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FULL PAPER

Epidemiology

Evaluating the antimicrobial use on dairy farms in Chiba Prefecture in Japan using the antimicrobial treatment incidence, an indicator based on Japanese defined daily doses from 2014–2016

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ABSTRACT. The use of antimicrobial agents in food-producing animals may lead to the emergence and spread of antimicrobial resistance in bacteria of animal origin. However, there is a paucity of data on the quantity of antimicrobials use on dairy farms in Japan. This study describes antimicrobial use on dairy farms from 1 January 2014 to 31 December 2016 in five administrative districts (central, eastern, western, southern and northern) of Chiba Prefecture. The use of antimicrobial agents in dairy cattle over these three years was evaluated in terms of the antimicrobial treatment incidence (ATI; theoretical number of animals per 1,000 animal-days subjected to antimicrobial treatment) using data collected from a total of 442 dairy farms in that prefecture. Our results revealed that the average ATI on these farms for these years ranged from 38.7 to 39.4 with no significant difference between years and that the average ATI for these administrative districts varied between 32.9 and 43.2 with a significant variation between some of the districts. Approximately 84% of antimicrobials were administered intramammarily, 13–14% by injection and 1–2% orally. Scenario analyses were performed to assess the effect of changes in some of the defined daily dose (DDDjp) values used to calculate the ATI. Our results revealed that the calculated ATI is considerably affected by the changes in the long-acting factor used for assigning the DDDjp values of intramammary products for dry cows and the way in which DDD values are assigned for combination products.

KEYWORDS: antimicrobial treatment incidence (ATI), antimicrobial use, defined daily dose (DDD), dry cow therapy (DCT), mastitis

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The use of antimicrobials in humans and animals is considered a primary cause of antimicrobial resistance in bacteria [24]. Currently, 700,000 people die of antimicrobial resistant infections every year. If no proactive solutions are taken to reduce the rise of drug resistance, by 2050, some 10 million lives could be at risk each year from drug-resistant infections [36]. Bacterial resistance arises through complex mechanisms, like mutations or the acquisition of genetic information that encodes resistance from other bacteria and is selected by antimicrobial exposure [16]. Therefore, diminishing the selection pressure by reducing antimicrobial use is considered to be one of the important strategies to prevent and control the emergence and spread of antimicrobial resistance [16]. Dairy farms constitute an ideal environment in which bacteria are subjected to antimicrobial exposure, and the subsequent selection pressure might favor selection and dissemination of resistant strains [1, 44, 49, 51]. The loss of efficacy of antimicrobials due to the presence of resistant bacteria, as seen in human medicine, will also arise in veterinary medicine. Therefore, prudent use of antimicrobials to prevent

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the emergence of antimicrobial resistance is not only important from a public health perspective but also for animal health and welfare.

On dairy farms in Japan, antimicrobials are administered mostly by veterinarians and partly by farmers under prescription or instruction by veterinarians, subject to the guidelines on prudent use issued by the Ministry of Agriculture, Forestry and Fisheries in 2013 [29]. Under these guidelines, veterinarians are supposed to use antimicrobials based on diagnosis using sensitivity test where possible and second-choice antimicrobials (3rd generation cephalosporins, macrolides, polymyxins and quinolones) only when first choice antimicrobials failed to take effect [31]. The 4th and 5th generation cephalosporins are not allowed to be used in cattle in Japan [31].

There is no global consensus on the collection of antimicrobial use data and reporting methods but many activities in this field are in progress [4]. Under the European Surveillance for Veterinary Antimicrobial Consumption (ESVAC) project of the European Medicines Agency (EMA), the European Union (EU) member states routinely report total quantities of antimicrobials sold for use in food-producing animals as mg of active ingredient, adjusted by animal biomass (population correction unit: PCU) [8], allowing for comparison between member states. The use of antimicrobial agents in food-producing animals in Japan has been investigated previously in terms of mg of active ingredient sold per kg of biomass using sales data at national level obtained from market authorization holders [26, 47]. This metric is simple to calculate and easy to understand. However, the use of this metric might encourage favoring the use of high potency antimicrobials for their lower mg per dose [28].

In Denmark, the Netherlands and some other European countries and Canada, dosage-based indicators are used to monitor antimicrobial usage at the farm level [22, 34, 42, 45]. In the Netherlands, the animal daily defined dose of antimicrobials per farm per year ($DDDA_F$), a dosage-based indicator representing the number of treatment days per year per animal is used to monitor antimicrobial use at farm level. This indicator is calculated by dividing the number of DDDs (quantity of treated biomass) by the average number of kilograms of animals on the farm [34]. In Denmark, the number of defined animal daily doses (ADDs), an indicator denoting the average number of treated animals per 100 animal-days is used to monitor antimicrobial use at farm level. This indicator is calculated by dividing the number of treated animals per month by the number of animal-days in that month and multiplying it by 100 days [45].

Dose-based indicators have the advantage of correcting the dosage differences between active ingredients and formulations and measuring developments over time, despite changes in which active ingredients are used [18]. In 2016, the EMA published average defined daily dose (DDDvet) values for antimicrobial agents used in food-producing animals as a tool to facilitate the standardized collection and presentation of antimicrobial use among EU member states [9]. These values were defined by calculating the mean dose of antimicrobial products registered in nine EU member states. The DDDvet values were assigned in mg of active ingredient per kg of body weight per day (mg/kg/day) for antimicrobials for oral and injection use, and the DDDvet values for intramammary and intrauterine antimicrobials were assigned in unit dose per teat, udder and animal (UD/teat, UD/udder and IUP/animal) [9]. In Canada, DDD values (DDDbovCA) were assigned for antimicrobials by administration route (injectable, oral, intramammary, intrauterine and topical) with the objective of establishing a harmonized and transparent quantification system of antimicrobial use in cattle [23]. The Canadian team defined DDD values for some of the antimicrobials for which the EMA had not assigned DDD values in mg/kg/day including those for lactating and dry-cow products and intrauterine products, with an idea to propose DDD values for all antimicrobials without exception in order to include them in antimicrobial usage reports using a daily-based indicator [23].

To establish a monitoring system using an indicator based on daily dosage in Japan, the DDD values (DDDjp) for veterinary antimicrobial agents used in antimicrobial products approved and marketed for use in cattle in Japan have been established recently [12].

This study aims to describe the antimicrobial use on dairy farms in Chiba Prefecture in Japan from 2014–2016 using the antimicrobial treatment incidence (ATI), an indicator based on Japanese daily doses (DDDjp) and presenting a theoretical number of animals per 1,000 animal-days subjected to antimicrobial treatment on the farm [6, 46, 50]. To the best of our knowledge, this is the first study to elucidate antimicrobial use at the farm level on Japanese dairy farms employing a dose-based indicator. We also investigated the temporal and geographical variations in antimicrobial use and variations by farm size, using a liner mixed effects model. As DDD values vary greatly depending on the assumed efficacy duration of long-acting antimicrobials such as intramammary antimicrobial products for dry cows and on the way in which DDD values are assigned for combination products [22], we performed scenario analyses. By using these analyses, we aimed to assess the effect of changes in some of the DDDjp values assigned for intramammary antimicrobial products for dry cows and combination products on ATI.

MATERIALS AND METHODS

Dairy farms subjected to the analysis in this study

The present study was approved by the Animal Research Ethics Committee of Chiba Prefectural Agricultural Mutual Aid Association (NOSAI Chiba) (approval number: CNS140101). NOSAI is a nationwide agricultural insurance scheme established and financially supported by the Japanese government. This scheme provides contracted farmers of dairy and beef cattle, horse and breeding pigs with life insurance for dead and culled animals as well as veterinary service. Chiba is one of the 47 prefectures of Japan located in the Kanto region (Fig. 1). There were 720 dairy farms keeping 25,100 cows as of February 2016 in Chiba Prefecture [30]. These farms supply consumers in Tokyo and other prefectures in the Kanto region with milk and dairy products. NOSAI Chiba has eight veterinary clinics in five districts in Chiba Prefecture. These clinics had contracts to provide veterinary services to 596 farms as of December 2016. Of these contracted farms, 442 farms for which data on antimicrobial use and number of cows were available throughout the years 2014–2016 were selected and analyzed in this study. The antimicrobials used on these farms were provided exclusively by the veterinary clinics of NOSAI Chiba, either during direct treatment or through prescriptions by NOSAI Chiba veterinarians.

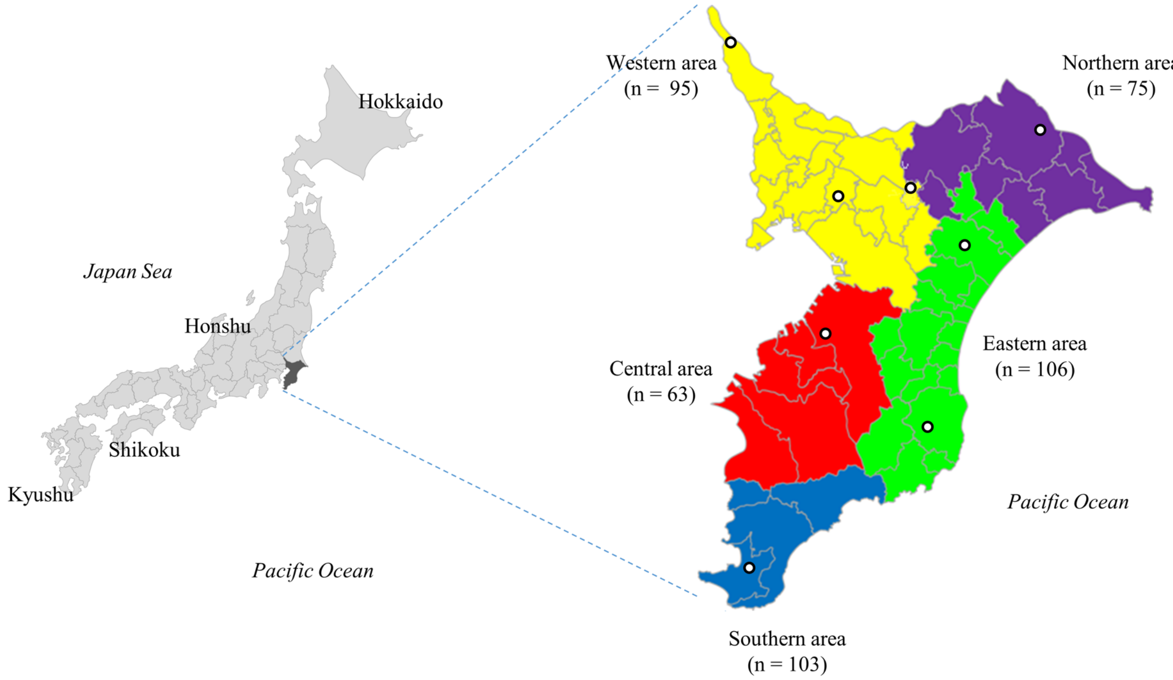

Fig. 1. Location of Chiba Prefecture and its five jurisdictional districts. Open circles indicate the location of NOSAI Chiba veterinary clinics. The numbers of farms in each district analyzed in this study are shown in parentheses. Blank map used for preparation of this figure was reprinted from https://www.freemap.jp/ under a CC BY license, with permission from Keisuke Inoue, original copyright 2006.

Database

A database was constructed by entering, for each farm, the data on the use of antimicrobials for 2014, 2015 and 2016 by antimicrobial agent and administration route (injection, intramammary, oral and intrauterine) in mg of active ingredient. In addition, data on the average number of cows kept on each farm and the administrative district (central, eastern, western, southern and northern) connected to the farm ID were entered into the database (see Fig. 1 for the locations of these districts and the number of farms in each district included in the analysis). The latter was calculated by adding the number of cows over two years old present on the farm at the beginning of every 21-day period (i.e., the average length of the bovine estrous cycle) and dividing the sum by 18 (the number of cycles in a year).

Indicator used to measure antimicrobial use on each dairy farm

The ATI was used as an indicator to measure the amount of antimicrobial use on each dairy farm. This indicator presents a theoretical number of animals per 1,000 animal-days subjected to antimicrobial treatment on the farm, assuming that the antimicrobial products are used in a cow of standard weight according to the dosage specified in the summary of product characteristics. To determine this indicator, we first calculated the number of DDDjps of antimicrobial agent (a) administered by route (r) on farm (i) in year (y) by dividing the weight of active ingredient of the antimicrobial agent (a), as follows:

$$\text{Number of DDDs}_{a,r,i,y}\ (\text{kg}\cdot\text{days}) = \frac{\text{Weight of active ingredient of antimicrobial agent } a \text{ administered by route } r \text{ in year } y \text{ on farm } i \text{ (mg)}}{\text{DDD value of the antimicrobial agent } a \text{ administered by route } r \text{ (mg/kg/day)}}$$

where the DDDjp value of the antimicrobial agent (a) administered by route (r) was available from the list of Japanese DDDjp values of antimicrobial agents established by Fujimoto *et al*. [12]. In preparing this list, the DDDjp values for intramammary products for lactating cows and intrauterine products were assigned by dividing the daily dose per teat by 635 kg (standard weight of dairy cows assigned based on the average weight of dairy cows in 2014 in Japan [25]). The DDDjp values for intramammary products for dry cows were assigned by multiplying the course dose per teat by four (number of teats per cow), following the principles applied by EMA [10], and dividing it by 635 kg and an assumed long-acting factor of four days based on expert opinions [13]. The DDDjp values were assigned separately to each constituent antimicrobial agent that make up combination products (Supplementary Tables 1 and 3).

Then the ATI for an antimicrobial agent (a) administered by route (r) on farm (i) in year (y) was calculated by dividing the number of $\text{DDDjps}_{a,r,i,y}$ by the average number of cows on farm (i) in year (y) and the standard weight of dairy cow (635 kg) as follows:

$$\text{ATI}_{a,r,i,y} = \frac{\text{Number of DDDs}_{a,r,i,y}\ (\text{kg}\cdot\text{days})}{\text{Average number of cows on the farm (animals)}\times 635(\text{kg})\times 365(\text{days})} \times 1{,}000 \text{ (animals)}$$

The ATI for administration route (r) on farm (i) in year (y) was calculated by summing up ATI $_{y,r,a,i}$ for each administration route over different antimicrobial agents, as follows:

$$ATI_{r,i,y} = \sum_{a} ATI_{a,r,i,y}$$

The overall ATI on farm (i) for year (y) was calculated by summing up $ATI_{r,i,y}$ over different administration routes, as follows:

$$ATI_{i,y} = \sum_{r} ATI_{r,i,y}$$

where r is a different administration route (intramammary, injection, oral and intrauterine).

Statistical analysis

Before subjecting the data to statistical analysis, we performed a square-route transformation of the antimicrobial use data to correct the right skewedness and normality assumptions of this variable. Then in order to analyze which factors are associated with the ATI at farm level, a linear mixed-effects model was built using the herd size (average number of cows on each farm), the year (2014, 2015 and 2016) and the administrative districts (central, eastern, western, southern and northern) as independent variables with fixed effects. A random effect for farm was included in the model, to account for the existence of three-year observations within farms. We applied a backward stepwise selection procedure to identify a model with highest predictability based on AIC values. For multiple comparisons, Bonferroni adjustment was made by dividing the alpha-levels by the number of comparisons and using the value so calculated as the P-value for determining significance. Confidence intervals of the coefficient estimate were also adjusted to match the adjusted alpha-level.

In the scenario analysis, comparisons between the effects on the ATI caused by the changes in some of the DDDjp values were evaluated using scatterplots and correlation analysis performed by Spearman's Rho test after the data were tested and denied for normality by Shapiro-Wilk's test.

All statistical analyses were conducted using R Statistical Software (version 3.6.1; R Foundation for Statistical Computing, Vienna, Austria). The lme4 package in R was used to fit the linear mixed-effect model.

RESULTS

Antimicrobial use of the 442 dairy farms analyzed in this study

The average antimicrobial use on the 442 farms in the Chiba Prefecture in terms of ATI by antimicrobial agent and administration route over three years are shown in Fig. 2 and Table 1. Distributions of the number of cows and ATI of the 442 dairy farms are shown in Supplementary Table 4. The average antimicrobial use on 442 dairy farms in terms of ATI in 2014, 2015 and 2016 were 38.7 (standard deviation (SD)=21.8), 38.9 (SD=20.3) and 39.4 (SD=20.3), respectively (Table 1 and Supplementary Table 4). Approximately 84% of

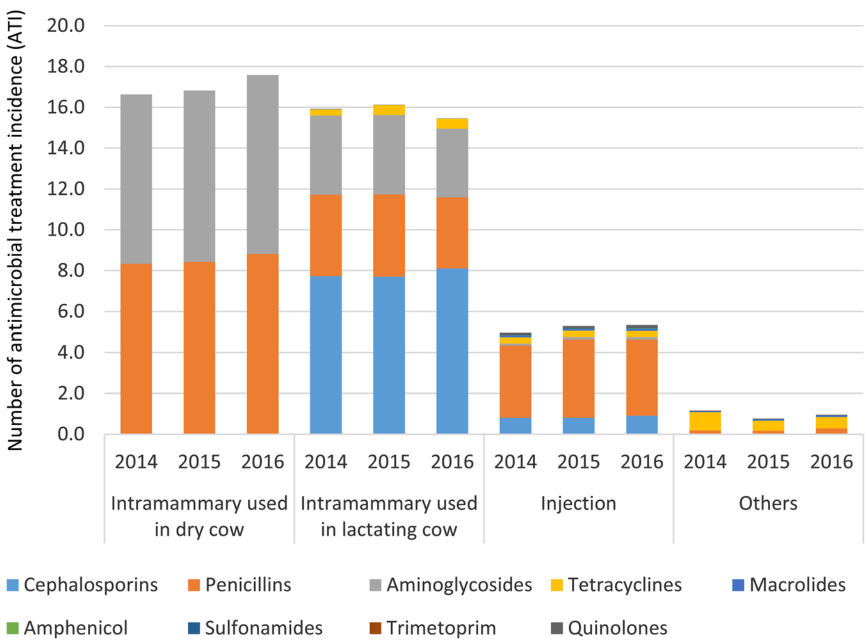

Fig. 2. The average of antimicrobial use of the 442 farms in Chiba Prefecture in Japan in terms of the antimicrobial treatment incidencs (ATI) by antimicrobial class and administration route in 2014, 2015 and 2016. Intrauterine and oral route are categorized as others.

Table 1. Average and median of antimicrobial use of the 442 farms in Chiba Prefecture in Japan in terms of the antimicrobial treatment incidence (ATI) by antimicrobial agent and administration route in 2014–2016

Antimicrobial class	Antimicrobial agent	Administration route	Antimicrobial treatment incidence (ATI) (proportion in percentage)								
			2014			2015			2016		
			Mean		Median	Mean		Median	Mean		Medxan
Tetracyclines	Oxytetracycline	Intramammary	0.34	(0.9%)	0.00	0.51	(1.3%)	0.00	0.47	(1.2%)	0.00
		Oral	0.47	(1.2%)	0.00	0.27	(0.7%)	0.00	0.53	(1.3%)	0.00
		Injection	0.28	(0.7%)	0.15	0.28	(0.7%)	0.08	0.30	(0.8%)	0.14
	Chlortetracycline	Intrauterine	0.40	(1.0%)	0.12	0.16	(0.4%)	0.00	0.05	(0.1%)	0.00
Amphenicol	Florfenicol	Injection	0.02	(0.05%)	0.00	0.01	(0.03%)	0.00	0.02	(0.05%)	0.00
Penicillins	Amoxicillin	Injection	0.00003	(0.0001%)	0.00	0.0002	(0.0005%)	0.00	0.0003	(0.001%)	0.00
	Ampicillin	Injection	2.62	(6.8%)	2.32	2.87	(7.4%)	2.48	2.93	(7.4%)	2.63
		Intrauterine	0.12	(0.3%)	0.00	0.12	(0.3%)	0.00	0.15	(0.4%)	0.00
		Oral	0.08	(0.2%)	0.00	0.07	(0.2%)	0.00	0.09	(0.2%)	0.00
	Diclo-xacillin	Intramammary	0.09	(0.2%)	0.00	0.13	(0.3%)	0.00	0.12	(0.3%)	0.00
	Mecillinam	Injection	0.03	(0.08%)	0.00	0.03	(0.08%)	0.00	0.03	(0.08%)	0.00
	Procaine benzylpenicillin	Intramammary (used in lactating cow)	3.89	(10.0%)	1.93	3.90	(10.0%)	1.91	3.37	(8.6%)	1.60
		Intramammary (used in dry cow)	8.33	(21.5%)	9.20	8.42	(21.6%)	9.60	8.81	(22.4%)	9.69
		Injection	0.90	(2.3%)	0.47	0.95	(2.4%)	0.52	0.78	(2.0%)	0.44
Sulfonamides	Sulfadimethoxine	Injection	0.02	(0.05%)	0.00	0.03	(0.08%)	0.00	0.03	(0.08%)	0.00
	Sulfa-monomethoxine	Oral	0.0008	(0.002%)	0.00	0.00	(0.0%)	0.00	0.004	(0.01%)	0.00
		Injection	0.00005	(0.0001%)	0.00	0.00	(0.0%)	0.00	0.00	(0.0%)	0.00
Macrolides	Erythromycin	Intramammary	0.02	(0.05%)	0.00	0.007	(0.02%)	0.00	0.007	(0.02%)	0.00
	Tilmicosin	Oral	0.07	(0.2%)	0.00	0.07	(0.2%)	0.00	0.10	(0.3%)	0.00
		Injection	0.004	(0.01%)	0.00	0.004	(0.01%)	0.00	0.007	(0.02%)	0.00
	Tylosin	Injection	0.08	(0.2%)	0.00	0.08	(0.2%)	0.00	0.09	(0.2%)	0.00
Aminoglycosides	Dihydro-streptomycin	Intramammary (used in lactating cow)	3.15	(8.1%)	1.59	3.26	(8.4%)	1.51	3.10	(7.9%)	1.35
		Intramammary (used in dry cow)	8.30	(21.4%)	9.17	8.40	(21.6%)	9.57	8.78	(22.3%)	9.66
		Injection	0.09	(0.2%)	0.00	0.12	(0.3%)	0.01	0.13	(0.3%)	0.03
	Kanamycin	Intramammary	0.74	(1.9%)	0.00	0.63	(1.6%)	0.00	0.26	(0.7%)	0.00
		Injection	0.021	(0.05%)	0.00	0.02	(0.05%)	0.00	0.02	(0.05%)	0.00
Cephalosporins	Cefapirin	Intramammary	0.029	(0.07%)	0.00	0.002	(0.005%)	0.00	0.001	(0.004%)	0.00
	Cefazolin	Intramammary	7.05	(18.2%)	4.23	7.13	(18.3%)	4.45	7.43	(18.9%)	5.01
		Injection	0.79	(2.0%)	0.50	0.77	(2.0%)	0.61	0.92	(2.3%)	0.66
	Ceftiofur	Injection	0.008	(0.02%)	0.00	0.006	(0.01%)	0.00	0.004	(0.01%)	0.00
	Cefuroxime	Intramammary	0.70	(1.8%)	0.00	0.57	(1.5%)	0.00	0.69	(1.8%)	0.00
Trimetoprim	Ormetoprim	Oral	0.0009	(0.002%)	0.00	0.004	(0.01%)	0.00	0.004	(0.01%)	0.00
Quinolones	**Danofloxacin**	Injection	0.0002	(0.0005%)	0.00	0.007	(0.02%)	0.00	0.0007	(0.002%)	0.00
	Enrofloxacin	Injection	0.06	(0.2%)	0.00	0.07	(0.2%)	0.00	0.13	(0.3%)	0.00
	Marbofloxacin	Injection	0.00009	(0.0002%)	0.00	0.00	(0.0%)	0.00	0.00	(0.0%)	0.00
	Orbifloxacin	Injection	0.03	(0.08%)	0.00	0.03	(0.08%)	0.00	0.04	(0.1%)	0.00
Subtotal of intramammary products used in dry cows			16.63	(42.9%)	18.38	16.82	(43.2%)	19.16	17.59	(44.7%)	19.35
Subtotal of intramammary products used in lactating cows			16.01	(41.3%)	11.15	16.14	(41.5%)	11.92	15.45	(39.2%)	10.77
Total			38.72	(100.0%)	35.51	38.94	(100.0%)	36.18	39.38	(100.0%)	36.76

Antimicrobials in bold letters are Highest Priority Critically Important Antimicrobials (HPCIAs) which are only allowed to be used as second-choice antimicrobials in Japan.

antimicrobials were administered intramammarily, followed by injection (13–14%), oral use (1–2%) and intrauterine use (1%). The antimicrobial agent most frequently used in the intramammary route was procaine benzylpenicillin with an ATI of 12.2–12.3, followed by dihydrostreptomycin (ATI of 11.5–11.9), cefazolin (ATI of 7.1–7.4), cefuroxime (ATI of 0.6–0.7), kanamycin (ATI of 0.3–0.7) and tetracycline (ATI of 0.3–0.5). The antimicrobial agent most frequently used for injection was ampicillin (ATI of 2.6–2.9), procaine benzylpenicillin (ATI of 0.8–1.0), and cefazolin (ATI of 0.8–0.9). The antimicrobial agents used orally were oxytetracycline (ATI of 0.3–0.5), followed by ampicillin, sulfamonomethoxine, tilmicosin and ormetoprim. The average ATI of antimicrobials used for dry cow therapy in 2014, 2015 and 2016 were 16.6, 16.8 and 17.6 respectively, representing 42.9–44.7% of the total use. Antimicrobials in bold letters are Highest Priority Critically Important Antimicrobials (HPCIAs) which are only allowed to be used as second-choice antimicrobials in Japan [31].

Result of the regression analysis

After square-route transformation, the antimicrobial use data was corrected for skewness and normality assumptions. The linear mixed-effect model using square-route transformed ATI as dependent variable and the herd size (average number of cows), year and administrative districts as independent variables with fixed effects and farm as random effects resulted in the interclass correlation coefficient (ICC) for farm 0.779 (95% confidence interval: 0.747–0.808) and no significant effect (P>0.05) from herd size and year on the ATI (Table 2).

After a backward stepwise selection procedure, the following model with the administrative district as fixed effect and farm as random effect independent variables was identified as the one with the highest predictability based on AIC value:

ATI ~ administrative district + (1 | farm ID)

This model revealed significant difference between western and southern districts (P<0.01) and between eastern and southern districts (P<0.001) in terms of their effect on antimicrobial use at farm level (Fig. 3 and Supplementary Table 5). Antimicrobial use in 2014, 2015 and 2016 showed a large variation between farms particularly in the eastern, western and northern districts, with a positive skewed distribution with several farms with high ATI affecting the mean value (Supplementary Table 4).

Table 2. Results of the regression analysis investigating the effect of administrative districts on the antimicrobial treatment incidence (ATI) # of the 442 dairy farms using a linear mixed-effect model with farm as random effect variable

Fixed effects	Coefficient estimate	Standard Error	95% Confidence interval	*P*-value
(Intercept)	5.734	0.224	5.286–6.182	<2e-16***
Farm size (number of cows)	0.0005	0.003	–0.006–0.007	0.883
Yesr (reference: 2014)				0.285
2015	0.028	0.053	–0.078–0.133	0.604
2016	0.083	0.053	–0.023–0.189	0.119
Administrative district (reference: Central)				0.0002***
Eastern	0.594	0.241	0.111–1.076	0.014*
Northern	0.259	0.259	–0.260–0.777	0.319
Southern	−0.278	0.243	–0.208–0.763	0.253
Western	0.510	0.247	0.016–1.003	0.039*

Square-route transformed data of the ATI were used for the analysis. ***: P<0.001, **: P<0.01, *: P<0.05, .: P<0.1.

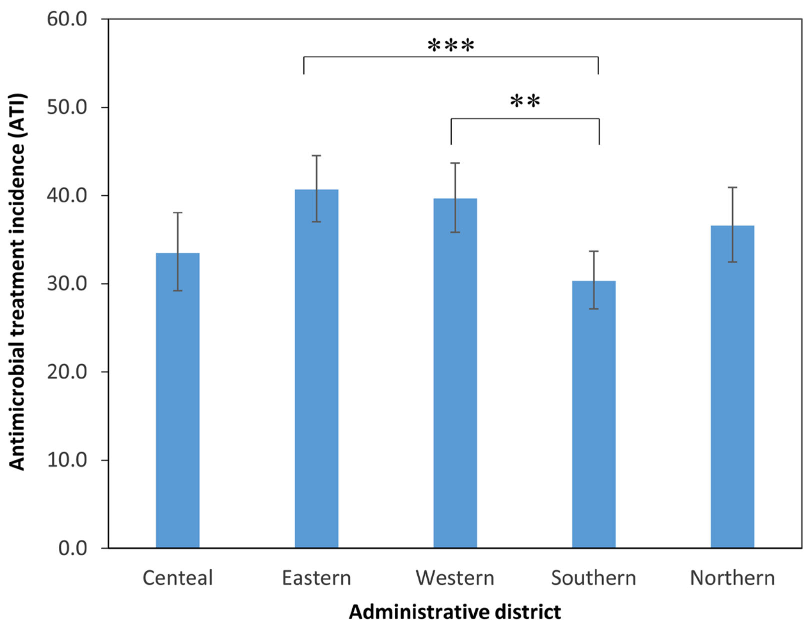

Fig. 3. The antimicrobial treatment incidence (ATI) in 2014 to 2016 on dairy farms in different administrative districts (central, eastern, western, southern and northern) of Chiba Prefecture, based on the result of regression analysis using a linear mixed-effect model. The details of the results are shown in Supplementary Table 5. The error bars represent 95% confidence interval. Single, double and triple asterisk indicates a statistically significant difference with P<0.05, P<0.01 and P<0.001, respectively.

Result of the scenario analysis

Changes in the long-acting factor used to assign DDDjp values for intramammary products for dry cows produced a notable change in the relative distribution of antimicrobial use by antimicrobial class (Fig. 4a) and in the average ATI (Fig. 4b). The proportion of aminoglycosides increased while the proportions of cephalosporins and tetracyclines decreased, and the ATI of most antimicrobial classes, in particular penicillins, aminoglycosides and cephalosporins increased as the number of days used as long-acting factor increased. The average ATI increased more than three times as the number of days used as long-acting factor increased from one to ten.

Changes in the long-acting factor in assigning DDDjp values for intramammary products for dry cows had a different extent of effect on the ATI at farm level, resulting in changes in the places of some farms in their ranking (Supplementary Fig. 1).

The effect of the changes in the way in which DDDjp values are assigned for combination products is shown in Fig. 5. By using single DDDjp values for combination products instead of assigning separate DDDjp values for constituent antimicrobial agents, the numbers of ATI decreased by 0.73 times. The extent of this effect differs between farms, resulting in changes in the places of some farms in their ranking (Supplementary Fig. 2).

DISCUSSION

This study is the first attempt to describe antimicrobial use at farm level on Japanese dairy farms using a dosage-based indicator. This study provides an overview of antimicrobial use in one prefecture in Japan. Chiba Prefecture is one of the major dairy production prefectures in Japan (ranked fourth in terms of the quantity of milk produced). The average herd size of the dairy farms analyzed in this study was 35.6 cows (Supplementary Table 4), which is similar to the average herd size in mainland Japan (38.0 cows as of February 2016 [30]). Despite these facts, the dairy farms analyzed in this study might not be representative of the national dairy herds, and therefore the findings of this study on Chiba Prefecture cannot be generalized to the dairy herds in Japan. However, this study provides valuable insights into understanding the antimicrobial use in Japanese dairy production.

This study revealed that the most common administration route was intramammary (84%). This is consistent with previous studies which reported that mastitis is the primary reason for antimicrobial use in dairy cattle [38, 46]. Penicillin, dihydrostreptomycin and cefazolin were the most commonly used antimicrobials with this administration route. At the time when the data were collected for this study, blanket dry cow treatment (BDCT), a prophylactic method for drying-off cows by administrating intramammary antimicrobials to all cows kept on the farm, was commonly practiced in Japan, contributing to the increased use of antimicrobials on dairy farms. The selective dry cow treatment (SDCT), which has been commonly practiced in European countries since the early 2010s [2, 7, 20], was only introduced into Japan in the late 2010s. As the SDCS is a treatment that can effectively reduce the antimicrobial use on dairy farms by restricting the administration of intramammary antimicrobials only to mastitic udders at dry-off [41, 43], the replacement of BDCT with SDCT might result in the reduced use of antimicrobials.

This study also revealed that ampicillin, penicillin and cefazolin were the antimicrobials most commonly used for injection.

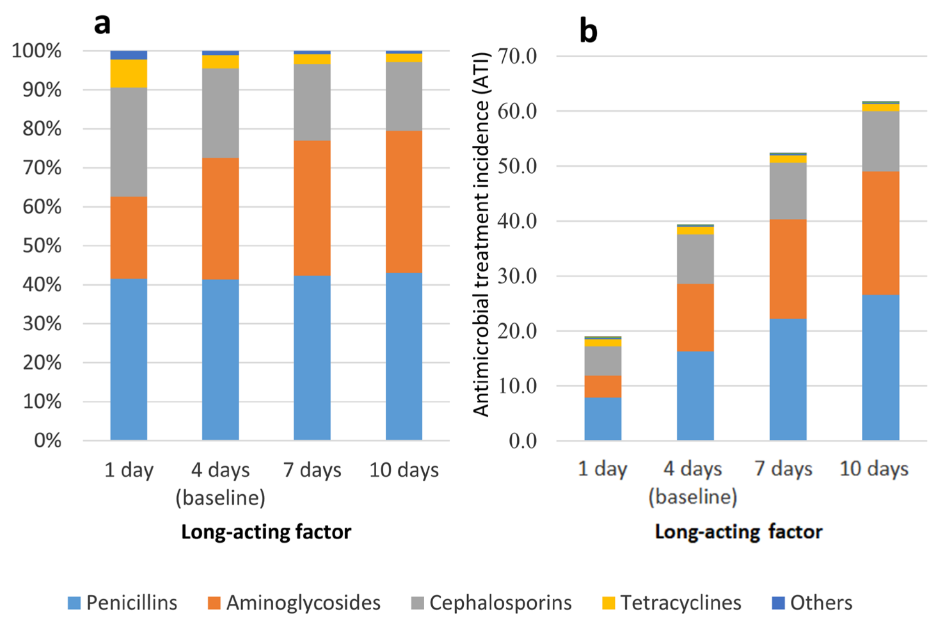

Fig. 4. Relative distribution of antimicrobial use (**a**) and the average antimicrobial use in terms of the antimicrobial treatment incidence (ATI) (**b**) by antimicrobial class on 442 dairy farms in Chiba Prefecture, Japan in 2016, calculated using Japanese defined daily dose (DDDjp) values assigned assuming a long-acting factor of one, four, seven and ten days for intramammary products for dry cows. Macrolides, quinolones, sulfonamides, thiamphenicol and trimethoprim are categorized as others.

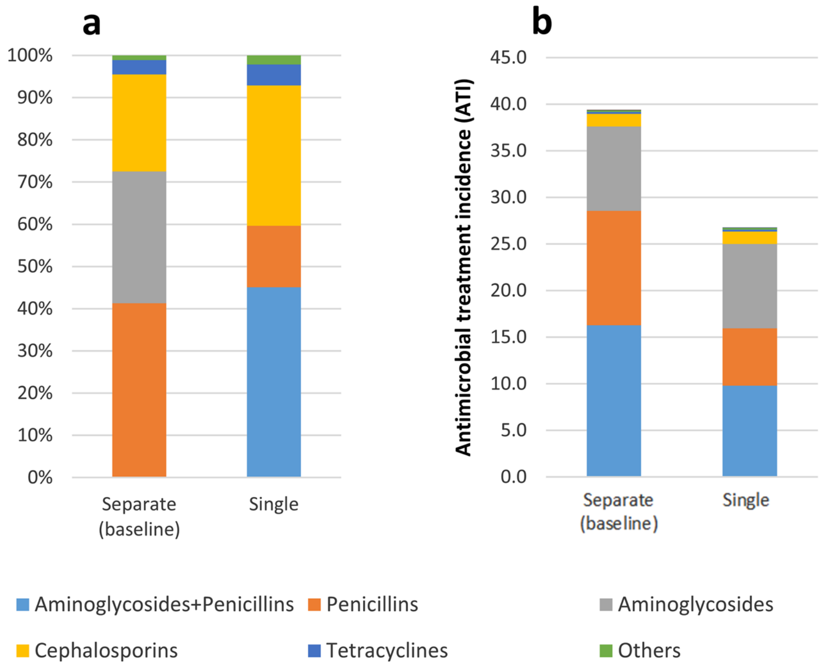

Fig. 5. Relative distribution of antimicrobial use (**a**) and the average antimicrobial use in terms of the antimicrobial treatment incidence (ATI) (**b**) by antimicrobial class on 442 dairy farms in Chiba Prefecture, Japan in 2016, calculated using two different sets of Japanese defined daily dose (DDDjp) values assigned for constituent antimicrobial agents in combination products. Separate (baseline): DDDjp values assigned separately to each constituent antimicrobial agent of combination products as the weight of active ingredient of each antimicrobial administered per day per kg of animal; Single: single DDDjp values assigned to the combination products as the total weight of active ingredient of the two constituent antimicrobial agents administered per day per kg of animal. See Supplementary Table 3 for detailed way of calculation. Macrolides, quinolones, Sulfonamides, thiamphenicol and trimethoprim are categorized as others.

These injection products are mainly used for the treatment of respiratory, gastrointestinal and infectious genital diseases and clinical mastitis as the first-choice antimicrobial on dairy farms in Chiba Prefecture in contrast to the Netherlands where ampicillin and 1st and 2nd cephalosporins are defined as the second-choice antimicrobials [33, 34]. According to the survey conducted by the Ministry of Health, Labor and Welfare, *Escherichia coli* and *Klebsiella pneumoniae* isolated from human patients have become increasingly resistant to cefazolin over the few several years [32]. Additionally, Nobrega *et al.* (2018) reported that penicillin, 3rd generation cephalosporin and macrolide for injection rather than intramammary administration are more strongly associated with the emergence of milk-derived resistant Staphylococci [35]. Thus, given that ampicillin, penicillin and cefazolin are used in both human medicine and dairy production, measures should be taken to reduce resistance of these bacteria to these antimicrobials. Prudent use of these antimicrobials based on diagnosis combined with biosecurity measures and vaccination programs to protect dairy farms from the introduction of respiratory, gastrointestinal and genital diseases might result in effective reduction of resistance to these antimicrobials.

Investigation of the large variation in antimicrobial use between farms observed in this study revealed a total of ten farms remained above the 75 percentiles of distribution over the three-year period, indicating that these farms persistently used high quantities of antimicrobials. According to the NOSAI Chiba veterinarians, none of these farms had experienced serious outbreaks of infectious diseases. This suggests that subjecting the farms with high ATI to intensive reduction measures might result in a successful reduction in antimicrobial use in Chiba Prefecture.

In this study, no significant effect of herd size on antimicrobial use was observed, which was consistent with the results from dairy farms in Argentina [14]. However, our findings on the relationship between farm size and antimicrobial use in dairy cattle were not consistent with those from Denmark, Canada and the United States [19, 22, 40]. This inconsistency might be because of the difference in the herd size of the dairy farms in our study (35.6 cows per farm) to those from these studies (65–199 cows per farm) [19, 22, 40]. The cause of the positive correlation between farm size and antimicrobial use was not well described in these previous studies. However, Hill *et al.* found that herd-level prevalence of disease such as mastitis or lameness and the selection of broad-spectrum preparations increased as herd size increased in dairy operations in the United States, suggesting a correlation between the herd size and antimicrobial treatment for diseases [15]. Further studies using data on large farms are needed to assess farm size effect on antimicrobial use.

In this study, no significant temporal change of ATI was observed over the three-year study period. We speculate that the ATI remained unchanged because of two reasons. Firstly, the patterns of prescription of antimicrobials or the direction of antimicrobial use by veterinarians in each district did not possibly change in the short term. Secondly, ATI might not be a sensitive index for antimicrobial use on dairy farms in Chiba Prefecture because of the large proportion of antimicrobials used for dry cow therapy, which is independent of the occurrence of diseases. Given the fact that our data were collected before the implementation of the national action plan on antimicrobial resistance by the Japanese government [48] and SDCT was introduced in Japan, the results of our study might not be readily applicable in the current situation in Japan.

With regard to geographical variation of antimicrobial use, the ATI on dairy farms in eastern and western districts were significantly higher than those on dairy farms in southern district. In the context of climate characteristics, high temperature and humidity in summer increase the risk of mastitis [39], leading to higher antimicrobial use. However, geographical variation of antimicrobial use in this study cannot be directly attributable to climate variation because average temperatures in summer were relatively higher in western and central districts than in other districts, whereas it was moderate in the eastern district [3]. Moreover, herd size is seemingly not associated with geographical variation of antimicrobial use because the average herd size in the eastern district (32.6 cows) is the second smallest of the five districts, despite having the mean number of ATI. Previous quantitative studies have shown that veterinarians and farmers play important roles in antimicrobial use. Information from veterinarians, farmers' personal experiences, and feedback about perceived efficacy or prescribing habit of veterinarians are considered to affect antimicrobial use [5, 27]. As shown in Supplementary Fig. 3, the way antimicrobials are administered seemed to differ between districts. Further studies should investigate the factors associated with geographical variation of antimicrobial use.

In this study, as a baseline calculation, DDDjp values assuming a long-acting factor of four days for intramammary products for dry cows were used to calculate the ATI. The result of scenario analysis revealed that depending on the long-acting factor used, there was a considerable change in the relative distribution of antimicrobial use by antimicrobial class as well as in the calculated value of ATI on each farm (even with changes of ranking in terms of the ATI). This is because a large proportion of antimicrobials used on dairy farms in Chiba Prefecture is intramammary for dry cows. This is consistent with previous reports [34, 38]. This scenario analysis, rather than demonstrating the ideal long-acting factor, suggests that the choice of long-acting factor used for intramammary products should always be kept in mind when making a comparison of antimicrobial use using different dosage-based monitoring systems. In addition, one should note the importance of the long-acting factor in establishing a benchmarking system for dairy farms because it may affect the ranking of farms.

In regard to the way of assigning single DDDjp values or separate DDDjp values for combination products, our scenario analysis revealed a 0.73-fold difference between the values of ATI calculated by the respective methods. This difference is largely due to the fact that the most frequently used intramammary antimicrobials (for clinical mastitis and dry cow treatment) on the dairy farms analyzed in this study were combination products that contained penicillin and dihydrostreptomycin. This finding was consistent with the result of a previous study that examined dairy farms in the United States [38]. This study did not aim to identify a better way of assigning DDD values for combination products: this should be done considering other factors including which way of assigning DDD values better reflects the selection pressure. However, considering the non-negligible difference observed in this study, one should keep in mind the way in which DDD values are assigned for combination products when making comparisons of antimicrobial use using different dosage-based monitoring systems.

Monitoring of antimicrobial use on dairy farms using a dosage-based metric is undertaken in many countries [6, 11, 17, 37, 46, 50]. For example, in Belgium, the US and Pakistan, ATI, a metric identical to the metric used in this study is used to monitor antimicrobial use on dairy farms [6, 46, 50]. According to these studies, the ATI on dairy farms in Flanders was calculated to be 20.8 for the years 2012–2013 [46]; the ATI on dairy farms in Wisconsin was calculated to be17.2 for the year 2017 [6]; and the ATI on dairy farms in Pakistan was calculated to be 47.7 for the year 2018 [50]. These values indicate that the antimicrobial use in dairy cattle in Chiba Prefecture is at face-value nearly twice as high as that in Flanders and Wisconsin, and that it is more or less the same as the antimicrobial use in dairy cattle in Pakistan. The Netherlands uses $DDDA_F$, another dosage-based indicator, presenting the average number of treatment days that a cow is subjected to in a year, to monitor antibiotic use at farm level. The average $DDDA_F$ of dairy farms in the Netherlands was calculated to be 2.2 in 2015 [34]. This value corresponds to 6.0 of ATI, indicating that the antimicrobial use in cows in the Netherlands is much lower than the value of ATI (38.9) observed on dairy farms in the Chiba Prefecture in that year in this study. However, this gap is partly due to the different ways the two countries assign DDD values for combination products: single DDD values are used for combination products in the Netherlands [21, 37] while in this study separate DDD values were assigned for combination products to calculate the ATI.

In conclusion, international comparison of the antimicrobial use monitoring results on dairy farms using a dosage-based metric should be done with caution. In making comparisons, one should keep in mind that not only the difference in dosing recommendations but also the choice of long-acting factor used to assign DDD values for dry cow products and the way in which the DDD values are assigned for combination products, because all these factors have a significant impact on the calculated results. Despite such limitations, this study has revealed that intramammary antimicrobial products, in particular intramammary products for dry cow therapy represent a large proportion of the total antimicrobial use in dairy cattle in Chiba Prefecture. Mastitis control and reducing dry cow therapy would effectively reduce antimicrobial use in dairy cattle. Herd-level monitoring of antimicrobial use using ATI and veterinary support tailored to individual farms are paramount to reducing antimicrobial use and bacterial resistance in dairy cattle.

CONFLICT OF INTEREST. The authors declare no conflicts of interest associated with this manuscript.

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