



# Prevalence and fluoroquinolone resistance of *Campylobacter* spp. isolated from beef cattle in Japan

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## Abstract

Beef is a source of human *Campylobacter* infections. Antimicrobial treatment is needed when patients are immunocompromised or have other comorbidities. Therefore, we investigated the prevalence and antimicrobial resistance of *Campylobacter* spp. in beef cattle in Japan. Rectal swab samples were collected from 164 beef cattle at an abattoir between March 2021 and August 2021, and *Campylobacter* spp. were isolated from 94 (57.3%) cattle. *C. jejuni* and *C. coli* were isolated from 68 and 26 cattle, respectively. For *Campylobacter jejuni*, the resistant rates against ampicillin, tetracycline and ciprofloxacin were 20.6, 75.0 and 64.7%, respectively. For *C. coli*, the resistant rates against ampicillin, tetracycline and ciprofloxacin were 53.8, 76.9 and 88.5%, respectively. No *Campylobacter* isolates were resistant to erythromycin. By multilocus sequence typing, *C. jejuni* and *C. coli* isolates were classified into 22 and 2 sequence types (STs). The top three STs of *C. jejuni* were ST806 (12 isolates), ST21 (nine isolates), and ST459 (eight isolates). The most frequent ST of *C. coli* was ST1068 (23 isolates). The results suggest that *Campylobacter* spp. are prevalent in the gastrointestinal tract of beef cattle slaughtered at abattoirs. Furthermore, the administration of erythromycin is effective against human campylobacteriosis caused by beef consumption. Monitoring the prevalence and antimicrobial resistance of *Campylobacter* spp. in beef cattle could be useful for managing the risk of human campylobacteriosis.

**Keywords:** Antimicrobial resistance, Beef cattle, *Campylobacter*, Fluoroquinolone resistance, Multilocus sequence typing

## Main text

*Campylobacter* spp. are one of four key global causes of diarrheal diseases in humans worldwide, and the two most frequent species isolated from human campylobacteriosis are *C. jejuni* and *C. coli*, according to the World Health Organization (WHO 2020). Antimicrobial therapy is effective in patients with severe symptoms (Yang et al. 2019; WHO 2020). Erythromycin is often used as first-line treatment for human campylobacteriosis; however, in the absence of a microbiological diagnosis,

fluoroquinolones, which are classified as one of “critically important antimicrobials” by WHO (2019), are used to treat human campylobacteriosis (Japanese Association for Infectious Disease/Japanese Society of Chemotherapy 2018). Therefore, fluoroquinolone-resistance in *Campylobacter* spp. poses an important issue in the chemotherapy treatment of humans. In *Campylobacter* spp., fluoroquinolone resistance is mainly caused by chromosomal mutation in the *gyrA* gene, which results in a Thr86-Ile substitution (Zirnstein et al. 1999; Zirnstein et al. 2000).

In Japan, Kumagai et al. (2020) investigated foodborne outbreaks caused by *Campylobacter* spp. between 2007 and 2018 and estimated that the first and second most important sources of the disease were chicken (80.3%)

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and beef products (10.5%). The Japanese Ministry of Agriculture, Forestry and Fisheries has identified chicken meat as the principal source of human campylobacteriosis and monitors the prevalence of *Campylobacter* spp. in broiler flocks (Sasaki et al. 2011; Haruna et al. 2012). However, there are only few reports on the prevalence of *Campylobacter* spp. in beef cattle in Japan. Therefore, we investigated the prevalence and antimicrobial resistance of *Campylobacter* spp. in beef cattle. The results of our study could be useful for managing the risk of human campylobacteriosis caused by the consumption of beef.

Rectal swabs were collected from 164 beef cattle shipped from 34 farms in seven regions (Hokkaido, Tohoku, Kanto, Tokai, Kinki, Chugoku, and Kyushu) between March 2021 and August 2021. Of the 164 cattle, 130 were Japanese Black (JB) and 33 were crossbred (JB × Holstein Friesian) (HF). The average ages of the 130 JB and 33 crossbred cattle were 30.4 (26.7 to 44.4) and 26.9 (24.0 to 32.1) months, respectively, with significant differences between the ages of JB and JB × HF crossbred cattle (Mann-Whitney U test:  $P < 0.01$ ). The remaining cattle aged 28.6 months old was another crossbred (JB × Japanese Shorthorn) (JS). Of the 130 JB cattle, 17 and 4 were born from HF and JB × HF crossbred cows, respectively, by embryo transferring. Whereas 16 (9.8%) cattle (15 JB cattle and one JB × JS crossbred cattle) were shipped to an abattoir directly from beef farms where they were born, the remaining 148 (90.2%) were raised on at least two farms (average of 2.5 farms, 2 to 6 farms).

*Campylobacter* spp. were isolated from 94 (57.3%) cattle shipped from 29 (85.3%) beef farms in all seven regions. The prevalence of *Campylobacter* spp. was statistically higher (Fisher's exact test:  $P < 0.05$ ) in JB × HF crossbred cattle (72.7%, 24/33) than in JB cattle (53.1%, 69/130). The prevalence of *Campylobacter* spp. in JB cattle born from HF cows by embryo transferring was 52.9% (9/17) and was reflected similarly (53.1%, 60/113) in other JB cattle. Thirty-two JB cattle were older than the eldest JB × HF crossbred cattle (32.1 months old). The prevalence of *Campylobacter* spp. in them was 40.6% (13/32) and tended to be lower than that (57.1%, 56/98) of JB cattle younger than 32.1 months.

*C. jejuni* was isolated from 68 cattle and *C. coli* was isolated from 26 cattle (Table 1). No animals carried both *C. jejuni* and *C. coli*. *C. jejuni* resistant rates against ampicillin, streptomycin, tetracycline, nalidixic acid, and ciprofloxacin were 20.6, 5.9, 75.0, 64.7 and 64.7%, respectively. Resistance to both tetracycline and ciprofloxacin was observed in 54.4% (37/68) of the *C. jejuni* isolates in this study. All the *C. jejuni* isolates were susceptible to erythromycin, chloramphenicol and gentamicin. *C. coli* resistant rates against ampicillin, tetracycline, chloramphenicol, nalidixic acid, and ciprofloxacin were 53.8, 76.9, 11.5, 88.5, and 88.5%, respectively. Resistance to both tetracycline and ciprofloxacin was observed in 73.1% (19/26) of the *C. coli* isolates. All the *C. coli* isolates were susceptible to erythromycin and gentamicin. By multilocus sequence typing (MLST), *C. jejuni* was classified into 22 sequence types (STs) and *C. coli* isolates were classified into two STs (Table 2). For *C. jejuni*, the top three STs were ST806 (12 isolates), ST21 (nine isolates), and ST459 (eight isolates). These STs were obtained from 10 farms located in four regions (Hokkaido, Tohoku, Kanto and Chugoku), eight farms in four regions (Hokkaido, Tohoku, Kanto and Kyushu), and six farms in four regions (Hokkaido, Tohoku, Kanto and Chugoku), respectively. The *C. jejuni* isolates classified into these top three STs accounted for 45.3% (29/64) of the *C. jejuni* isolates, and resistance to both tetracycline and ciprofloxacin was observed in 79.3% (23/29). For *C. coli*, ST1068 isolates accounted for 88.5% (23/26) of the *C. coli* isolates and were obtained from nine farms in all regions. Resistance to both tetracycline and ciprofloxacin was observed in 78.3% (18/23) of ST1068 *C. coli* isolates. Ciprofloxacin resistance was observed in 16 (72.7%, ST21, ST487, ST596, ST656, ST806, ST982, ST4526, ST11029, ST1739, ST9078, ST459, ST6532, ST9681, ST61, ST929 and ST922) and two (100%, ST827 and ST1068) of 22 STs of *C. jejuni* and two STs of *C. coli*, respectively.

The Thr-86-Ile change (mediated by the C257T mutation in the *gyrA* genes) in all ciprofloxacin-resistant isolates was detected using two mismatch amplification mutation assay PCR methods. Moreover, in one ciprofloxacin-resistant strain per sequence type, the mutation

**Table 1** Antimicrobial resistance in *C. jejuni* and *C. coli* isolates

Species	No. of isolates	No. of resistant isolates (%)					
		ABPC	SM	TC	CP	NA	CPFX
Total	94	28 (29.8)	4 (4.3)	71 (75.5)	3 (3.2)	67 (71.3)	67 (71.3)
<i>C. jejuni</i>	68	14 (20.6)	4 (5.9)	51 (75.0)	0 (0.0)	44 (64.7)	44 (64.7)
<i>C. coli</i>	26	14 (53.8)	0 (0.0)	20 (76.9)	3 (11.5)	23 (88.5)	23 (88.5)

Abbreviations: ABPC Ampicillin, SM Streptomycin, TC Tetracycline, CP Chloramphenicol, NA Nalidixic acid, CPFX Ciprofloxacin

**Table 2** Sequence types and antimicrobial resistance profiles of *C. jejuni* and *C. coli* isolates

Species	CC (No.)	ST	Antimicrobial resistance profile	No.		
<i>C. jejuni</i>	21 (30)	21	SM+TC+NA+CPFX	1		
			TC+NA+CPFX	6		
			SM+TC	1		
			ABPC+TC	1		
			50	ABPC	1	
			487	NA+CPFX	1	
			596	NA+CPFX	1	
			656	ABPC+TC+NA+CPFX	1	
			806	ABPC+TC+NA+CPFX	4	
				ABPC+TC	1	
			TC+NA+CPFX	5		
			TC	2		
			982	ABPC+SM+TC+NA+CPFX	1	
				ABPC+TC+NA+CPFX	1	
				ABPC+TC	1	
			4526	TC+NA+CPFX	1	
			11,029	NA+CPFX	1	
			22 (4)	1739	TC+NA+CPFX	2
				9078	NA+CPFX	2
			42 (10)	42	Susceptible	1
				459	TC+NA+CPFX	7
					Susceptible	1
				6532	ABPC+SM+TC+NA+CPFX	1
			45 (1)	9681	NA+CPFX	1
			61 (14)	61	NA+CPFX	2
					TC	1
					Susceptible	4
					1244	TC
				10,369	ABPC+TC	1
					TC	3
	257 (1)	929	TC+NA+CPFX	1		
	403 (2)	933	TC	2		
	Unassigned (6)	922	ABPC+TC+NA+CPFX	1		
			TC+NA+CPFX	4		
		3502	Susceptible	1		
<i>C. coli</i>	828 (26)	827	TC+NA+CPFX	1		
			NA+CPFX	1		
			TC	1		
			1068	ABPC+TC+NA+CPFX	13	
		TC+CP+NA+CPFX		3		
		ABPC+NA+CPFX		1		
		TC+NA+CPFX		2		
		NA+CPFX		2		
		Susceptible	2			

**Abbreviations:** CC Clonal complex, ST Sequence type, ABPC ampicillin, SM Streptomycin, TC tetracycline, CP Chloramphenicol, NA Nalidixic acid, CPFX ciprofloxacin

was confirmed by directly sequencing of partial *gyrA* gene.

In the present study, the prevalence of *Campylobacter* spp. was statistically higher in JB × HF crossbred cattle than in JB cattle. One of the differences between the two was the age at the time of slaughter. Several studies conducted at dairy and beef farms and abattoirs have shown that young cattle had a higher prevalence of *Campylobacter* spp. than older cattle (Giacoboni et al. 1993; Beach et al. 2002; Mielsen 2002; Ramonaitė et al. 2013). Therefore, the higher prevalence of *Campylobacter* spp. in JB × HF crossbred cattle compared to JB cattle, might be attributed to the fact that the crossbred cattle were slaughtered at a younger age than the JB cattle.

A decade ago, we investigated the prevalence of *Campylobacter* spp. in beef and dairy farms and reported that the prevalence of both *Campylobacter* spp. in beef and dairy farms were approximately 90% (Haruna et al. 2013; Sasaki et al. 2013). In Japan, beef cattle are rarely transported directly from the farms where they were born to abattoirs. Since JB × HF crossbred cattle and JB cattle by embryo transferring are generally born in dairy farms, they are transported to beef farms to fatten. In the present study, 90.2% of cattle was raised on at least two farms (maximum 6 farms). In some cases, the distance between farms where cattle were transported was 900 km or more. The long-term shedding of *Campylobacter* spp. in beef and dairy cattle has been reported (Ingliš et al. 2004; Hakkinen and Hänninen 2009). As a result of the transportation of cattle colonized with *Campylobacter* spp. between farms, it could spread to beef farms throughout Japan and the prevalence in beef farms could remain high.

The top three frequent STs of *C. jejuni* STs (ST806, ST21 and ST459) and the most frequent *C. coli* ST (ST1068) were obtained from at least six farms. It was previously reported that prevalent STs of *C. jejuni* and *C. coli* isolated from cattle in Japan were ST806 and ST21, and ST1068 (Asakura et al. 2012; Asakura et al. 2019; Sasaki et al. 2020). ST21 of *C. jejuni* and ST1068 of *C. coli* are also prevalent in cattle in Italy, USA., and the UK, demonstrated that the host associations of *Campylobacter* genotypes are more robust than their geographic variations (Kwan et al. 2008; Sanad et al. 2011; Roux et al. 2013; Bianchini et al. 2014; Jonas et al. 2015; Cha et al. 2017; Sheppard et al. 2010). The top three STs of *C. jejuni* and the most frequent ST (ST1068) of *C. coli* would be adaptable to beef cattle and widely spread to beef farms in Japan. Moreover, these prevalent STs might be selected by use of tetracyclines and fluoroquinolones, since approximately 80% of them were resistant to both tetracycline and ciprofloxacin. In Japan, tetracyclines and

**Table 3** List of primers used in this study

Name	Sequence (5'–3')	Target	Size (bp)	Reference
<b>Multiplex PCR for <i>C. jejuni</i>, <i>C. fetus</i>, <i>C. coli</i>, <i>C. upsaliensis</i>, <i>C. hyointestinalis</i>, and <i>C. lari</i></b>				Kamei et al. 2016
Cj-spBU5	ATC TTT TAA CCT TGC TTT TGC	<i>C. jejuni</i>	714	
Cj-spBR6	GCA AGC ATT AAA ATC GCA GC			
Cf-spBU6	GGC TTT GCA AAA CCA GAA G	<i>C. fetus</i>	553	
Cf-spBR3	CAA GAG TTC CTC TTA AAC TC			
Cc-spBU10	CTG TAT CAA GAC CTA GCT C	<i>C. coli</i>	433	
Cc-spBR9	TAT AAA GCT GCA GTG TTG G			
Cu-spBU5	GCC TTA GCT TTC TTT GGG	<i>C. upsaliensis</i>	342	
Cu-spBR5	CAT CGG CTT GGA CGC GAC			
ChII-spBU8	CCT AGT AGC GCT ACT TAG	<i>C. hyointestinalis</i>	215	
ChII-spBR8	CAA ATA CCC TAC CTG TAGC			
Cl-spBU4	GTA TCC ATG CTT TAT CAA GA	<i>C. lari</i>	141	
Cl-spBR4	GTA GGC CTA TAA GAG AAC C			
<b>Amplification and sequencing of partial <i>gyrA</i> for <i>C. jejuni</i></b>				Zirnstien et al. 1999
GZgyrA5	ATT TTT AGC AAA GAT TCT GAT	<i>gyrA</i>	673	
GZgyrA6	CCA TAA ATT ATT CCA CCT GT			
<b>Detection of the Thr-86-to-Ile mutation for <i>C. jejuni</i></b>				Zirnstien et al. 1999
CampyMAMAgryA1	TTT TTA GCA AAG ATT CTG AT	Thr-86-to-Ile mutation	265	
CampyMAMAgryA5	CAA AGC ATC ATA AAC TGC AA			
<b>Amplification and sequencing of partial <i>gyrA</i> for <i>C. coli</i></b>				Zirnstien et al. 2000
GZgyrACcoli3F	TAT GAG CGT TAT TAT CGG TC	<i>gyrA</i>	505	
GZgyrACcoli4R	GTC CAT CTA CAA GCT CGT TA			
<b>Detection of the Thr-86-to-Ile mutation for <i>C. coli</i></b>				Zirnstien et al. 2000
GZgyrACcoli3F	TAT GAG CGT TAT TAT CGG TC	Thr-86-to-Ile mutation	192	
CampyMAMAgryA8	TAA GGC ATC GTA AAC AGC CA			

fluoroquinolones are used for treating beef cattle with bacterial diarrhea or pneumonia.

According to the report of the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) (National Veterinary Assay Laboratory of Japan 2020), resistance rates against ciprofloxacin in *C. jejuni* and *C. coli* isolated from cattle slaughtered at abattoirs in 2017 were 50.5 and 81.4%, respectively, slightly lower than those found in this study. However, no erythromycin resistance was observed in *C. jejuni* and *C. coli* isolated from cattle, which is reflected in the results of this study. Erythromycin is often the first drug of choice for the treatment of human campylobacteriosis (JAID/JSC 2018). This study indicate that erythromycin administration remains effective in treating human campylobacteriosis caused by the consumption of beef.

## Conclusion

The Approximately 60% of beef cattle slaughtered at abattoirs carry *Campylobacter* spp. in the gastrointestinal tract and approximately 70% of the isolates are resistant

to fluoroquinolones. Thus, it is necessary to monitor the prevalence and antimicrobial resistance of *Campylobacter* spp. in beef cattle.

## Methods

Sampling was conducted at an abattoir in Tokyo between March 2021 and August 2021. We sampled rectal swabs from 164 beef cattle diagnosed as healthy upon visual inspection by veterinarians. The individual cattle identification number was recorded to obtain information about the production history of the cattle. After evisceration, a rectal sample was taken of the opened rectal lumen of the cattle using a cotton swab (SEEDSWAB No. 1; Eiken Chemical, Tokyo, Japan) and transported to the National Institute of Health Sciences at 4°C. At the laboratory, the sample was refrigerated at 4°C until examination, which was performed within 4 h of sampling. Each swab head was rubbed over the surface of modified charcoal cefoperazone deoxycholate agar (Oxoid, Basingstoke, United Kingdom) and incubated at 42°C for 48 h. A maximum of

two suspected isolates per sample were identified using a multiplex PCR (Kamei et al. 2016). The primers used for the multiplex PCR are shown in Table 3. One *Campylobacter* species per sample was subjected to antimicrobial susceptibility testing and MLST (<http://pubmlst.org/campylobacter/>). Antimicrobial susceptibility testing was conducted against ampicillin (0.12–256 mg/L), streptomycin (0.12–128 mg/L), erythromycin (0.12–128 mg/L), tetracycline (0.12–128 mg/L), chloramphenicol (0.12–256 mg/L), nalidixic acid (0.12–128 mg/L), ciprofloxacin (0.03–64 mg/L), and gentamicin (0.12–256 mg/L). Antimicrobial minimal inhibitory concentrations were determined using the broth microdilution method with dried plates (Eiken Chemical), as per the guidelines of the Clinical and Laboratory Standards Institute (2016). The antimicrobial susceptibility breakpoints (ampicillin: 32 mg/L, streptomycin: 32 mg/L, erythromycin: 32 mg/L, tetracycline: 16 mg/L, chloramphenicol: 16 mg/L, nalidixic acid: 32 mg/L, and ciprofloxacin: 4 mg/L) adopted by Clinical and Laboratory Standards Institute (2020) and JVARM (2020) were used; except for the tests involving gentamicin (2 mg/L), which was specified by the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (2000). *Campylobacter jejuni* ATCC33560 were used as the quality control strain. To detect the Thr-86-Ile mutation (*C. jejuni*: ACA → ATA, *C. coli*: ACT → ATT) in *gyrA* genes of ciprofloxacin-resistant isolates, two mismatch amplification mutation assay PCR methods (Zirnstein et al. 1999; Zirnstein et al. 2000) were used. Moreover, one strain per sequence type was determined by MLST, partial *gyrA* genes were amplified by PCR (Zirnstein et al. 1999; Zirnstein et al. 2000), and the PCR products were directly sequenced to detect the Thr-86-Ile mutation. The primers used for the detection of the mutation of *gyrA* are shown in Table 3. All statistical analyses were performed using R V. 4.1.2.

#### Abbreviations

WHO: World Health Organization; JAID/JSC: Japanese Association for Infectious Disease/Japanese Society of Chemotherapy; JB: Japanese Black; HF: Holstein Friesian; JS: Japanese Shorthorn; MLST: multilocus sequence typing; ST: sequence type; JVARM: Japanese Veterinary Antimicrobial Resistance Monitoring System; CLSI: Clinical and Laboratory Standards Institute.

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#### Authors' contributions

Conceptualization, Y.S.; methodology, Y.S., T.A.; investigation, Y.S.; supervision, T.A., H.A.; writing-original draft preparation, Y.S.; writing-review and editing, T.A. All authors have read and agreed to the published version of the manuscript.

#### Availability of data and materials

Data are contained within the article.

## Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no conflict of interest.

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## References

- Asakura, H., J. Sakata, H. Nakamura, S. Yamamoto, and S. Murakami. 2019. Phylogenetic diversity and antimicrobial resistance of *Campylobacter coli* from humans and animals in Japan. *Microbes Environ* 34: 146–154. <https://doi.org/10.1264/jsm2.ME18115>.
- Asakura, H., H. Brüggemann, S.K. Sheppard, T. Ekawa, T.F. Meyer, S. Yamamoto, and S. Igimi. 2012. Molecular evidence for the thriving of *Campylobacter jejuni* ST-4526 in Japan. *Plos One* 7: e48394. <https://doi.org/10.1371/journal.pone.0048394>.
- Beach, J.C., E.A. Murano, and G.R. Acuff. 2002. Prevalence of *Salmonella* and *Campylobacter* in beef cattle from transfer to slaughter. *J Food Protect* 65: 1687–1693. <https://doi.org/10.4315/0362-028X-65.11.1687>.
- Bianchini, V., M. Luini, L. Borella, A. Parisi, R. Jonas, S. Kittl, and P. Kuhnert. 2014. Genotypes and antibiotic resistances of *Campylobacter jejuni* isolates from cattle and pigeons in dairy farms. *Int J Environ Res Public Health* 11: 7154–7162. <https://doi.org/10.3390/ijerph110707154>.
- Cha, W., R.E. Mosci, S.L. Wengert, C.V. Vargas, S.R. Rust, P.C. Bartlett, D.L. Grooms, and S.D. Manning. 2017. Comparing the genetic diversity and antimicrobial resistance profiles of *Campylobacter jejuni* recovered from cattle and humans. *Front Microbiol* 8: 818. <https://doi.org/10.3389/fmicb.2017.00818>.
- Clinical and Laboratory Standards Institute. 2016. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. In *CLSI guideline M45*, 3rd ed. Wayne: Clinical and Laboratory Standards Institute.
- Clinical and Laboratory Standards Institute. 2020. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. In *CLSI document VET01S*, 5th ed. Wayne: Clinical and Laboratory Standards Institute.
- Danish Integrated Antimicrobial Resistance Monitoring and Research Programme. 2000. NANMAP 2000 (use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods, and humans in Denmark) (ISSN 1600-2032). <https://www.danmap.org/reports/2020>.
- Giacoboni, G.I., K. Itoh, K. Hirayama, E. Takahashi, and T. Mitsuoka. 1993. Comparison of fecal *Campylobacter* in calves and cattle of different ages and area in Japan. *J Vet Med Sci* 55: 555–559. <https://doi.org/10.1292/jvms.55.555>.
- Hakkinen, M., and M.L. Hänninen. 2009. Shedding of *Campylobacter* spp. in Finnish cattle on dairy farms. *J Appl Microbiol* 107: 898–905. <https://doi.org/10.1111/j.1365-2672.2009.04269.x>.
- Haruna, M., Y. Sasaki, M. Murakami, A. Ikeda, M. Kusukawa, Y. Tsujijama, K. Ito, T. Asai, and Y. Yamada. 2012. Prevalence and antimicrobial susceptibility of *Campylobacter* in broiler flocks in Japan. *Zoonoses Public Health* 59: 241–245. <https://doi.org/10.1111/j.1863-2378.2011.01441.x>.
- Haruna, M., Y. Sasaki, M. Murakami, T. Mori, T. Asai, K. Ito, and Y. Yamada. 2013. Prevalence and antimicrobial resistance *Campylobacter* isolates from beef cattle and pigs in Japan. *Journal of Veterinary Medical Science* 75: 625–628. <https://doi.org/10.1292/jvms.12-0432>.

- Inglis, G.D., L.D. Kalischuk, and H.W. Busz. 2004. Chronic shedding of *Campylobacter* species in beef cattle. *Journal of Applied Microbiology* 97: 410–426. <https://doi.org/10.1111/j.1365-2672.2004.02313.x>.
- Japanese Association for Infectious Disease/Japanese Society of Chemotherapy. 2018. JAID/JSC guidelines for infection treatment 2015 – Intestinal infections. *Journal of Infection and Chemotherapy* 24: 1–17. <https://doi.org/https://doi.org/10.1016/j.jiac.2017.09.002>.
- Jonas, R., S. Kittl, G. Overesch, and P. Kuhnert. 2015. Genotypes and antibiotic resistance of bovine *Campylobacter* and contribution to human campylobacteriosis. *Epidemiol Infect* 143: 2373–2380. <https://doi.org/10.1017/S0950268814003410>.
- Kamei, K., H. Kawabata, M. Asakura, W. Samosornsuk, A. Hinenoya, S. Nakagawa, and S. Yamasaki. 2016. A cytolethal distending toxin gene-based multiplex PCR assay for *Campylobacter jejuni*, *C. fetus*, *C. coli*, *C. upsaliensis*, *C. hyointestinalis*, and *C. lari*. *Japan J Infect Diss* 69: 256–258. <https://doi.org/10.7883/yoken.JJID.2015.182>.
- Kumagai, Y., S.M. Pires, K. Kubota, and H. Asakura. 2020. Attributing human foodborne diseases to food sources and water in Japan using analysis of outbreak surveillance data. *J Food Protect* 83: 2087–2094. <https://doi.org/10.4315/JFP-20-151>.
- Kwan, P.S.L., A. Birtels, F.J. Bolton, N.P. French, S.E. Robinson, L.S. Newbold, M. Upton, and A.J. Fox. 2008. Longitudinal study of the molecular epidemiology of *Campylobacter jejuni* in cattle on dairy farms. *Appl Environ Microbiol* 74: 3626–3633. <https://doi.org/10.1128/AEM.01669-07>.
- Mielsen, E.M. 2002. Occurrence and strain diversity of thermophilic campylobacters in cattle of different age groups in dairy herds. *Lett Appl Microbiol* 35: 85–89. <https://doi.org/10.1046/j.1472-765X.2002.01143.x>.
- National Veterinary Assay Laboratory of Japan. 2020. Report on the Japanese veterinary antimicrobial resistance monitoring system 2016–2017. [https://www.maff.go.jp/nval/yakuzai/pdf/200731\\_JVARMReport\\_2016-2017.pdf](https://www.maff.go.jp/nval/yakuzai/pdf/200731_JVARMReport_2016-2017.pdf).
- Ramonaitė, S., A. Rokaitytė, E. Tamulevičienė, A. Malakauskas, T. Alter, and M. Malakauskas. 2013. Prevalence, quantitative load, and genetic diversity of *Campylobacter* spp. in dairy cattle herds in Lithuania. *Acta Veterinaria Scandinavica* 55: 87 <https://actavetscand.biomedcentral.com/articles/10.1186/1751-0147-55-87>.
- Roux, F., E. Sproston, O. Rotariu, M. MacRac, S.K. Sheppard, P. Bessell, A. Smith-Palmer, J. Cowden, M.C.J. Maiden, K.J. Forbes, and N.J.C. Strachan. 2013. Elucidating the aetiology of human *Campylobacter coli* infections. *Plos One* 8: e64504. <https://doi.org/10.1371/journal.pone.0064504>.
- Sanad, Y.M., I.I. Kassem, M. Abley, W. Gebreyes, J.T. Lejeune, and G. Rajashekara. 2011. Genotypic and phenotypic properties of cattle-associated *Campylobacter* and their implications to public health in the USA. *Plos One* 6: e25778. <https://doi.org/10.1371/journal.pone.0025778>.
- Sasaki, Y., T. Iwata, M. Uema, and H. Asakura. 2020. Prevalence and characterization of *Campylobacter* in bile from bovine gallbladders. *Shokuhin Eiseigaku Zasshi* 61: 126–131 (in Japanese). <https://doi.org/10.3358/shokueishi.61.126>.
- Sasaki, Y., M. Murakami, M. Haruna, N. Maruyama, T. Mori, K. Ito, and Y. Yamada. 2013. Prevalence and characterization of foodborne pathogens in dairy cattle in the eastern part of Japan. *Journal of Veterinary Medical Science* 75: 543–546. <https://doi.org/10.1292/jvms.12-0327>.
- Sasaki, Y., Y. Tsujiyama, H. Tanaka, S. Yoshida, T. Goshima, K. Oshima, S. Katayama, and Y. Yamada. 2011. Risk factors for *Campylobacter* colonization in broiler flocks in Japan. *Zoonoses Public Health* 58: 350–356. <https://doi.org/10.1111/j.1863-2378.2010.01370.x>.
- Sheppard, S.K., F. Colles, J. Richardson, A.J. Cody, R. Elson, A. Lawson, G. Brick, R. Meldrum, C.L. Little, R.J. Owen, M.C.J. Maiden, and N.D. McCarthy. 2010. Host association of *Campylobacter* genotypes transcends geographic variation. *Appl Environ Microbiol* 76: 5269–5277. <https://doi.org/10.1128/AEM.00124-10>.
- World Health Organization. 2019. Critically Important Antimicrobials for Human Medicine, 6th Revision. <https://www.who.int/publications/i/item/9789241515528>. Accessed 12 April.
- World Health Organization. 2020. Factsheet, *Campylobacter*. <https://www.who.int/news-room/fact-sheets/detail/campylobacter>. Accessed 12 April.
- Yang, Y., K.M. Feye, Z. Shi, H.O. Pavlidis, M. Kogut, A.J. Ashworth, and S.C. Ricke. 2019. A historical review on antibiotic resistance of foodborne *Campylobacter*. *Front Microbiol* 10: 1509. <https://doi.org/10.3389/fmicb.2019.01509>.
- Zirnstein, G., L. Helsel, Y. Li, B. Swaminathan, and J. Besser. 2000. Characterization of *gyrA* mutations associated with fluoroquinolone resistance in *Campylobacter coli* by DNA sequence analysis and MAMA PCR. *FEMS Microbiol Lett* 190: 1–7. <https://doi.org/10.1111/j.1574-6968.2000.tb09253.x>.
- Zirnstein, G., Y. Li, B. Swaminathan, and F. Angulo. 1999. Ciprofloxacin resistance in *Campylobacter jejuni* isolates: Detection of *gyrA* resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. *J Clin Microbiol* 37: 3276–3280. <https://doi.org/https://doi.org/10.1128/JCM.37.10.3276-3280.1999>.

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