracycline resistance between various farm management types and results of other studies[32,47-50] suggested that the issue of antimicrobial resistance in food animals warrants investigation of modifiable herd and individual animal risk factors to aid in the planning and implementation of interventions that will promote food safety and a healthy livestock population.

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c. Becton-Dickinson Microbiology Systems (formerly Difco), Sparks, Md.
d. Bolton broth, Oxoid USA (Remel Inc), Lenexa, Kan.
e. Campylobacter agar with 5 antimicrobics and 10% sheep blood (Blaser), BD Diagnostics, Franklin Lakes, NJ.
f. *Haemophilus* testing medium, TREK Diagnostics Systems Inc, Cleveland, Ohio.
g. SensiTitre, TREK Diagnostics Systems Inc, Cleveland, Ohio.
h. TREK Diagnostic Systems Inc, Cleveland, Ohio.
i. SAS, version 8.2, SAS Institute Inc, Cary, NC.

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Selected abstract for *JAVMA* readers from the *American Journal of Veterinary Research*

Histomorphologic evaluation of extracorporeal shock wave therapy of the fourth metatarsal bone and the origin of the suspensory ligament in horses without lameness

Andrea S. Bischofberger et al

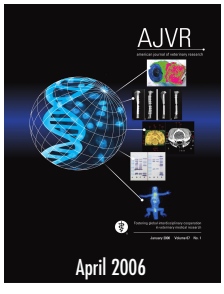
Objective—To determine via histologic examination and scintigraphy the effect of focused extracorporeal shock wave therapy (ESWT) on normal bone and the bone-ligament interface in horses.

Animals—6 horses without lameness.

Procedure—Origins of the suspensory ligament at the metacarpus (35-mm probe depth) and the fourth metatarsal bone (5-mm probe depth) were treated twice (days 0 and 16) with 2,000 shocks (energy flux density, 0.15 mJ/mm^2). One forelimb and 1 hind limb were randomly treated, and the contralateral limbs served as nontreated controls. Bone scans were performed on days –1 (before ESWT), 3, 16, and 19. Histomorphologic studies of control and treated tissues were performed on day 30.

Results—ESWT significantly increased the number of osteoblasts but caused no damage to associated soft tissue structures and did not induce cortical microfractures. A significant correlation between osteoblast numbers and radiopharmaceutical uptake was noticed on lateral views of the hind limb on days 3 and 16 and on caudal views of the forelimb on day 3.

Conclusions and Clinical Relevance—Results suggested that ESWT has the potential to increase osteoblast numbers in horses. The correlation between increased osteoblast numbers and radiopharmaceutical uptake 3 days and 16 days after the first ESWT suggested that stimulation of osteogenesis occurred soon after ESWT. No damage to bone or the bone-ligament interface should occur at the settings used in this study, and ESWT can therefore be administered safely in horses. (*Am J Vet Res* 2006;67:577–582)



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