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Prevalence and antimicrobial susceptibility of *Salmonella* in Japanese broiler flocks

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SUMMARY

This study determined the prevalence and antimicrobial susceptibility of *Salmonella* isolated from broiler flocks in Japan. Caecal dropping samples were collected from 288 broiler flocks between November 2007 and February 2010. *Salmonella* was prevalent in 248 (86·1%) broiler flocks. The top three serovars were *S.* Infantis, *S.* Manhattan and *S.* Schwarzengrund. *S.* Infantis was found in all regions tested in this study. However, *S.* Manhattan and *S.* Schwarzengrund were frequently found only in the western part of Japan. High antimicrobial resistance rates were observed against oxytetracycline (90·2%), dihydrostreptomycin (86·7%) and ampicillin (36·5%), and 258 (90·5%) of 285 isolates were resistant to two or more antimicrobial agents. Interestingly, 26·3% of isolates were resistant to ceftiofur, especially 38·1% of *S.* Infantis isolates, although its use in broilers has not been approved in Japan. This study showed that *Salmonella* is highly prevalent (86·1%) in Japanese broiler flocks, that 90·5% of *Salmonella* isolates were multidrug-resistant, and that *S.* Infantis frequently exhibited resistance to cephalosporin antimicrobial agents.

Key words: Antibiotic resistance, foodborne infections, *Salmonella*, surveillance.

INTRODUCTION

Salmonella is an enteric bacterial pathogen that causes foodborne illness in humans. In Japan, more than 10 000 cases of foodborne salmonellosis were reported annually between 1996 and 1999 [1]. During this period, *Salmonella enterica* subsp. *enterica* serovar Enteritidis (hereafter *S.* Enteritidis) was the most frequently isolated serovar. *S.* Enteritidis infections are frequently associated with the consumption of eggs, therefore the Enforcement Regulations of the Food Sanitation Law were partially amended in 1998 to ensure the safe distribution of raw shell eggs and liquid egg products. Although the number of foodborne salmonellosis cases has significantly decreased since 2000, *Salmonella* remains one of the two main causative agents of bacterial food poisoning [2].

The percentage of *S.* Enteritidis has decreased in *Salmonella* isolates from human salmonellosis cases,

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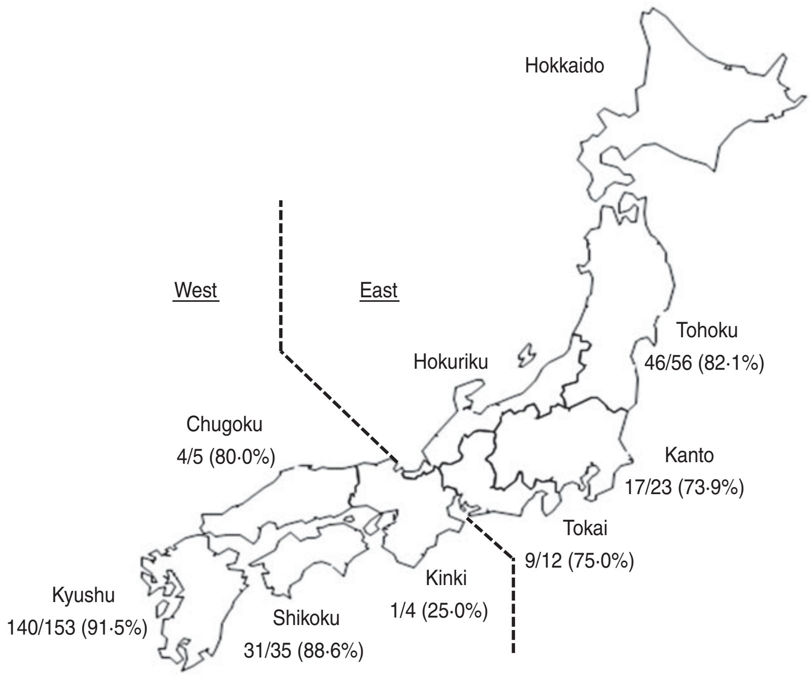

Fig. 1. Map of the study regions. Samples were obtained from seven regions (Tohoku, Kanto, Tokai, Kinki, Chugoku, Shikoku, Kyushu). Below each region label is the number of *Salmonella*-positive flocks and the total number of flocks tested.

whereas the percentage of *S*. Infantis has increased [2]. In Japan, *S*. Infantis is frequently isolated from broilers [3–7], but rarely isolated from beef and dairy cattle and swine [4, 8, 9]. According to Asai *et al*. [10], 94·0% of *S*. Infantis isolates from Japanese broilers and chicken meat products between 2001 and 2003 were resistant to both oxytetracycline (OTC) and dihydrostreptomycin (DSM). Hamada *et al*. [11] reported that *S*. Infantis isolates from humans between 1994 and 2003 were resistant to both streptomycin and tetracycline. We recently reported that *S*. Infantis was isolated from 14 (4·1%) of 338 laying-hen farms in Japan between 2007 and 2008, and 13/14 *S*. Infantis isolates were susceptible to both DSM and OTC [12]. Noda *et al*. [13] reported that the genotypes of *S*. Infantis isolated from humans were similar to those isolated from chicken meat, whereas no common genotypes were found in human and chicken egg isolates. The results of these studies suggest that human *S*. Infantis infection cases in Japan originate from the consumption and handling of broiler meat.

Human salmonellosis is generally self-limiting, so antimicrobial therapy is not usually required. However, invasive infections require treatment with antimicrobial agents. It is important to monitor the rate of antimicrobial resistance in *Salmonella* isolated from broiler farms.

The purpose of this study was to determine the prevalence and antimicrobial susceptibility of *Salmonella* isolated from broiler flocks in Japan.

MATERIALS AND METHODS

Broiler flocks

Fourteen broiler production companies that account for more than 50% of domestic Japanese chicken production participated in this study. Samples were collected from 288 flocks engaged in conventional production between November 2007 and February 2010. A total of 265 broiler farms investigated were located in seven regions of Japan, i.e. Tohoku, Kanto, Tokai, Kinki, Chugoku, Shikoku and Kyushu (Fig. 1). A flock was defined as a group of birds raised in a broiler house during the same period of time.

Samples

Sampling of a selected flock was conducted on a farm only once during the final 2 weeks of the production cycle. In 23 farms, sampling was also conducted at the end of the next production cycle. Five pooled fresh caecal dropping samples were taken from a house in the respective farms. Each pooled fresh caecal

Table 1. *Frequency distribution of* Salmonella *serovars in broiler flocks*

Serovar	No. of *Salmonella*-positive flocks East ($n=91$)	West ($n=197$)	Total ($n=288$)
Infantis	56	120	176
Manhattan	0	34	34
Schwarzengrund	4	24	28
Typhimurium	10	0	10
Nigeria	1	6	7
Brezany	0	3	3
Agona	2	0	2
Anatum	1	0	1
Bareilly	0	1	1
Bovismorbicans	0	1	1
Derby	0	1	1
Enteritidis	0	2	2
Eppendorf	0	1	1
Isangi	1	1	2
Livingstone	1	1	2
Montevideo	0	1	1
Thompson	0	1	1
Untypable	4	8	12
Total	**80**	**205**	**285**

n, Number of flocks tested.
East (Tohoku, Kanto, Tokai); West (Kinki, Chugoku, Shikoku, Kyushu).

dropping sample (~10 g) consisted of caecal droppings from several birds. Samples were placed in plastic vials and transported under refrigeration to the Japan Food Research Laboratories (JFRL) or the Research Institute for Animal Science in Biochemistry and Toxicology (RIAS). The samples were kept refrigerated in the laboratories until examination, which was performed within 48 h of their arrival.

Bacteriological examination

Ten grams of pooled fresh caecal droppings were mixed with 90 ml buffered peptone water (Eiken Chemical, Japan) and maintained at room temperature before being cultured at 35 °C for 22±2 h. Subsequently, 0·1- and 1-ml aliquots of the culture were added to 10 ml Rappaport-Vassiliadis broth (bioMérieux Japan Ltd, Japan) and 10 ml tetrathionate broth (Merck Ltd, Japan), respectively. After incubation at 42 °C for 22±2 h, each culture was streaked onto two selective isolation agar plates, xylose-lysine-deoxycholate agar (bioMérieux) and Brilliant Green agar (bioMérieux). Candidate colonies were biochemically identified. *Salmonella* isolates were tested by slide agglutination with O antisera (Denka Seiken Co., Japan). One of each different O serogroup isolates was stored in tryptone soya broth containing 10% glycerol at −80 °C. All *Salmonella* isolates were sent to RIAS or the Machida Hygiene Control Laboratory (MHCL) for tube agglutination tests using H antisera (Denka Seiken). Serovars were determined on the basis of reaction with O and H group antigens according to the Kauffmann–White method [14].

Antimicrobial susceptibility testing

The minimum inhibitory concentration (MIC) was determined using the agar dilution method of the Clinical and Laboratory Standards Institute (CLSI) [15]. *Escherichia coli* ATCC 25922 was used as the quality control strain. The resistance breakpoints defined by CLSI were used [16]. Breakpoints not defined by CLSI were obtained from a previous report [17]. Antimicrobial susceptibility tests were conducted at RIAS or MHCL.

RESULTS

Salmonella prevalence and serovars

Salmonella was obtained from 248 (86·1%, 95% confidence interval 82·1–90·1) of 288 broiler flocks. The prevalence of *Salmonella* in broiler flocks in each of the investigated regions was greater than 73%, with the exception of Kinki region (Fig. 1). The prevalence (91·5%, 140/153) of *Salmonella* in Kyushu region was statistically ($P=0{\cdot}004$, Fisher's exact test) higher than that (80·0%, 108/135) in the other regions.

In all, 285 isolates were obtained from 248 flocks. They were serotyped into 17 serovars and 12 untypable *Salmonella* (Table 1). The three main serovars were *S.* Infantis, *S.* Manhattan and *S.* Schwarzengrund. *S.* Infantis was the most frequent serovar, and it was isolated from 176 (61·1%) flocks in all seven regions where the study was conducted. *S.* Manhattan and *S.* Schwarzengrund were frequently found only in the western part of Japan.

Sampling was conducted on a second occasion for 23 farms at the end of the next production cycle. Twenty of the 23 farms were *Salmonella*-positive both before and after the production gaps, while the remaining three farms were negative on both occasions. Moreover, *Salmonella* isolates obtained before and after the production gaps were identical in terms of serovars and antimicrobial resistance profiles on 14 (70·0%) of the 20 *Salmonella*-positive farms (data not shown).

Antimicrobial susceptibility

High antimicrobial resistance rates were observed against OTC (90·2%), DSM (86·7%) and ampicillin (ABPC) (36·5%) (Table 2). All of the isolates in this study were susceptible to gentamycin (MIC $\leqslant 1$), apramycin (MIC $\leqslant 8$), enrofloxacin (MIC $\leqslant 1$) and fosfomycin (MIC $\leqslant 32$). Sulphadimethoxine showed no activity against all isolates (MIC $\geqslant 128$). Of the 285 isolates, 258 (90·5%) were resistant to two or more of the antimicrobials tested in this study (Table 3). In particular, 243 (85·3%) isolates were resistant to both DSM and OTC. For *S*. Infantis, 160 (90·9%) of 176 isolates were resistant to both DSM and OTC. With regard to the β-lactam antimicrobial agents [ABPC, cefazolin (CEZ) and ceftiofur (CFT)], 81 (46·0%), 71 (40·3%) and 67 (38·1%) of 176 *S*. Infantis isolates were resistant to ABPC, CEZ and CFT, respectively.

DISCUSSION

Two previous reports of *Salmonella* prevalence in broiler flocks in Japan were based on samples taken in slaughterhouses [6, 18]. Surveillance in Tochigi Prefecture in Kanto region between 1995 and 1997 and in Kagoshima Prefecture in Kyushu region between 1998 and 2003 demonstrated a high prevalence of 64·3% (18/28) and 70·6% (178/252), respectively [6, 18]. Although their results are not comparable to our results due to differences in sampling and isolation methods, *Salmonella* prevalence in broiler flocks could remain high throughout the country. In the present study, *S*. Infantis was the most frequent serovar, isolated from 176 (61·1%) flocks. The prevalence of *Salmonella* was statistically higher in Kyushu region (91·5%), where 45% of Japanese broiler meat production takes place [19], than that in the other regions (80·0%). The most frequently isolated serovar in this region was also found to be *S*. Infantis. Therefore, efforts to reduce human salmonellosis caused by *S*. Infantis should focus on the establishment of control measures in broiler farms, especially, those located in Kyushu region. *Salmonella* isolates obtained before and after the production gaps were identical in terms of serovars and antimicrobial resistance profiles on 14 (70·0%) of the 20 *Salmonella*-positive farms. We recently showed that most broiler farms have adopted appropriate biosecurity measures, such as disinfection of vehicles and equipment before entering the farm, the practice of 'all-in/all-out' management at the farm level, changing of working clothes every day, and disinfection of footwear at each house entrance [20]. These results strongly suggest that the currently adopted biosecurity measures are not sufficiently effective or they have not been implemented properly, thereby allowing the persistence of *Salmonella* on broiler farms. Alternatively, *Salmonella* might be brought to broiler houses after production gaps from reservoirs located in the surrounding environment. Further studies are necessary to identify the mechanism of the persistence or re-introduction of *Salmonella* to broiler farms in Japan, and adequate countermeasures need to be established to address these problems.

Since the late 1990s, *S*. Infantis has been the main serovar isolated from broilers in Japan [3–7]. *S*. Infantis seems to have predominated in broiler flocks in Japan during this decade. Such predominance by *S*. Infantis has not been commonly observed in other countries [21, 22], the reasons for which cannot be explained from currently available information and require further investigation. *S*. Manhattan was the second most common serovar, but it was seldom isolated from broiler flocks before the present study. The National Veterinary Assay Laboratory (NVAL), which has managed the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM) System since 1999, reported that *S*. Manhattan was first obtained from the faecal samples of broilers in 2007 [23]. In the present study, *S*. Manhattan was isolated from broiler flocks located in the western part of Japan during the investigation period. The serovar may have settled in the western part of Japan. Since 2005, *S*. Schwarzengrund was previously detected only in the Kinki, Chugoku and Kyushu regions, which are located in the western part of Japan [23, 24]. *S*. Schwarzengrund isolates had never been detected in the eastern part of Japan before 2009, but four isolates were detected in the Tokai region in 2009 during this study, which is the westernmost region of the eastern part of Japan. These results suggest that this serovar has spread from the western parts to the eastern parts of Japan.

With the exception of the β-lactam antimicrobial agents (ABPC, CEZ, CFT) the patterns of antimicrobial resistance were very similar to those

Table 2. *Antimicrobial susceptibility of* Salmonella *isolates from broiler flocks*

		ABPC (32)*		CEZ (32)		CFT (4)		DSM (32)		KM (64)		OTC (16)		BCM (128)		CP (32)		CL (16)		NA (32)		TMP (16)	
Serovar	*N*	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Infantis	176	81	46·0	71	40·3	67	38·1	160	90·9	47	26·7	169	96·0	1	0·6	1	0·6	0	0·0	14	8·0	67	38·1
Manhattan	34	2	5·9	2	5·9	2	5·9	29	85·3	0	0·0	27	79·4	0	0·0	0	0·0	0	0·0	4	11·8	0	0·0
Schwarzengrund	28	1	3·6	0	0·0	0	0·0	27	96·4	20	71·4	27	96·4	28	100·0	0	0·0	0	0·0	6	21·4	17	60·7
Typhimurium	10	9	90·0	3	30·0	3	30·0	8	80·0	3	30·0	10	100·0	0	0·0	0	0·0	0	0·0	5	50·0	0	0·0
Nigeria	7	2	28·6	2	28·6	2	28·6	6	85·7	1	14·3	7	100·0	0	0·0	0	0·0	0	0·0	1	14·3	4	57·1
Brezany	3	0	0·0	0	0·0	0	0·0	3	100·0	3	100·0	3	100·0	3	100·0	0	0·0	0	0·0	1	33·3	3	100·0
Agona	2	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0
Anatum	1	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0
Bareilly	1	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0
Bovismorbicans	1	0	0·0	0	0·0	0	0·0	1	100·0	1	100·0	1	100·0	0	0·0	0	0·0	0	0·0	0	0·0	1	100·0
Derby	1	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0
Enteritidis	2	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	1	50·0	0	0·0	0	0·0	2	100·0	0	0·0	0	0·0
Eppendorf	1	0	0·0	0	0·0	0	0·0	1	100·0	1	100·0	1	100·0	1	100·0	0	0·0	0	0·0	0	0·0	1	100·0
Isangi	2	1	50·0	1	50·0	1	50·0	1	50·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0
Livingstone	2	0	0·0	0	0·0	0	0·0	1	50·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0
Montevideo	1	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	1	100·0	0	0·0	0	0·0	0	0·0	0	0·0
Thompson	1	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0	0	0·0
Untypable	12	8	66·7	2	16·7	0	0·0	10	83·3	5	41·7	11	91·7	0	0·0	0	0·0	0	0·0	0	0·0	3	25·0
Total	**285**	**104**	**36·5**	**81**	**28·4**	**75**	**26·3**	**247**	**86·7**	**81**	**28·4**	**257**	**90·2**	**34**	**11·9**	**1**	**0·4**	**2**	**0·7**	**31**	**10·9**	**96**	**33·7**

* Values in parentheses are the minimum inhibitory concentration (mg/l) of the breakpoint.
ABPC, Ampicillin; CEZ, cefazolin; CFT, ceftiofur; DSM, dihydrostreptomycin; KM, kanamycin; OTC, oxytetracycline; BCM, bicozamycin; CP, chloramphenicol; CL, colistin; NA, nalidixic acid; TMP, trimethoprim.

Table 3. *Antimicrobial resistance profiles of* Salmonella *isolates* (*n* = *285*)

Antimicrobial resistance profile	*n*	Serovars
Susceptible	15	Agona (2), Anatum (1), Bareilly (1), Derby (1), Infantis (1), Isangi (1), Livingstone (1), Manhattan (5), Thompson (1), Untypable (1)
DSM	3	Manhattan (2), Livingstone (1)
KM	2	Infantis (2)
OTC	3	Infantis (1), Typhimurium (1), Untypable (1)
BCM	2	Schwarzengrund (1), Montevideo (1)
CL	1	Enteritidis (1)
TMP	1	Infantis (1)
DSM + OTC	58	Infantis (35), Manhattan (21), Nigeria (1), Untypable (1)
KM + TMP	1	Infantis (1)
OTC + CL	1	Enteritidis (1)
KM + OTC	3	Infantis (2), Nigeria (1)
ABPC + CEZ + CFT	1	Infantis (1)
ABPC + DSM + OTC	4	Infantis (2), Untypable (2)
DSM + KM + OTC	14	Infantis (13), Untypable (1)
DSM + OTC + BCM	1	Schwarzengrund (1)
DSM + OTC + NA	8	Infantis (4), Manhattan (4)
DSM + OTC + TMP	15	Infantis (13), Nigeria (2)
ABPC + CEZ + CFT + DSM	1	Isangi (1)
ABPC + CEZ + CFT + OTC	1	Infantis (1)
ABPC + CEZ + CFT + TMP	1	Infantis (1)
ABPC + CEZ + DSM + OTC	3	Infantis (2), Untypable (1)
ABPC + DSM + KM + OTC	6	Infantis (4), Untypable (1), Typhimurium (1)
ABPC + DSM + OTC + NA	3	Typhimurium (3)
ABPC + DSM + OTC + TMP	3	Infantis (2), Untypable (1)
ABPC + KM + OTC + NA	1	Typhimurium (1)
DSM + KM + OTC + NA	1	Infantis (1)
DSM + KM + OTC + BCM	4	Schwarzengrund (4)
DSM + KM + OTC + TMP	18	Infantis (17), Bovismorbificans (1)
DSM + OTC + NA + TMP	4	Infantis (3), Nigeria (1)
DSM + OTC + BCM + NA	3	Schwarzengrund (3)
DSM + OTC + BCM + TMP	1	Infantis (1)
ABPC + CEZ + CFT + DSM + OTC	39	Infantis (33), Manhattan (2), Nigeria (1), Typhimurium (3)
ABPC + CEZ + CFT + OTC + NA	1	Infantis (1)
ABPC + CEZ + CFT + OTC + TMP	4	Infantis (4)
ABPC + CEZ + DSM + KM + OTC	3	Infantis (2), Untypable (1)
ABPC + DSM + KM + OTC + BCM	1	Schwarzengrund (1)
ABPC + DSM + KM + OTC + NA	2	Infantis (1), Typhimurium (1)
ABPC + DSM + KM + OTC + TMP	2	Untypable (2)
DSM + KM + OTC + BCM + NA	1	Schwarzengrund (1)
DSM + KM + OTC + BCM + TMP	17	Schwarzengrund (14), Brezany (2), Eppendorf (1)
DSM + OTC + BCM + NA + TMP	3	Schwarzengrund (3)
ABPC + CEZ + CFT + DSM + OTC + NA	3	Infantis (3)
ABPC + CEZ + CFT + DSM + OTC + TMP	20	Infantis (19), Nigeria (1)
ABPC + DSM + KM + OTC + NA + TMP	1	Infantis (1)
DSM + KM + OTC + BCM + NA + TMP	1	Brezany (1)
ABPC + CEZ + CFT + DSM + KM + OTC + TMP	3	Infantis (3)
ABPC + CEZ + CFT + DSM + OTC + CP + TMP	1	Infantis (1)

ABPC, Ampicillin; BCM, bicozamycin; CEZ, cefazolin; CFT, ceftiofur; CL, colistin; CP, chloramphenicol; DSM, dihydrostreptomycin; KM, kanamycin; NA, nalidixic acid; OTC, oxytetracycline; TMP, trimethoprim.

reported in previous studies [4, 7]. Resistance rates against β-lactam antimicrobial agents were higher than those reported in previous studies. In particular, the resistance rates against CEZ (a first-generation cephalosporin) and CFT (a third-generation cephalosporin) were notable because cephalosporins have not been approved for poultry disease treatment in Japan, under the Japanese Pharmaceutical Affairs Law. Ishihara *et al*. [7] reported that two (1·8%) of 111 isolates from broilers were resistant to CFT in 1999. According to JVARM, two (4·3%) of 47 *Salmonella* were resistant to CEZ in isolates from broilers during 2006, but no *Salmonella* resistant to CEZ were isolated between 2000 and 2005 [23]. Shahada *et al*. [25] recently reported that *S*. Infantis resistance to cephalosporins has emerged in broilers from 2005 based on a survey conducted at a slaughterhouse between 2004 and 2006, where 11 (9·1%) of 120 *S*. Infantis isolates were found to be resistant to cefotaxime (a third-generation cephalosporin). However, none of the 135 *S*. Infantis isolates sampled from broilers at a slaughterhouse between 1998 and 2003 were found to be resistant [6]. In this study, 71 (40·3%) and 67 (38·1%) of 176 *S*. Infantis isolates were resistant to CEZ and CFT, respectively. These results suggest that cephalosporin resistance rates have increased in *Salmonella* isolated from broilers since 2006. *S*. Infantis resistant to cephalosporins might be already causing human salmonellosis via broiler meat consumption although there is no way to gauge the real impact on human health due to lack of studies on antimicrobial susceptibility of *S*. Infantis in human salmonellosis cases. Therefore, the impact of a growing resistance to cephalosporins in broilers cannot be dismissed. Further studies are needed to clarify the reasons why *Salmonella* resistance to cephalosporins is prevalent in broiler flocks in Japan.

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DECLARATION OF INTEREST

None.

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