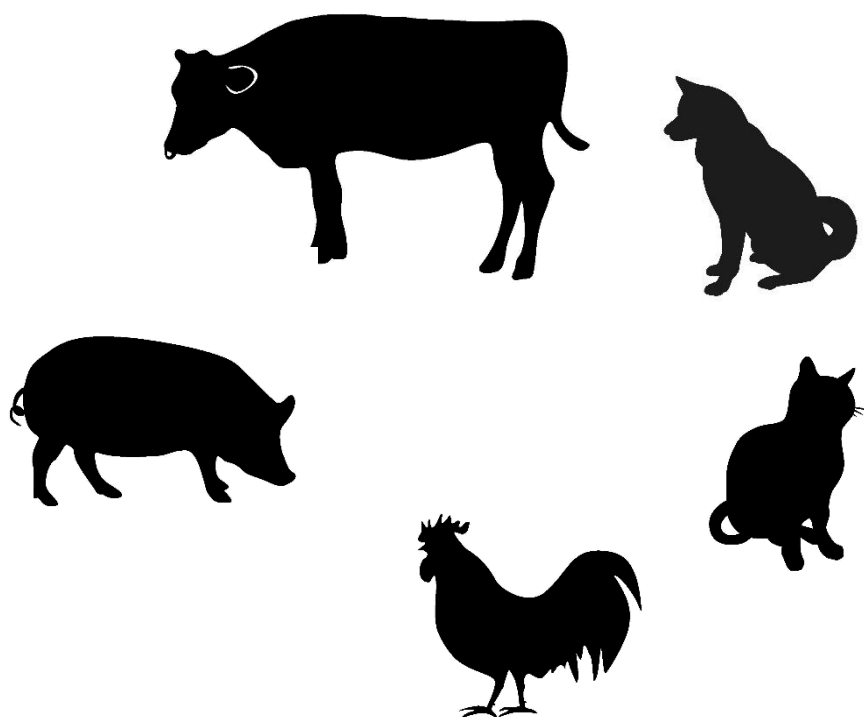


**2020
JVARM
Japanese Veterinary Antimicrobial Resistance Monitoring
Annual report**



**Veterinary AMR Center
National Veterinary Assay Laboratory,
Ministry of Agriculture, Forestry and Fisheries**



July 2024

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1.Introduction

- Antimicrobial agents are essential for protecting animal health, ensuring a stable supply of livestock and aquatic products, or treating infectious diseases in animals, but there is always a risk that antimicrobial resistant bacteria selected by antimicrobial use may affect the medical care of humans, livestock, companion animals, and aquatic animals. For this reason, the Ministry of Agriculture, Forestry and Fisheries (MAFF) conducts monitoring of antimicrobial resistant bacteria and, based on the results of the survey, formulates and implements risk management measures according to the assessed level of risk.
- The problem caused by antimicrobial resistant bacteria is not only a problem in Japan, but has become one of the most important international issues. In 2015, the World Health Organization (WHO) formulated the “Global Action Plan on Antimicrobial Resistance” and requested member countries to promote measures to combat antimicrobial resistance. In response, Japan formulated the “National Action Plan on Antimicrobial Resistance (AMR) (2016-2020)” in 2016, and has been promoting measures against antimicrobial resistance in cooperation with related parties and ministries.
- The nationwide monitoring of antimicrobial resistant bacteria is considered one of the key pillars of control measures outlined in the National Action Plan on AMR (2016-2020). This function has been carried out by the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM) division of the MAFF since 1999 in conjunction with the Food and Agricultural Materials Inspection Center (FAMIC) as well as other related public and private institutions.
- This report provides an overview of the monitoring of antimicrobial resistant bacteria isolated from healthy and diseased animals in 2020.
- The number of samples/species, resistance rates and trends in antimicrobial sales are published on our website (https://www.maff.go.jp/nval/yakuzai/yakuzai_p3.html).

2. Overview of Monitoring Results for 2020

List of bacteria collected in 2020

Category	Health/ disease	Animal species	Species				
Livestock	Health (3-1)	Cattle	Enterococcus spp.	Campylobacter spp. / C. jejuni, C. coli			
		Pigs					
		Chickens			Salmonella spp.		
	Disease (clinical isolates) (3-2)	Cattle	Salmonella spp.	Staphylococcus aureus	Mannheimia haemolytica		
		Pigs			Streptococcus suis		
		Chickens					
	Health (3-3)	Dogs and Cats	Enterococcus spp.	Escherichia coli			
		Dogs and Cats			Klebsiella spp.	Enterobacter spp.	Acinetobacter spp.
	Disease (3-4)	Dogs and Cats					Coagulase positive Staphylococcus spp.

Appendix: Class and abbreviations of the tested antimicrobials

Antimicrobial class		Drug	Abbreviations
β-lactam	Penicillins	Ampicillin	ABPC
		Benzylpenicillin	PCG
		Oxacillin	MPIPC
	Cephalosporins	Cefazolin	CEZ
		Cephalexin	CEX
		Cefoxitin	CFX
		Cefotaxime	CTX
		Cefquinome	CQN
		Ceftiofur	CTF
	Carbapenems	Meropenem	MEPM
Aminoglycosides		Streptomycin	SM
		Dihydrostreptomycin	DSM
		Gentamicin	GM
		Kanamycin	KM
Macrolides		Erythromycin	EM
		Azithromycin	AZM
		Tylosin	TS
		Tilmicosin	TMS
		Tulathromycin	TUM
Lincosamides		Lincomycin	LCM
		Clindamycin	CLDM
Tetracyclines		Tetracycline	TC
		Oxytetracycline	OTC
Amphenicols		Chloramphenicol	CP
		Florfenicol	FFC
		Thiamphenicol	TP
Polypeptides		Colistin	CL
		Bacitracin	BC
Glycopeptides		Vancomycin	VCM
Quinolones		Nalidixic acid	NA
Fluoroquinolones		Ciprofloxacin	CPFX
		Enrofloxacin	ERFX
Polyethers		Salinomycin	SNM
Sulfonamides		Trimethoprim	TMP
Other		ST (sulfamethoxazole/trimethoprim)	ST (SMX/TMP)

2. Overview of Monitoring Results for 2020

Healthy livestock

- In 2020, the resistance rates of *E. coli* to fluoroquinolones (CPFX) and third-generation cephalosporins (CTX), which were defined as outcome indices in the National Action Plan on antimicrobial Resistance (AMR) (2016-2020) as antimicrobial agents of critically important antimicrobials in human medicine, remained low. In addition, resistance rates to meropenem (MEPM) and vancomycin (VCM), antimicrobials not approved for veterinary use but considered last-resort treatments for multidrug-resistant bacteria in human medicine, were both 0.0% across all animal species. On the other hand, tetracyclines, the most frequently used antimicrobials in the animal sector failed to achieve target resistance rate values of 33% remaining at 45% despite the decreasing trend in sales volume.
- In chickens(broilers), the sale of fluoroquinolone has trended upward in recent years, coinciding with an observed rising trend in the antimicrobial resistance rate of CPFX in *E.coli*. In addition, the sales volumes of KM for use in chickens(broilers) increased since 2012, leading to elevated resistance rates in *E. coli* and *Salmonella*.
- In cattle, although antimicrobial sales were generally lower compared to pigs, there was an increasing trend in macrolide sales and a corresponding rise in macrolide resistance rates in *Enterococcus* spp. Additionally, increasing trends in sales of tetracyclines and fluoroquinolones were observed, correlating with rising resistance rates to TC and CPFX in *Campylobacter*. While the proportion of sales to the overall livestock population was low, continued caution is necessary to prevent their indiscriminate use.
- In pigs, while there was a decline in the sales volumes of tetracyclines, an increase in macrolide sales since 2016 led to a rise in resistance rates in *Enterococcus* spp.

Diseased livestock: clinical strains

- In *E. coli*, the resistance rate to TC has leveled off, while resistance rate to CL has decreased. The resistance rate to CPFX and CTX tended to decrease in pigs, while it tended to increase in cattle. In chickens, the resistance rate to CPFX increased in 2020.
- In *M. haemolytica*, the second-line drug, CQN, showed a low resistance rate of 5% or less in cattle, but a high resistance rate of 50% or more in DSM and ABPC.
- In *S. suis*, veterinary drugs approved for streptococci in pigs showed a resistance rate to ST, GM and PCG of less than 20% and the susceptibility was maintained. On the other hand, a high resistance rate of more than 90% in TC and more than 80% in EM was observed.
- *Salmonella* spp. have a high resistance rate of more than 40% in TC approved for cattle, pigs and chickens. Dublin isolated from cattle showed higher resistance rates to CL and multidrug resistance.
- In *S. aureus*, the susceptibility was generally maintained in cattle and chickens, but the resistance rate was more than 80% in PCG and more than 50% in TC in pigs.

Healthy companion animals (dogs and cats)

In *E. coli* and *Enterococcus* spp. derived from healthy dogs and cats, the resistance rate including the second-line drug was 20% or less in many of the tested drugs. Resistance rates for carbapenems and VCM resistant *Enterococcus* spp. remained at 0.0% as there is no approved veterinary drug in circulation. It was confirmed that the antimicrobial resistance rate of bacteria from healthy companion animals was lower than that of bacteria from diseased animals for many tested drugs. Additionally, it was noted commensal bacteria in healthy dogs and cats were well maintained as antimicrobial susceptibility was retained.

Diseased companion animals (dogs and cats)

The trends for *E. coli*, *Klebsiella* spp., coagulase-positive *Staphylococcus* spp. and *Enterococcus* spp., which have been continuously collected since the start of the survey, were generally similar to the previous results. *Enterobacter* spp. collected in 2020 showed higher resistance rates to third-generation cephalosporins and fluoroquinolones, whereas *Acinetobacter* spp. resistance rates to the tested drugs were less than 20% in dogs. The resistance rates to carbapenems in the gram-negative bacteria and to VCM in enterococci was both 0.0%. For second-line drugs, resistance rates to CTX were high among *Klebsiella* spp., *Enterobacter* spp. and *S. aureus* from cats, but less than 30% in other bacteria. It showed a broad resistance rate of 5.9-97.6% to CPFX and more than 70% to AZM of 15-membered ring macrolides (only coagulase-positive *Staphylococcus* spp.). For CL, few strains showed resistance except *Enterobacter* spp. and no *mcr* gene was detected in all CL-resistant strains isolated in 2020.

It is important to ensure the prudent use of antimicrobial agents such as selecting effective antimicrobial agents by performing susceptibility testing prior to treatment and considering measures other than the administration of antimicrobial agents, such as washing and disinfection for dermatitis, so that they can continue to be used effectively in the treatment of bacterial infections in the future.

Antimicrobial sales

The sales volume of veterinary antimicrobials in 2020 was 842.9t, declining by about 20% from 2001, but has remained around 800t in recent years. Tetracyclines account for the largest percentage of the total, approximately 40%, but the sales volume of tetracyclines has been decreasing, reaching its lowest in 2020. While the volume of veterinary antimicrobial agents sold to pigs remains the largest among all animal species, sales have been declining in recent years, primarily due to reduced tetracycline sales; 2020 being almost half of 2001's sales. In fisheries (seawater), macrolides (EM) have increased since 2015 with the occurrence and treatment of streptococcal infections (type II alpha-hemolytic *Streptococcus*) that differ from conventional serotypes. However, in 2020, macrolides have changed from an increasing to decreasing trend, potentially the result of effective vaccine implementation.

Among second-line drugs, CL was the highest sales volume and followed by fluoroquinolones. Almost all CL were sold for pigs, and about 80% of the second-line drugs for pigs were CL, which increased after the revocation of the designation as a feed additive in 2018. As a vaccination for oedema disease, an indication for CL, was recently developed, it is expected that the sales of CL will decline in the future. The amounts of second-line drugs sold for broilers was the second highest following pigs and most of them were fluoroquinolones. The amount of fluoroquinolones sold for broilers has increased in recent years, correlation with the escalation in the resistance rate to fluoroquinolones. It is crucial to monitor this situation carefully.

The volume of antimicrobial feed additives showed little change over the decade, hovering around 200t. The volume of polyether (not used for humans) was on the rise with the usage percentage increasing from 59.7% in 2007 to 89.6% in 2020.

With regard to antimicrobials for human use marketed to animal clinics, in 2020, the volume sold to small animal clinics was at its lowest (4.8t) since the beginning of the survey in 2016. But there was no major change in the kinds of antimicrobials and the ratio of each drug to the total number of human antimicrobials. Factors for the use of human antimicrobials may have been influenced not only by anticipated necessity and usefulness, but also economics, availability and experience. It was found that the drugs penems, carbapenem and glycopeptides, which are not approved and not sold as a veterinary drug and which are used as a last resort for the treatment of the multidrug resistant bacteria in the human medicine, are being used in companion animals. At present, in JVARM, bacteria resistant to these drugs have not been identified in dogs and cats. However, caution should be taken due to the likelihood of the emergence of antimicrobial resistant strains, and the use of these drugs should be avoided.

3-1 Healthy livestock

A summary of the results of monitoring antimicrobial-resistant bacteria, including *Escherichia coli* and *Enterococcus* spp. which are indicator bacteria isolated from healthy livestock (cattle, pigs, and chickens (broilers)), as well as *Campylobacter* and *Salmonella* spp., which are foodborne pathogens of public health concern, during the 2020 fiscal year.

3-1-1 Outcome indices of National Action Plan on antimicrobial Resistance (AMR) (2016-2020)

The resistance rate of *E. coli* to tetracyclines, which are the most commonly used in domestic animals, and to fluoroquinolones and third-generation cephalosporins, which are critically important in human medicine, are specified as animal-related outcome indices in AMR Action Plan (2016-2020) (Table 3-1-1).

Table 3-1-1 Outcome indices in the Action Plan (2016-2020)

Outcome indices (resistance rate of <i>E. coli</i> *)	2020 (target value)
Tetracycline	33% or less
3rd generation cephalosporins	Same level as the figures in each G7
Fluoroquinolone	Same level as the figures in each G7

*Resistance rates of *E. coli* in healthy livestock:
average of resistance rate of cattle, pigs and chickens (broilers)

3-1-1-1 Outcome indices and resistance rates of National Action Plan on AMR (2016-2020)

The resistance rates of *E. coli* isolated from healthy livestock in 2020 to third-generation cephalosporins (CTX) and fluoroquinolones (CPFX) remained low, indicating the achievement of the target (Fig. 3-1-1). However, despite a decrease in sales after 2018 (Fig. 3-1-2), the resistance rate to tetracyclines (TC) did not decline and remained higher than the target percentage (Fig.3-1-1).

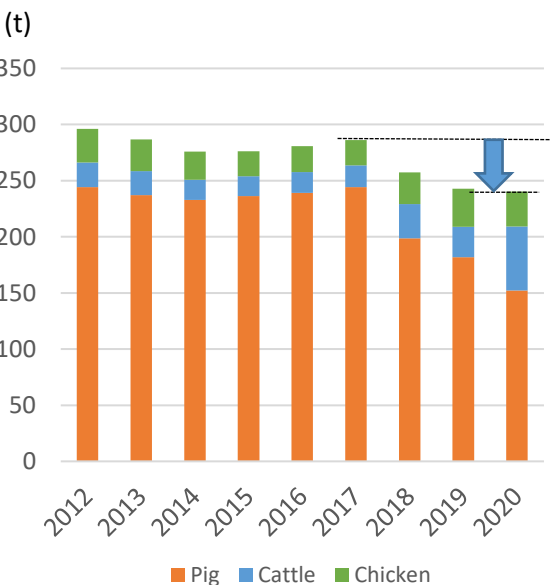
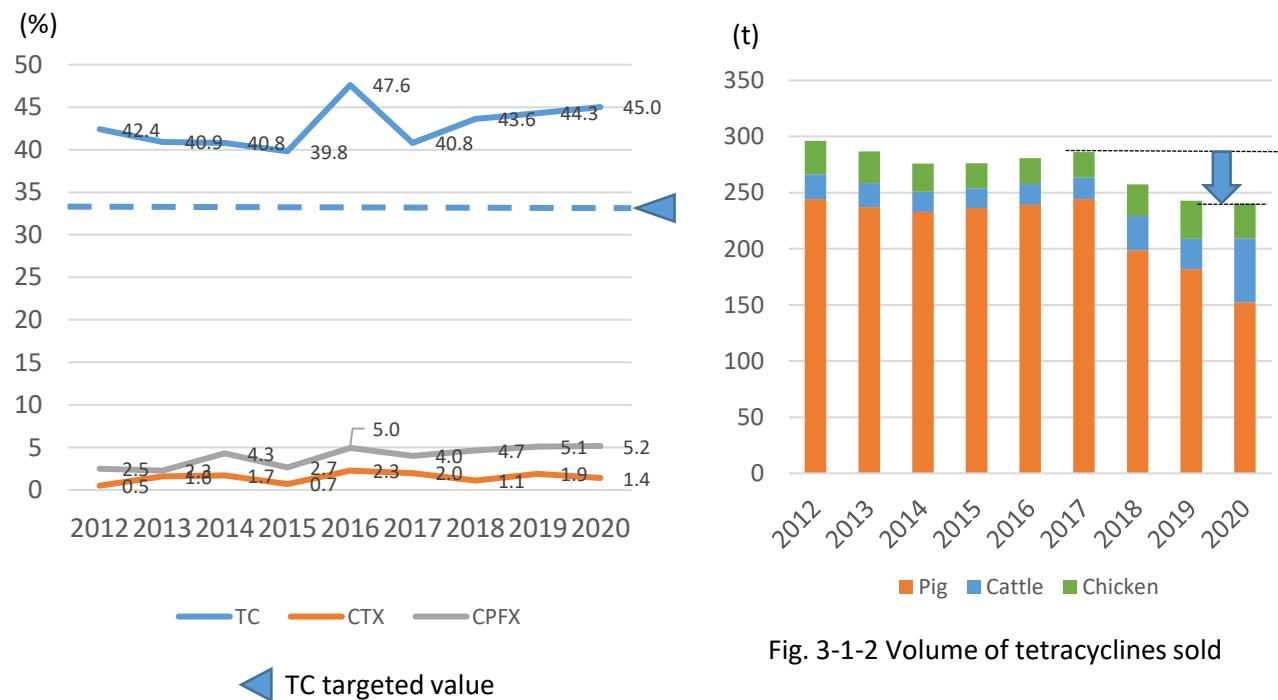


Fig. 3-1-2 Volume of tetracyclines sold

Fig. 3-1-1 Resistance rates of healthy livestock-derived *E. coli*.
(Average resistance rate of cattle, pigs and chickens(broilers))

3-1-1-2 Outcome indices and resistance rates of National Action Plan on AMR (2023-2027)

Based on the outcome indicator achievements in the previous action plan, the Action Plan on AMR (2023-2027) has adopted the resistance rates of *E. coli* to tetracyclines, third-generation cephalosporins, and fluoroquinolones as outcome indices in the veterinary sector for continuity. Furthermore, considering variations in resistance status and hygiene management of different animal species, target values have been set for each animal species to be achieved by 2027 (Fig. 3-1-3 to 5). These targets serve as indicators of the response outcomes specific to each animal species. Additionally, a target was set to reduce the total sales of veterinary antimicrobials in the livestock sector by 15% from 2020 to 2027, along with a goal to limit the use of second-line drugs to below 27 tones (Table 3-1-2).

Fig. 3-1-3 Tetracycline resistance rates and targets values

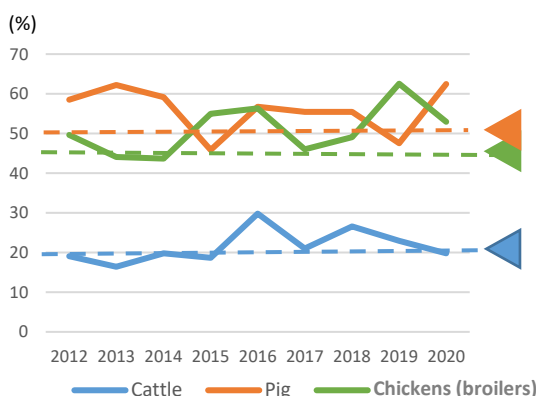


Fig. 3-1-4 Third-generation cephalosporins Resistance rates and targets values

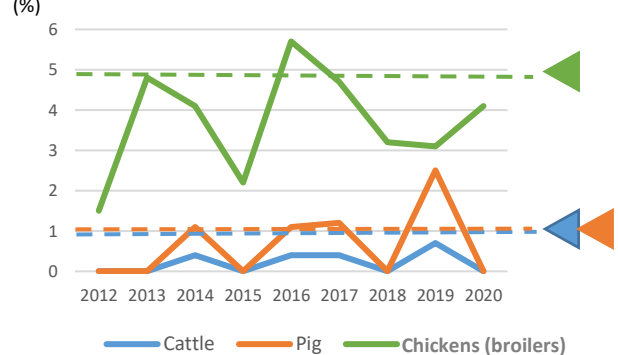
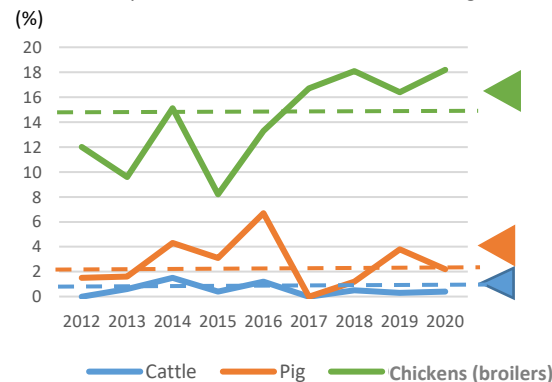


Fig. 3-1-5 Fluoroquinolones resistance rates and targets values



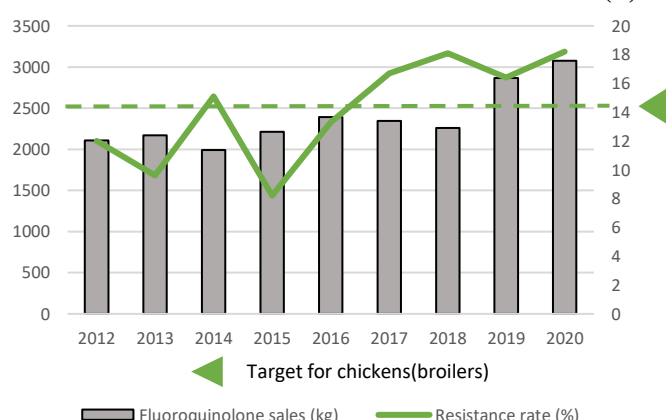
Target for cattle Target for pigs Target for chickens (broilers)

Table 3-1-2 Outcome indices in the Action Plan (2023-2027)

Indices (resistance rate of <i>E. coli</i>)	2027 (target values)		
	Cattle	Pig	Chicken (Broiler)
Tetracycline	20% or less	50% or less	45% or less
Third generation Cephalosporin	1% or less	1% or less	5% or less
Fluoroquinolone	1% or less	2% or less	15% or less
In the livestock sector Total use of veterinary antimicrobials		Reduce by 15% from 2020 levels by 2027.	
In the livestock sector Total dose of second-line drugs		27 t or less in 2027.	

Prudent use of antibacterial agents across all species, along with a reduction in unnecessary usage, is crucial. Specifically, the sales of fluoroquinolones in chickens (broilers) has increased recently. Consequently, the antimicrobial resistance rate in *E. coli* isolated from chickens (broilers) has also increased (Fig. 3-1-6). Given its designation as an outcome indicator, prudent use of fluoroquinolones as a second-line drug is imperative.(Table 3-1-2).

Fig. 3-1-6 Resistance rates to fluoroquinolones in chickens (broilers) and sales volume of fluoroquinolone (%)



3-1-2 Resistance rates by species

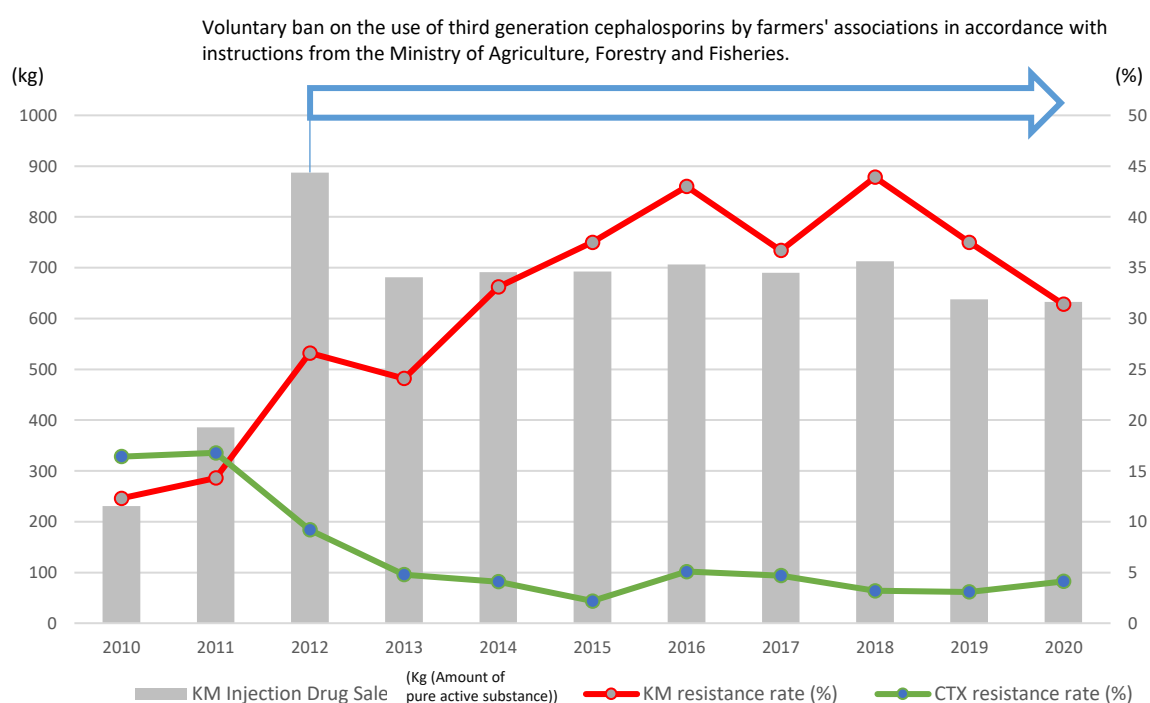
The main changes in antimicrobial resistance in each species other than the outcome indices of National Action Plan on AMR are described below. For specific numbers and trends in resistance rates, please refer to the following URL (https://www.maff.go.jp/nval/yakuzai/yakuzai_AMR_2.html).

3-1-2-1 *Escherichia coli*

Based on the risk assessment conducted by the Food Safety Commission, CL was reclassified as a second-line drug for veterinary use in 2019 and its designation as a feed additive was revoked. In 2020, its resistance rate remained below 5% in all animal species. Additionally, the resistance rate of MEPM, one of Carbapenems[※], critically important antimicrobial agents in human medicine, was 0.0% across all animal species.

※ It is not approved for veterinary use.

Fig. 3-1-7 Resistance rates of KM in Chickens (broilers) and sales of KM injection antimicrobials



In chicken(broiler)-derived *E. coli*, there has been an upward trend in the resistance rate to KM since 2012 (Fig. 3-1-7). The resistance rate to third-generation cephalosporins in chickens (broilers) exceeded 15% around 2010. This was considered to be due to the mixed administration of third-generation cephalosporins (CTF) with in-egg vaccines in some hatcheries, aimed at reducing post-hatch mortality rates. Since the use of third-generation cephalosporins was off-label and these drugs are critically important antimicrobial agents in human medicine, the Ministry of Agriculture, Forestry and Fisheries instructed farmers' associations to cease their use, following the indication of increasing resistance rates, and notifications were issued from producer organizations to their members to stop usage. Subsequently, the resistance rate to third-generation cephalosporins decreased. On the other hand, since 2012, there has been an increase in the sales of KM, coinciding with the rise in resistance rates. This could be attributed to the potential impact of KM being used as an alternative to CTF. It is necessary to consider and implement measures after comprehensively considering the actual use of antimicrobial agents for animals in production sites and the availability of veterinary medicinal products that can be used as alternatives to antimicrobial agents.

3-1-2-2 *Enterococcus* spp.

In *Enterococcus* spp. isolated from all animal species, the resistance rate to vancomycin (VCM) ※, considered critically important in human medicine, was 0.0%. For macrolide antibiotics EM and TS, for which the antimicrobial susceptibility status in *E. coli* could not be determined, resistance rates in *Enterococcus* spp. trended higher in chickens(broilers) and pigs than in cattle. In chickens(broilers), the sales of macrolids have decreased since 2012, and a decreasing trend in resistance rates of EM and TS has been observed. Notably, TS showed a significant decrease in 2020 compared to 2012, 2014, 2016, and 2017 (Fig. 3-1-8). Although the designation of the feed additive tylosin phosphate used in pigs was revoked in 2019 based on the results of the Food Safety Commission's risk assessment, the use of macrolides as veterinary medicinal products in pigs has been on the increase since 2015 and the resistance rate has not shown a decreasing trend until 2016 after an increasing trend was observed(Fig. 3-1-9).

※ It is not approved for veterinary use.

Fig. 3-1-8 Chickens(broilers) : Resistance rates of EM and TS and sales volumes of macrolides

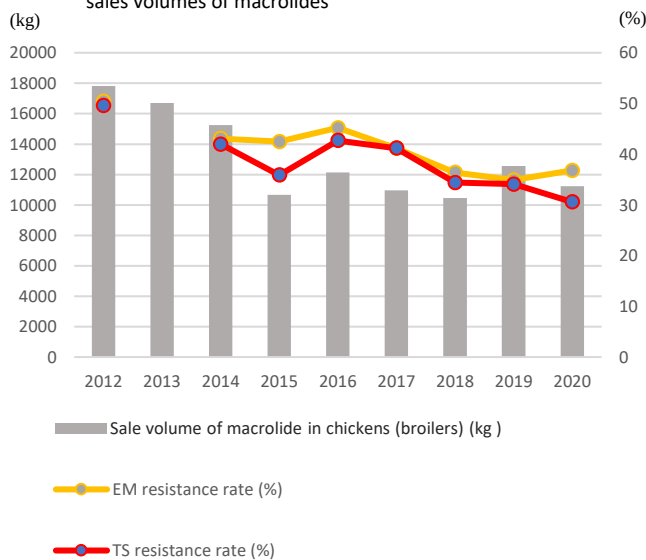


Fig. 3-1-9 Pigs: Resistance rates of EM and TS and sales volumes of macrolides

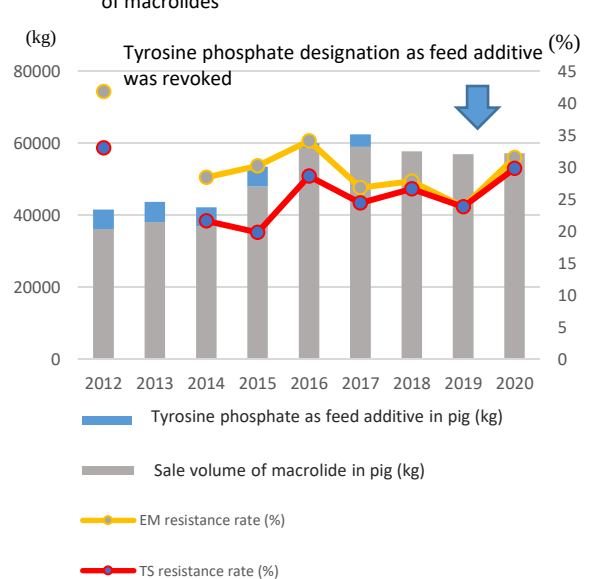


Fig. 3-1-10 Cattle: Resistance rates of EM and TS and sales volumes of macrolides

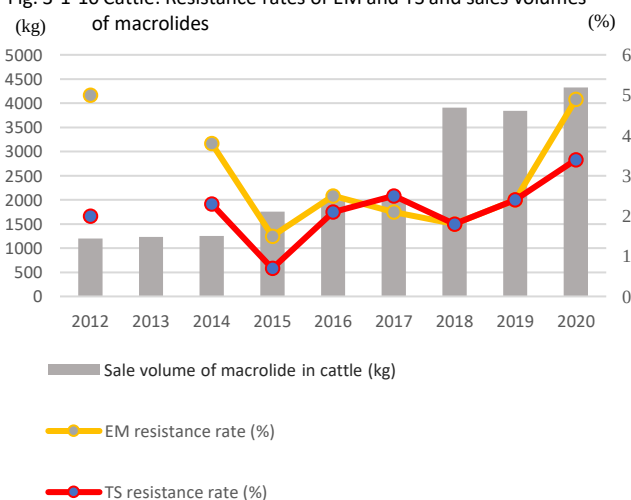
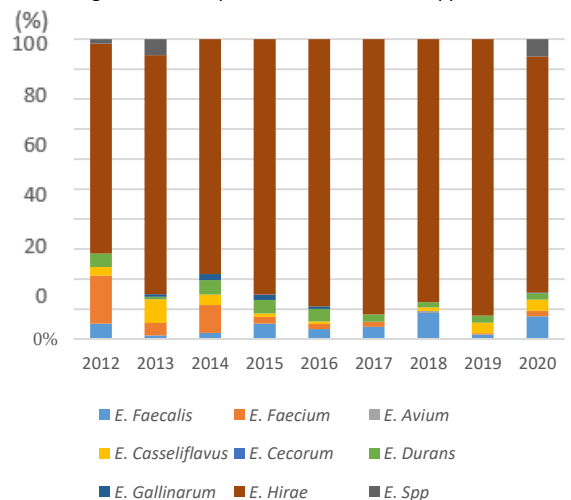


Fig. 3-1-11 Proportion of *Enterococcus* spp. in cattle



In addition, sales of antimicrobials in cattle are lower than in chickens (broilers) and pigs, but there is an increasing trend, and resistance rates are also on the rise, with resistance rates for EM and TS in 2020 being significantly higher than in 2015 (Fig. 3 - 1- 10). As *Enterococcus* spp. include many species, it is important to check changes in the proportion of species isolated, as the trend in resistance rates may differ depending on the species. In cattle, *E. hirae* has predominated (70.1-92.2%) in all years since 2012, and no significant changes in species have been observed since 2019, when the resistance rate of EM and TS increased, suggesting that the increase in the resistance rate of EM and TS is influenced by the use of macrolides rather than changes in species composition. In human medicine, among the species of *Enterococcus*, *E. faecalis* and *E. faecium* are considered opportunistic pathogens, while *E. hirae* is not regarded as problematic in human medicine. In pigs, *E. hirae* (38.1% to 73.8%) predominates, while in chickens (broilers), *E. faecalis* (22.3% to 70.2%) accounts for a significant proportion, and no significant changes in species composition have been observed since 2012.

3-1-2-3 *Campylobacter* spp.

In *Campylobacter* spp., which are foodborne pathogens, antimicrobial susceptibility is being investigated primarily for *C. jejuni* in cattle and chickens (broilers), and for *C. coli* in pigs. In cattle, *C. jejuni* showed high resistance rates of over 30% to TC, NA, and CPFX, with an increasing trend observed (Fig. 3-1-12). The resistance rate to TC in 2020 was significantly higher than that from 2012 to 2015, while the resistance rates to NA and CPFX were significantly higher than those from 2012 to 2018 (except for 2017) (Fig. 3-1-12).

Fig. 3-1-12 Cattle: resistance rate of each antimicrobial agent in *C. jejuni*

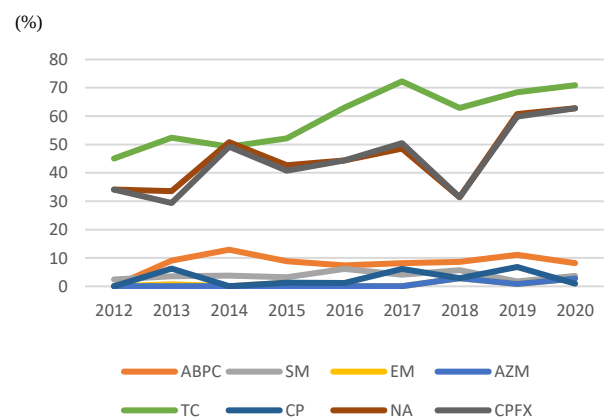


Fig. 3-1-13 Cattle: Resistance rates of CPFX and sales volumes of fluoroquinolone sales

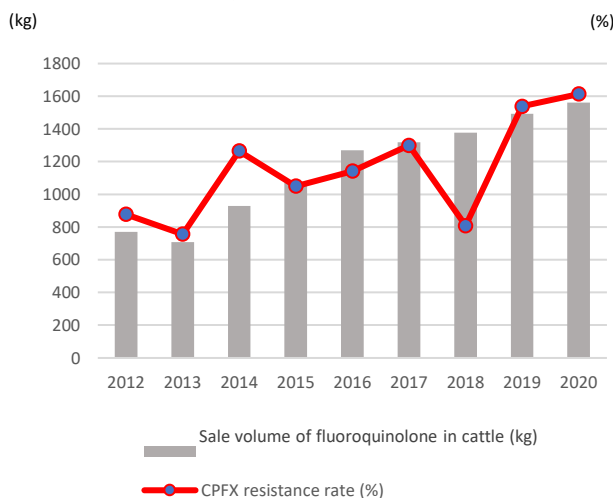
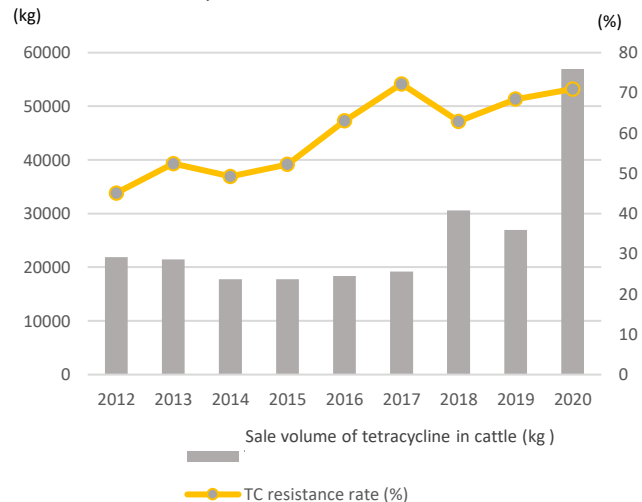


Fig. 3-1-14 Cattle: Resistance rates of TC and sales volumes of tetracycline



The increasing trend in antimicrobial resistance rates corresponds to the rising sales of fluoroquinolones (Fig. 3-1-13) and tetracyclines (Fig. 3-1-14) in cattle. Although the sales volumes of these formulations are lower in cattle compared to fluoroquinolones in chickens (broilers) and tetracyclines in pigs, both resistance rates and sales volumes are increasing in each formulation for cattle. Therefore, it is crucial to ensure prudent use by limiting the use of fluoroquinolones strictly as second-line drugs when first-line drugs are ineffective and refraining from prophylactic administration of tetracyclines. On the other hand, resistance rates to other drugs in cattle showed low values of less than 10%.

C. coli in pigs exhibited higher resistance rates to all drugs compared to *C. jejuni* in cattle and chickens (broilers). Resistance rates to various drugs in *C. coli* in pigs and *C. jejuni* in chickens (broilers) showed fluctuations without a consistent increasing or decreasing trend (Fig. 3-1-15, 16).

Fig. 3-1-15 Pigs: Resistance rates of *C. coli*

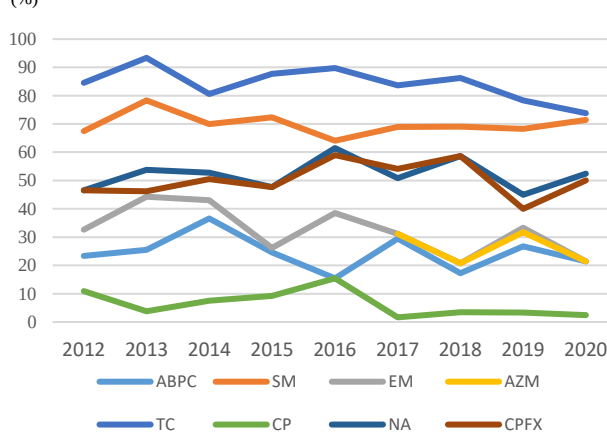
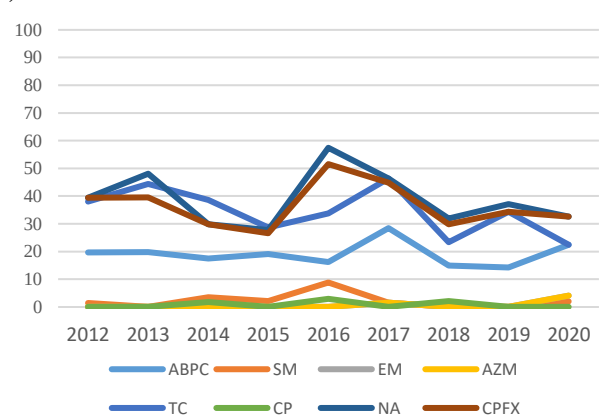


Fig. 3-1-16 Chickens (broilers): Resistance rates of *C. jejuni*



3-1-2-4 *Salmonella* spp.

Salmonella spp. are rarely isolated from healthy domestic cattle and pigs, whereas they can be isolated from chickens (broilers), so antimicrobial susceptibility surveys are conducted on strains isolated from chickens (broilers) in monitoring healthy livestock. As for serotypes of *Salmonella* from poultry slaughterhouses, the proportion of Schwarzengrund isolates is increasing year by year. On the other hand, Enteritidis, the most common human food poisoning-derived strain, is rarely isolated (Fig. 3-1-17). Antimicrobial resistance rates for the second-line drugs CL, CPFX and CTX showed low values, with 0.0% for the carbapenem drug MEPM. Conversely, the resistance rate of TC remained high at 69.2-85.2%, and the resistance rate of KM showed an increasing trend from 2012 onwards, as in the case of *E. coli*, and the use of the KM may have had an impact (Fig. 3-1-18).

Fig. 3-1-17 *Salmonella* serotypes

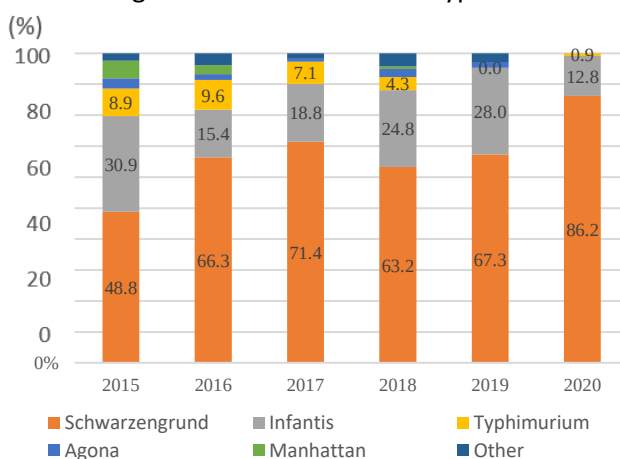
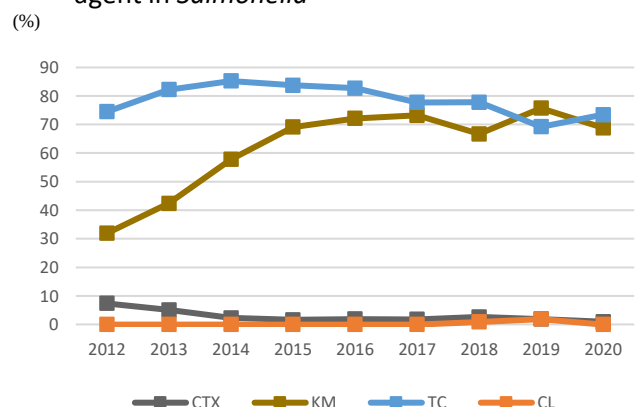


Fig. 3-1-18 Resistance rate of each antimicrobial agent in *Salmonella*



3-1-3 Summary of results

The resistance rates of *E. coli* to fluoroquinolones (CPFX) s and third-generation cephalosporins (CTX), which were defined as outcome indices in the National Action Plan on AMR (2016-2020) as antimicrobial agents of critically important antimicrobial agents in human medicine, remained low, reflecting their continued prudent use by stakeholders, such as livestock farmers and veterinarians. Additionally, the resistance rates to CL, another critically important antimicrobial agent, remained low, indicating successful efforts in its prudent use as a second-line drug. Furthermore, resistance rates to MEPM and VCM, antimicrobials not approved for veterinary use and considered last-resort treatments for multidrug-resistant bacteria in human medicine, were both 0.0% across all animal species.

However, regarding tetracyclines, which were the most frequently used antimicrobials in the animal sector and an outcome index in the action plan, despite a decreasing trend in sale volumes, the resistance rate remained high at 45.0%, failing to achieve the target values of 33%.

Furthermore, concerning specific bacterial species and animal species, an increasing trend in the sales of fluoroquinolones in chickens (broilers) corresponded with a rising trend in CPFX resistance rates in *E. coli*. Similarly, the sales volumes of KM in chickens (broilers) increased since 2012, leading to elevated resistance rates in *E. coli* and *Salmonella*.

In cattle, although antimicrobial sales were generally lower compared to pigs, there was an increasing trend in macrolide sales and a corresponding rise in macrolide resistance rates in *Enterococcus* spp. Additionally, increasing trends in sales of tetracyclines and fluoroquinolones were observed, correlating with rising resistance rates to TC and CPFX in *Campylobacter*. While the proportion of sales to the overall livestock population was low, continued caution is necessary to prevent their indiscriminate use.

In pigs, while there was a decline in the sales volumes of tetracyclines, an increase in macrolide sales since 2016 led to a corresponding rise in resistance rates in *Enterococci*, which have been sustained. To avoid the use of macrolides as an alternative to tetracyclines, stakeholders must exercise prudent use.

What is required of all stakeholders is to collectively engage in the efforts of the New Action Plan, which includes:

1. Promoting prevention of infectious diseases through appropriate livestock hygiene management, utilization of vaccines, etc., and
2. Ensuring prudent use of antibiotics through appropriate selection and refraining from prophylactic administration, aiming to establish a livestock production system that does not rely on antibiotics.

These actions are crucial for maintaining antimicrobial susceptibility to ensure that antibiotics can be used as therapeutic agents when needed in both veterinary and human medicine in the future, while still earning the trust of consumers in domestically produced livestock products. The Ministry of Agriculture, Forestry and Fisheries (MAFF) will continue to disseminate information on trends and issues related to antimicrobial resistance as well as devise and implement more effective specific measures. MAFF will further strengthen its collaboration with livestock farmers and veterinarians differently involved in livestock hygiene management and antimicrobial use.

3-1-4 Acknowledgement

In conducting this survey, we express our sincere gratitude to all the personnel at the livestock farms, poultry processing plants, and other collaborators who assisted with the sample collection. We appreciate and kindly ask your continued cooperation for future monitoring surveys.

3-2 Diseased Livestock

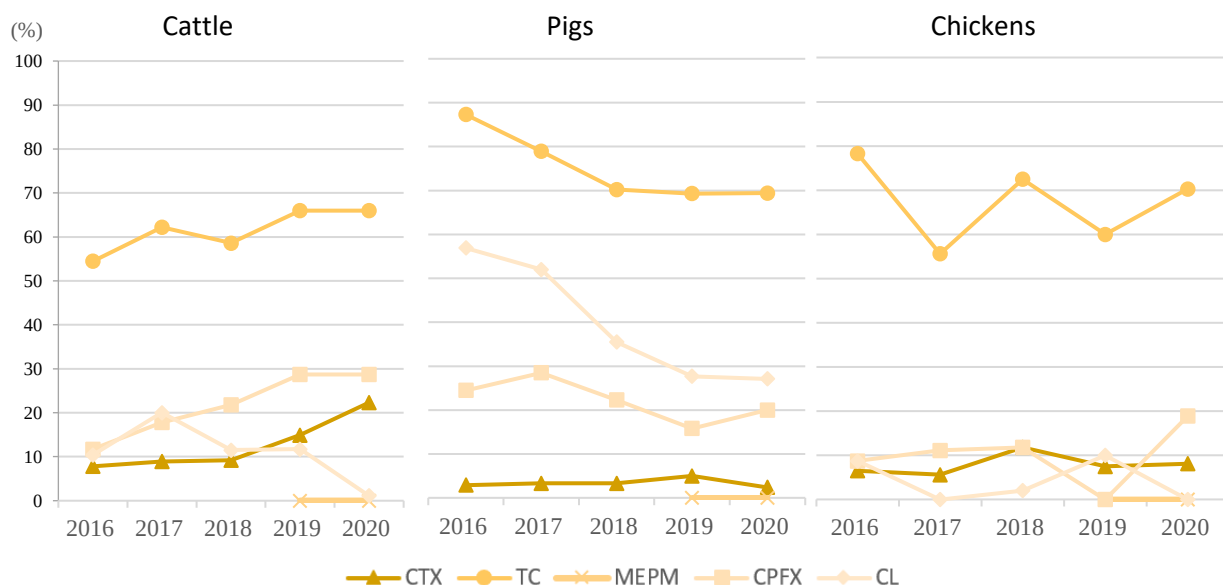
:Clinical isolates

In 2020, *Escherichia coli*, *Mannheimia haemolytica*, *Salmonella* spp. and *Staphylococcus aureus* were collected. As part of the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM), antimicrobial susceptibility test results are summarized for drugs approved for cattle, pigs or chickens for each bacterial species and belonging to the same class, as well as drugs of public health significance that are continuously monitored. Antimicrobial susceptibility testing was performed by broth microdilution according to the Clinical & Laboratory Standards Institute (CLSI), and the minimum inhibitory concentration (MIC) was determined and the values of the CLSI guidelines or microbiological BP were used as breakpoints (BP, resistance limits).

3-2-1 *Escherichia coli* (cattle, pigs, chickens)

A total of 216 isolates, 94 from cattle, 85 from pigs and 37 from chickens, were collected from 32 prefectures.

Fig. 3-2-1-1 Antimicrobial resistance rates of *E. coli* from diseased livestock



Between 2016 and 2020, the resistance rates to TC exceeded 50% in cattle, pigs, and chickens, with similar trends observed in 2020.

Resistance rates above 50% were observed for SM and ABPC, but not for TC. These drugs are used as veterinary drugs for cattle and pigs bacterial diarrhea caused by *E. coli* and colibacillosis in chickens. The results of this survey suggest that confirmation of antimicrobial susceptibility is essential for administration in clinical practice.

Although the resistance rate to CPFX and CTX was less than 30% in all species from 2016 to 2020, the resistance rate to CPFX and CTX in cattle and to CPFX in chickens increased in 2020. Therefore, it is necessary to continue to monitor the resistance rate.

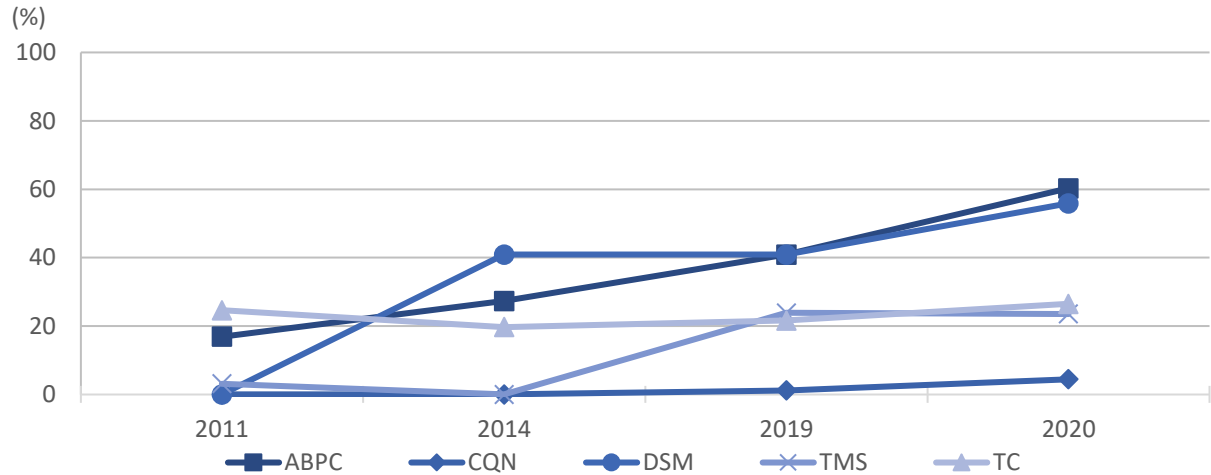
On the other hand, for CL, the resistance rate tended to decrease in both cattle and pigs, and it was 10.0% in chickens in 2019, but it was 0.0% again in 2020.

None of the strains isolated in any species were resistant to MEPM.

3-2-2 *Mannheimia haemolytica* (cattle)

From 22 prefectures, 68 isolates from cattle were collected.

Fig. 3-2-2-1 Antimicrobial resistance rates of *Mannheimia haemolytica* from cattle



M. haemolytica is one of the respiratory pathogens in cattle, and the main symptoms include pyrexia, nasal discharge, and cough, which are often caused by environmental stress sensitization. Approved drugs for bovine pneumonia or *M. haemolytica* include ABPC, CQN, DSM, TMS and TC.

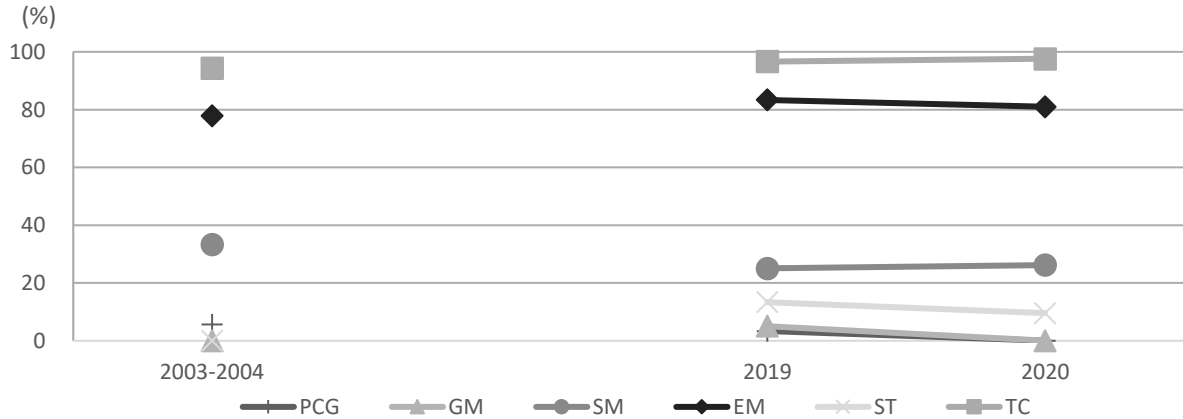
In *M. haemolytica* from diseased cattle, the resistance rates to ABPC and DSM exceeded 40% in 2019 and 50% in 2020, with an increasing trend.

The resistance rates to TC and TMS were less than 30% in both 2019 and 2020, but the tendency of resistance to TC was slightly increasing, so the future trend of resistance should be monitored. On the other hand, the resistance rate to CQN was 5% or less, but as a 2nd line drug its use should be kept under close observation.

3-2-3 *Streptococcus suis* (pigs)

From 22 prefectures, 42 isolates from pigs were collected.

Fig. 3-2-3-1 Antimicrobial resistance rates of *Streptococcus suis* from diseased pigs



Streptococcus suis, which causes pneumonia and arthritis, etc. in pigs, showed a high resistance rate of more than 90% for TC and more than 80% for EM. The resistance rate to SM was less than 30% in both 2019 and 2020.

On the other hand, the resistance rate to ST was around 10%, GM and PCG were less than 10%, and there were no resistant strains to CEZ, due to it not being approved for pigs.

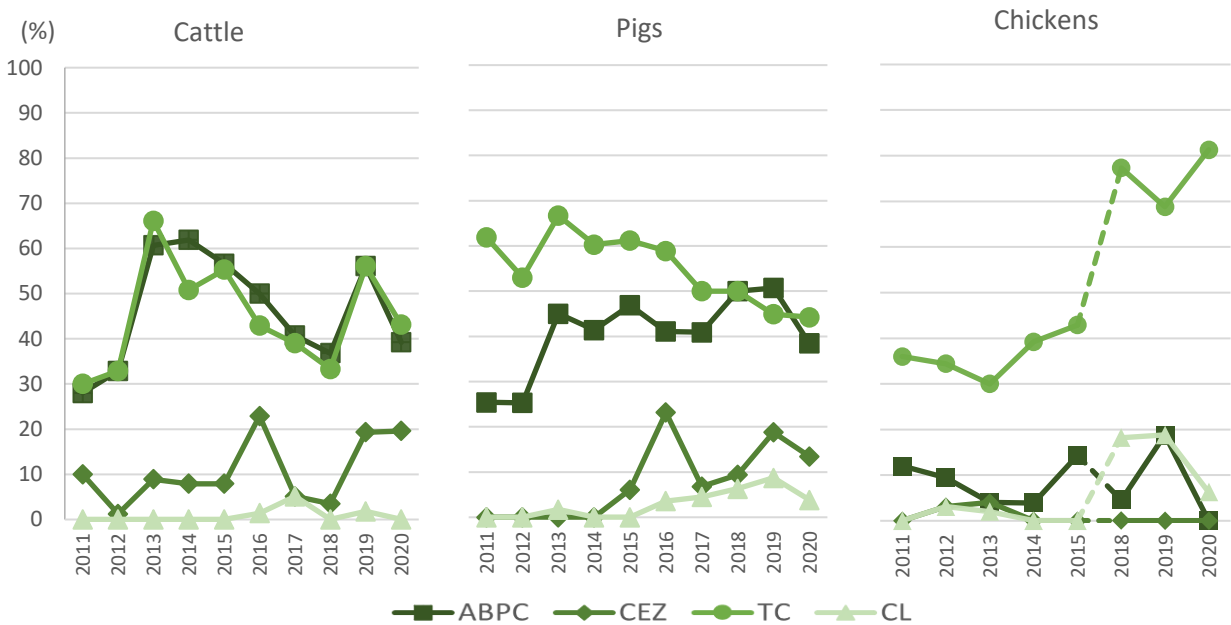
The results of 18 isolates from pigs collected and identified in 2003 and 2004 are shown in the figure for reference. Like the results in 2019-2020, the resistance rates, of TC and EM were high, and to PCG and GM were relatively low.

※In 2003-2004, MIC was measured with penicillin but not PCG, and OTC but not TC by agar plate dilutions.

3-2-4 *Salmonella* spp. (cattle, pigs, chickens)

A total of 120 isolates were collected from 25 prefectures, including 51 isolates from cattle, 53 isolates from pigs, and 16 isolates from chickens. The predominant serovars were 42 strains of 4:i:-, 24 strains of Typhimurium, followed by 16 strains of Dublin.

Fig. 3-2-4-1 Antimicrobial resistance rates of *Salmonella* spp. from diseased livestock



In *Salmonella* spp. from diseased livestock, the resistance rate to TC exceeded 30% in cattle, pigs and chickens from 2011 to 2020; the resistance rate decreased in cattle and pigs but increased to more than 80% in chickens from 2019 to 2020 .

The resistance rate to ABPC in cattle and pigs was more than 20% from 2011 to 2020, but decreased from 2019 to 2020. The resistance rate to CEZ in cattle decreased to less than 20% since 2017, but the resistance rate increased slightly from 2019 to 2020, so the future trend should be monitored.

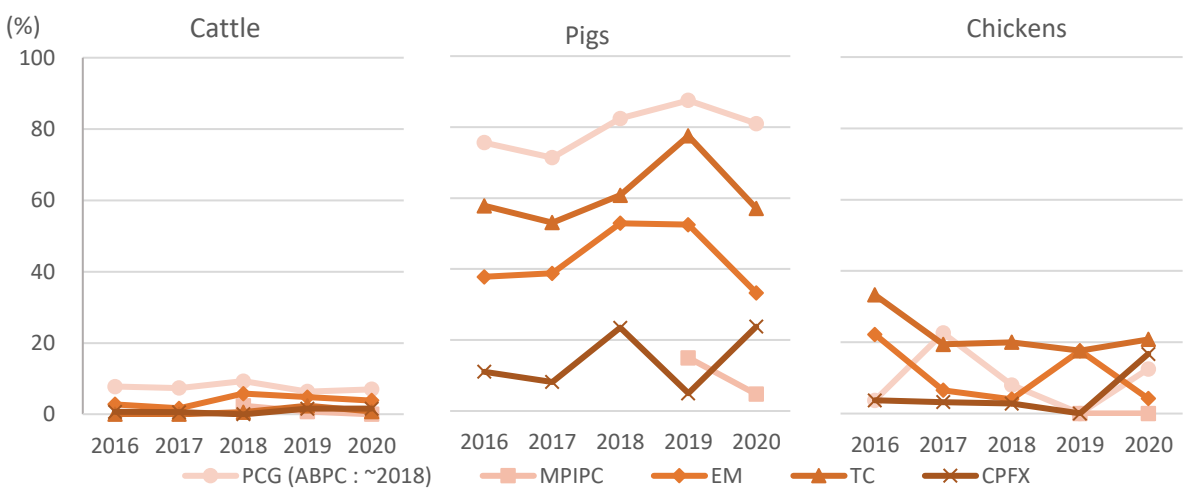
On the other hand, the CL resistance rate in cattle and pigs remained low, at less than 10% after 2011, while the resistance in chickens increased to more than 18% from 2018 to 2019, but that was less than 10% in 2020.

In addition, the isolation rate of Dublin tended to increase in cattle, and more than 80% of them were CL resistant strains.

3-2-5 *Staphylococcus aureus* (cattle, pigs, chickens)

A total of 173 isolates were collected from 39 prefectures, including 128 isolates from cattle, 21 isolates from pigs, and 24 isolates from chickens.

Fig. 3-2-5-1 Antimicrobial resistance rates of *S. aureus* from diseased livestock.



Resistance rates of *S. aureus* from diseased livestock from 2016 to 2020 are shown (Fig. 3-2-5-1).

In cattle, the resistance rate was maintained at less than 10% for all drugs. In pigs, the resistance rate to PCG (ABPC until 2018) exceeded 80%. EM in pigs resistance rate was above 50% in 2018 and 2019, but decreased to 33.3% in 2020. The resistance rate to TC in pigs was higher than 50%, although the resistance rate followed a decreasing trend, and the resistance rate in chickens has remained around 20% since 2017. The resistance rate to CPFX in chickens increased in 2020, and the future trend of resistance rates needs to be monitored.

The above 4 drugs are either approved for veterinary use or are of the same class, or medicines as those approved, and the sensitivity is generally maintained in cattle and chickens. On the other hand, the resistance rates to PCG and TC continue to do high in pigs, making it important to perform antimicrobial susceptibility testing for administration in clinical practice.

In addition, two strains which were resistant to MPIPC and identified as MRSA were isolated, one from cattle and one from pigs. Genomic analysis determined that these strains carry the *mecA* gene, indicating the necessity to monitor them closely in the future.

3-2-6 Summary

In *E. coli*, the resistance rate to TC has leveled off and the resistance rate to CL has decreased. The resistance rates to CPFX and CTX in pigs decreased, while those in cattle tended to increase. In chickens, the resistance rate to CPFX increased in 2020.

In *M. haemolytica*, the resistance rate to CQN, which is approved for cattle as a second-line drug, was 5% or less, while DSM and ABPC resistances were 50% or more.

S. suis showed higher resistance rates of more than 90% for TC and more than 80% for EM in drugs approved for pigs.

Salmonella spp. has a high resistance rate of more than 40% for TC approved for cattle, pigs and chickens. In addition, since Dublin isolated from cattle showed high resistance rates to CL and, exhibited multidrug resistance, it should be closely monitored.

In *S. aureus*, the sensitivity was generally maintained in cattle and chickens, but the resistance rate was more than 80% for PCG and more than 50% for TC in pigs.

The resistance rate to CPFX in chickens also increased in *S. aureus* in 2020. Since the same tendency has been observed in *E. coli*, it is necessary to pay attention to the trends including whether it is transiently rising or increasing based on a trend caused by the use of fluoroquinolones in chickens.

Overall, bacteria resistant to approved antimicrobial drugs were detected in diseased livestock. Although resistance rates against 2nd -line drugs were generally low, they exhibited concerning upward trends. Therefore, it is important to continue perform antimicrobial susceptibility testing and to be more discerning about when the use of antimicrobial drugs is necessary.

3-2-7 Acknowledgement

We would like to thank the livestock hygiene service center in providing strains for this project.

3-3 Healthy companion animals

In the AMR monitoring of healthy companion animal, rectal swab samples were collected from dogs and cats visited for medical checkups or vaccination, rather than treatment of the disease.The indicator bacteria, *Escherichia coli* and *Enterococcus* spp. were isolated. In 2020, samples from 172 dogs and 173 cats were collected. Table 3-3-1 shows the identification results and the number of isolated strains from the samples.

Table 3-3-1 Bacteria species and numbers of isolates

	Dogs	Number of strains	Cats	Number of strains
<i>Escherichia coli</i>	<i>E. coli</i>	146	<i>E. coli</i>	146
	<i>E. faecalis</i>	99	<i>E. faecalis</i>	64
	<i>E. gallinarum</i>	10	<i>E. hirae</i>	4
	<i>E. faecium</i>	9	<i>E. faecium</i>	3
	<i>E. hirae</i>	6	<i>E. gallinarum</i>	3
<i>Enterococcus</i> spp.	<i>E. avium</i>	2	<i>E. avium</i>	3
	<i>E. casseliflavus</i>	2		
	<i>E. durans</i>	1		
	Total	129	Total	77

3-3-1 *Escherichia coli*

Resistance rates of *E. coli* from dogs and cats from 2018 to 2020 are shown (Fig. 3-3-1-1, Fig. 3-3-1-2)

Fig. 3-3-1-1 Resistance rates of *E. coli* from dogs, 2018-2020.

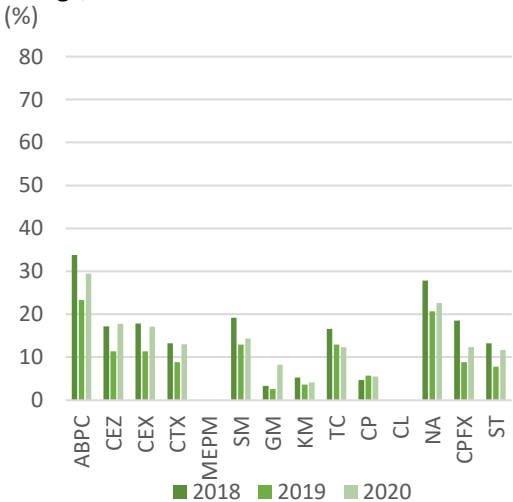
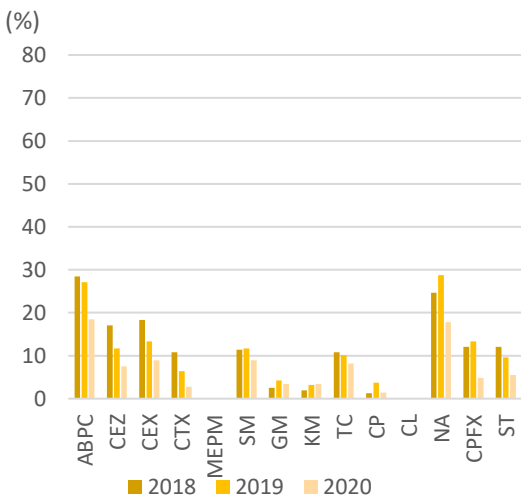


Fig. 3-3-1-2 Resistance rates of *E. coli* from cats, 2018-2020.



In 2020, the resistance rates of *E. coli* strains taken from dogs to the tested antimicrobials were less than 20% except ABPC and NA. Resistance rates to CTX (third generation cephalosporin) and CPFX (fluoroquinolones) were 12.3% and 13.0% respectively while MEPM (carbapenems) and CL (polypeptides) were both 0%. The resistance rate of *E. coli* from healthy dogs to GM was higher in 2020 than that in 2019 (Fig. 3-3-1-1). Resistance rates were significantly lower than those of *E. coli* from diseased dogs collected in the same year, except for GM, KM, CP and ST (Fig. 3-3-1-3).

The resistance rates of *E. coli* from cats were generally less than 20%, and the highest resistance rate was observed in ABPC followed by NA as in dogs (Fig. 3-3-1-2). The resistance tendency was similar to that of the dog-derived strains, being 4.8% for CPFX and 2.7% for CTX, and 0% for MEPM and CL. The resistance rates of NA and CPFX in 2020 were lower than that of healthy cat-derived *E. coli* in 2019. Resistance rates were all significantly lower than those of *E. coli* from diseased cats collected in the same year, except for KM and CP (Fig. 3-3-1-4).

Fig. 3-3-1-3 Resistance rates of *E. coli* from healthy and diseased dogs

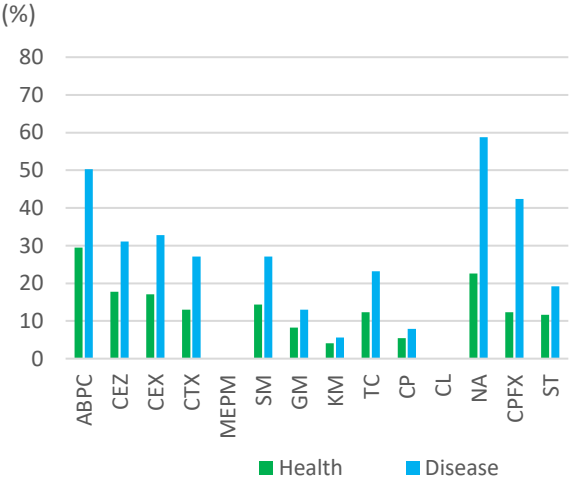
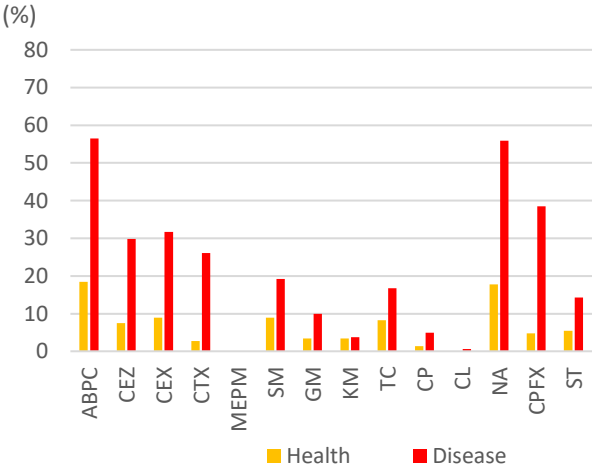


Fig. 3-3-1-4 Resistance rates of *E. coli* from healthy and diseased cats



3-3-2 *Enterococcus* spp.

Trends in resistance rates of *Enterococcus* spp. from dogs and cats from 2018 to 2020 are shown (Fig. 3-3-2-1, Fig. 3-3-2-2).

Fig. 3-3-2-1 Resistance rates of *Enterococcus* spp. from dogs, 2018-2020

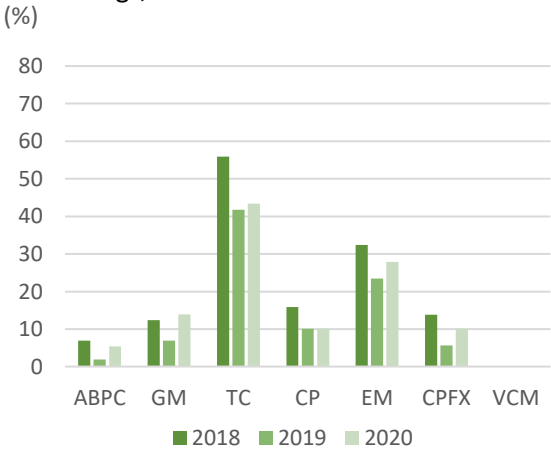
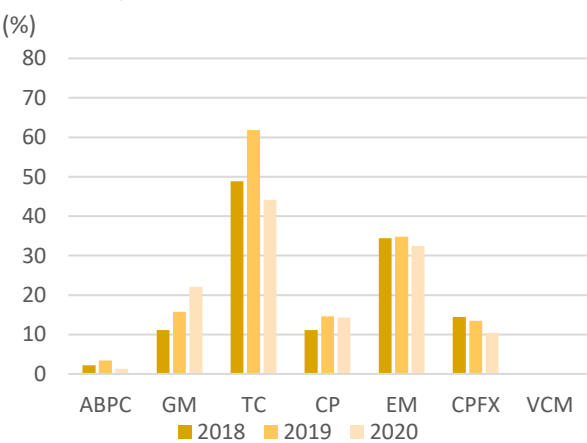


Fig. 3-3-2-2 Resistance rates of *Enterococcus* spp. from cats, 2018-2020



3-3 Healthy companion animals

In 2020, the resistance rates of dog-derived strains showed relatively high for TC and EM and less than 20% of all other drugs (Fig. 3-3-2-1). The resistance rate to CPFX was 10.1%, and to VCM was 0%. There were no differences in healthy dog derived *Enterococcus* spp. between 2019 and 2020. Resistance rates of all drugs were significantly lower than those of diseased dogs collected in the same year except for CP (Fig. 3-3-2-3).

The resistance rate of cat-derived strains were relatively high for TC, EM and GM, and less than 20% of the other drugs (Fig. 3-3-2-2). The resistance tendency was similar between the dog-derived strain and the cat-derived strain. The resistance rate to CPFX was 10.4%, and to VCM was 0%. Only TC was significantly lower when compared to healthy cat-derived strains in 2019. Resistance rates of all drugs were significantly lower than those of diseased cats collected in the same year except for GM and CP (Fig. 3-3-2-4).

Fig. 3-3-2-3 Resistance rates of *Enterococcus* spp. from healthy and diseased dogs

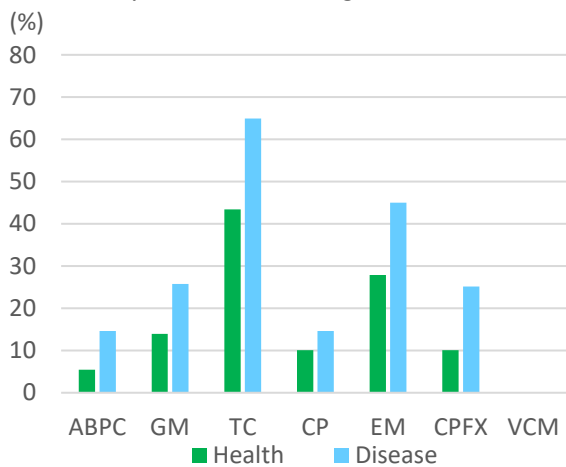
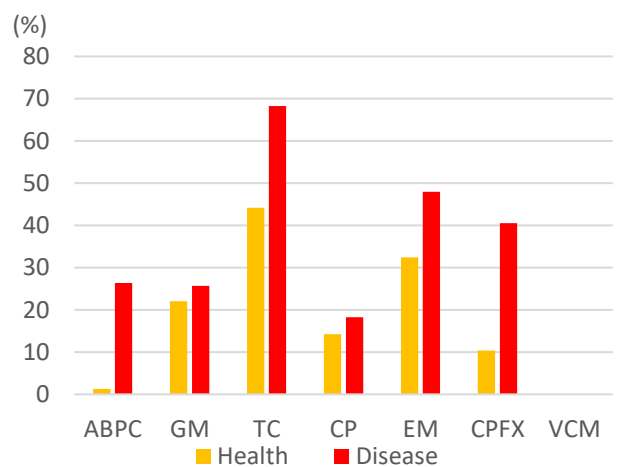


Fig. 3-3-2-4 Resistance rates of *Enterococcus* spp. from healthy and diseased cats



3-3-3 Questionnaire results

A questionnaire was administered to owners of 172 dogs and 173 cats sampled at animal clinics in 2020, and the results are shown on the following pages.

In terms of sex, about 50% of dogs were males and females, about 60% of cats were males and about 40% were females. In terms of age, the younger age group of 6-year-old or less in both dogs and cats occupied about 60%. About 60% of the dogs did not have other dogs living with them, but more than 60% of the cats lived with one or more other cats.

For both dogs and cats, the most common objective of the clinic visit was a medical checkup, followed by vaccination. More than 90% of both cats and dogs spent their daily lives indoors, except when walking their dogs or going out. The main diet was "commercial dry food" in more than 80% of dogs and cats, followed by "canine prescription diet" in dogs and "commercial wet food" in cats.

In the "3 month before sample collection" period, the 19% of dogs and 12% of cats had been administered antimicrobials. The most common administration route was oral administration in both dogs and cats. In addition, more than 90% of both dogs and cats had never been hospitalized and had no contact with a person who had suffered an infection or was hospitalized.

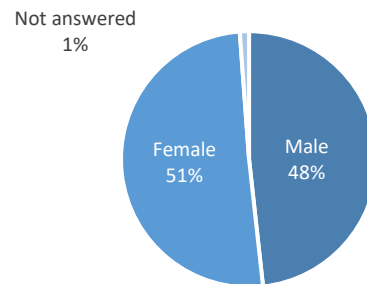
3-3 Healthy companion animals

Questionnaire results on dogs sampled in this survey in 2020 (number of responses 172)

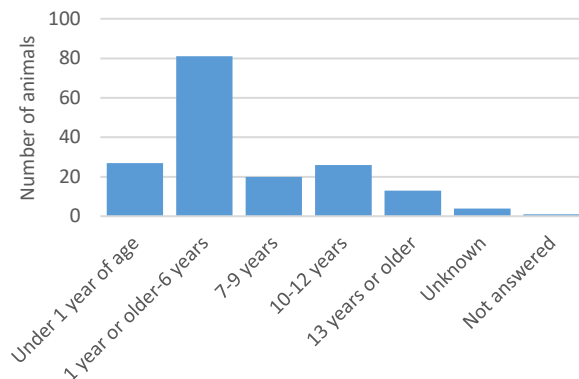
1. Species

Breed	Number of animals
Mongrel	34
Toy poodle	23
Dachshund	21
Chihuahua	13
Labrador retriever	11
Shiba dog	10
Maltese	7
Welsh corgi	6
Pomeranian	5
American cocker spaniel	4
Golden retriever	4
Yorkshire terrier	4
Italian greyhound	3
Shih tzu	3
Miniature schnauzer	3
Other	20
Not answered	1
Total	172

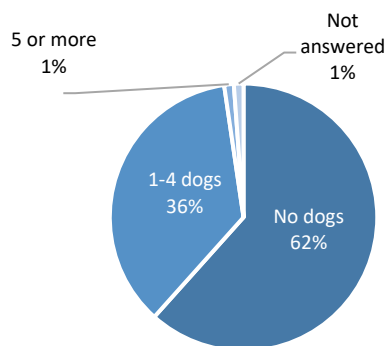
2. Sex



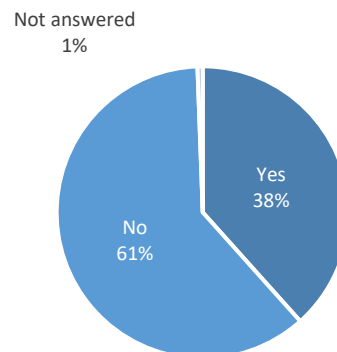
3. Age groups



4. Number of dogs living together



5. Cohabitation with animals other than dogs

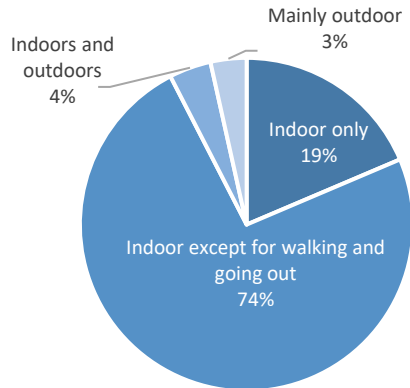


6. Purpose of the visit (multiple answers)

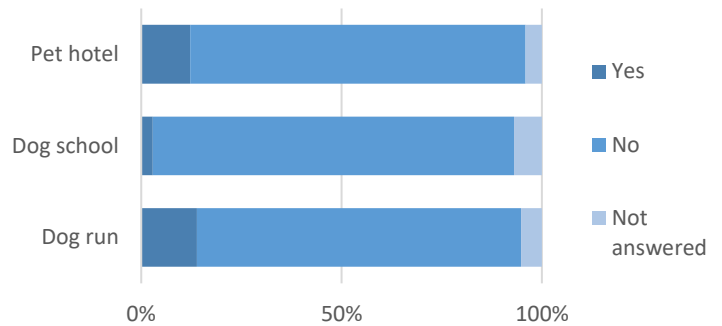
Purpose of the visit	Number of animals	%
Vaccination	50	29.1
Medical checkups	62	36.0
Trimming	17	9.9
Training Class	0	0
Pet hotel	7	4.1
Other	32	18.6
Not answered	7	4.1

<Questionnaire results: 3 months before sample collection>

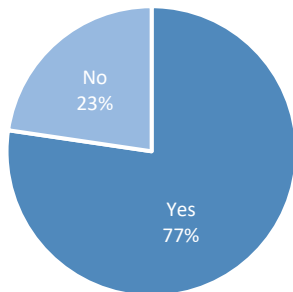
7. Where did you spend most of your time?



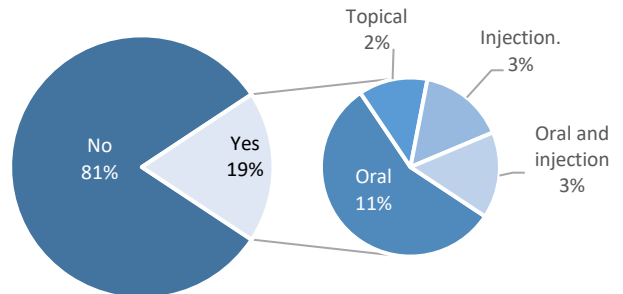
8. Have you used the following services?



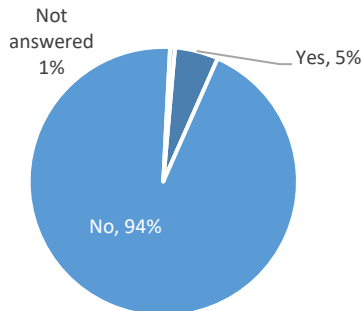
9. Have you visited animal clinic with a dog? (For any purpose of the visit)



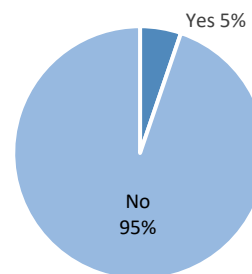
10. Has your dog administrated antimicrobials?
10-1. If yes, which was the route?



11. Has your dog been hospitalized?



12. Has your dog been in contact with anyone who has an infectious disease or is hospitalized?



13. What is the main diet?
(multiple answers)

Diet	Number of animals	%
Commercial dry food	143	83.1
Commercial wet food	21	12.2
Commercially semi-dried dog food	4	2.3
Canine prescription diet	36	20.9
The same as a human diet	4	2.3
Cooked for dogs at home	23	13.4
Human meals leftover	5	2.9
Raw vegetables	9	5.2
Raw meat, bone	0	0

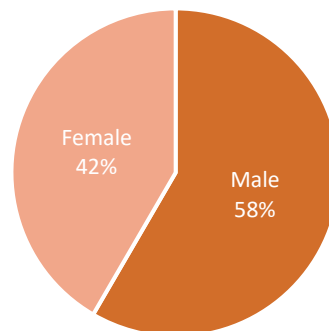
3-3 Healthy companion animals

Questionnaire results on cats sampled in this survey in 2020 (number of responses 173).

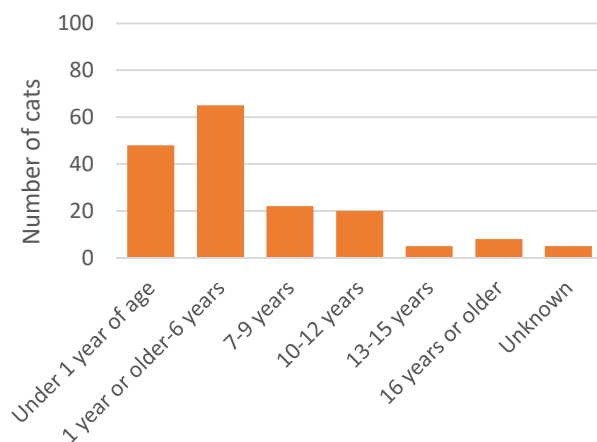
1. Species

Breed	Number of animals
Mongrel (Including those described as Mix, Japanese cats or cats in the answer)	152
American short hair	5
Scotish fold	2
British short hair	2
Munchkin	2
Minuet	2
Abyssinian	1
Bengal	1
Main coon	1
Ragamaffin	1
Not answered	4
Total	173

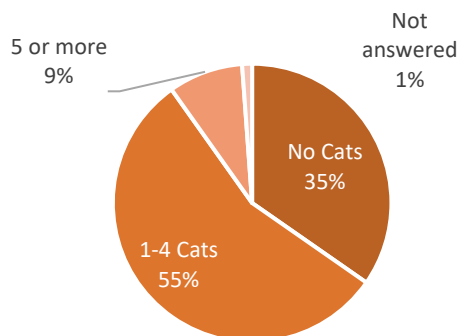
2. Sex



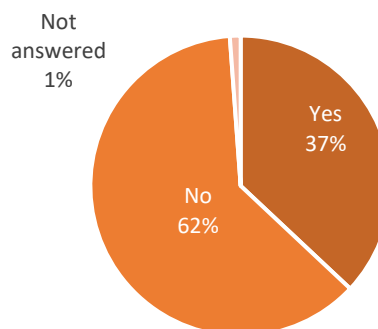
3. Age groups



4. Number of cats living together



5. Cohabitation with animals other than cats

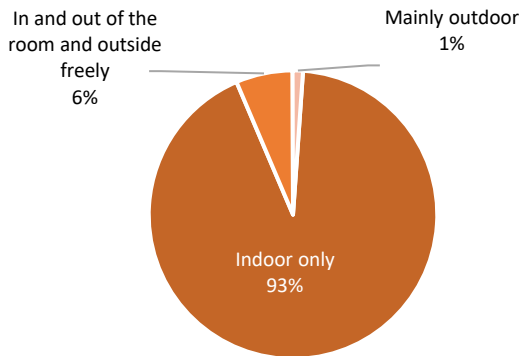


6. Objective of visit (multiple answers)

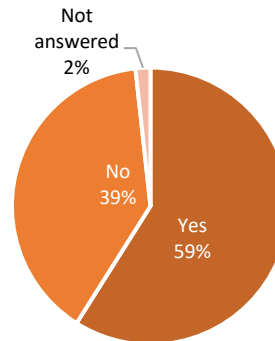
Purpose of the visit	Number of animals	%
Vaccination	51	29.5
Medical checkups	59	34.1
Trimming	3	1.7
Training class	1	0.6
Pet hotel	13	7.5
Other	45	26.0
Not answered	3	1.7

<Questionnaire results: 3 months before sample collection>

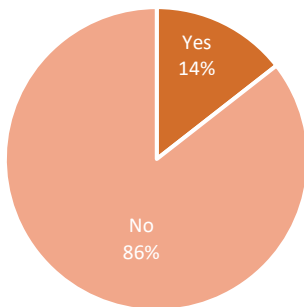
7. Where did you spend most of your time?



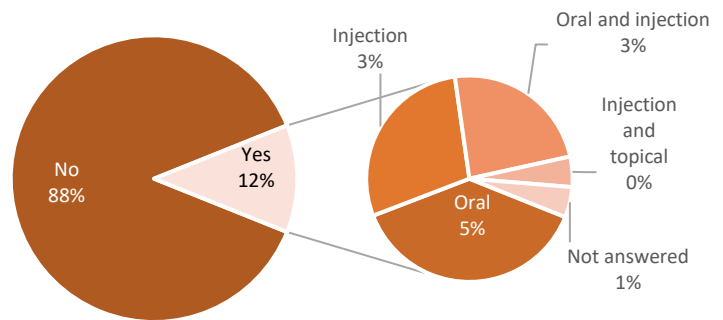
8. Have you visited a veterinary hospital with a cat? (For any purpose of the visit)



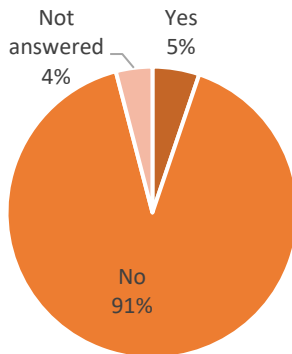
9. Did you use a pet hotel?



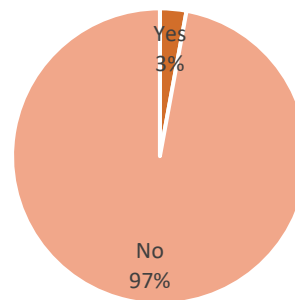
10. Has your cat administrated antimicrobials?
10-1. If yes, which was the route?



11. Has your cat hospitalized?



12. Has your cats been in contact with anyone who has an infectious disease or is hospitalized?



13. What is the main diet?
(Multiple answers)

Diet	Number of animals	%
Commercial dry food	142	82.1
Commercial wet food	57	32.9
Commercial semi-dried type cat food	7	4.0
Cat prescription diet	35	20.2
The same as a human diet	1	0.6
Cooked at home for cats	2	1.2
Human meals leftover	1	0.6
Raw vegetables	0	0
Raw meat	1	0.6
Not answered	1	0.6

3-3-4 Summary

Since companion animals have much closer contact with humans (owners) than livestock, there is a concern about the possibility of antimicrobial resistant bacteria transmission from humans to animals or vice versa. In the present, the resistance rate of *E. coli* for carbapenem, which is a critically important antimicrobial agent as a last treatment measure against multidrug resistant bacteria, and the resistance rate of *Enterococcus* spp. to VCM, which is a problem in the human nosocomial infection, were both 0%. Resistance rates to third generation cephalosporins and fluoroquinolones, which are very important antibacterial agents in human medicine, were also less than 20% for both *E. coli* and *Enterococcus* spp. The resistance rate of bacteria from healthy dogs and cats was lower than that of bacteria from diseased dogs and cats, and the antimicrobial susceptibility of commensal bacteria in healthy dogs and cats were at a well maintained low level. In the questionnaire results, about 60% of all healthy dogs and cats that collected samples in this time were in the young age group, and over 80% did not receive antimicrobials within 3 months.

3-3-5 Acknowledgement

We would like to thank the veterinarians and staff at animal hospitals throughout Japan who have cooperated in collecting the samples for this project, as well as the owners who have agreed to collecting samples, and dogs and cats who have provided samples. Additionally, the Japanese Veterinary Medical Association deserves special thanks for their devoted cooperation with our investigations.

3-4 Diseased companion animals

For the AMR monitoring of diseased dogs and cats, strains isolated from samples submitted to clinical laboratories in 2020 were collected and tested for antimicrobial susceptibility. The target bacterial species and the sampling sites are shown in Table 3-4-1, and the number of strains collected is shown in Table 3-4-2.

Table 3-4-1 Bacteria species and sampling sites collected in 2020

Species	Sampling site
<i>Escherichia coli</i> , <i>Klebsiella</i> spp.	urine, reproductive tract
<i>Enterobacter</i> spp.	urine
<i>Acinetobacter</i> spp., Coagulase-positive <i>Staphylococcus</i> spp.	urine, skin
<i>Enterococcus</i> spp	urine, ear

Table 3-4-2 Number of isolates

Species	Dogs	Number of strains	Cats	Number of strains
<i>Escherichia coli</i>		177		161
<i>Klebsiella</i> spp.	<i>K. pneumoniae</i>	71	<i>K. pneumoniae</i>	47
	<i>K. oxytoca</i>	6	<i>K. oxytoca</i>	10
	<i>K. aerogenes</i>	6	<i>K. aerogenes</i>	5
	Total	83	Total	62
<i>Enterobacter</i> spp.	<i>E. cloacae</i>	30	<i>E. cloacae</i>	30
	<i>E. asburiae</i>	1	<i>E. bugandensis</i>	1
	Total	31	Total	31
<i>Acinetobacter</i> spp.	<i>A. pittii</i>	10	<i>A. pittii</i>	3
	<i>A. radioresistens</i>	6	<i>A. baumannii</i>	2
	<i>A. baumannii</i>	5	<i>A. Johnsonii</i>	2
	<i>A. nosocomialis</i>	2	<i>A. ursingil</i>	2
	<i>A. guillouiae</i>	2	<i>A. lwoffii</i>	1
	<i>A. calcoaceticus</i>	1	<i>A. junii</i>	1
	<i>A. johnsonii</i>	1		
	<i>A. ursingil</i>	1		
	<i>A. lwoffii</i>	1		
	<i>A. baylyi</i>	1		
	Total	30	Total	11
Coagulase-positive <i>Staphylococcus</i> spp.	<i>S. pseudintermedius</i>	74	<i>S. pseudintermedius</i>	49
	<i>S. aureus</i>	4	<i>S. aureus</i>	26
	Total	78	Total	75
<i>Enterococcus</i> spp.	<i>E. faecalis</i>	130	<i>E. faecalis</i>	94
	<i>E. faecium</i>	22	<i>E. faecium</i>	38
	<i>E. gallinarum</i>	7	<i>E. gallinarum</i>	7
	<i>E. avium</i>	5	<i>E. casselifravus</i>	3
	<i>E. durans</i>	3	<i>E. hirae</i>	2
	<i>E. casselifravus</i>	3	<i>E. raffinosus</i>	2
	<i>E. hirae</i>	1	<i>E. avium</i>	1
			<i>E. durans</i>	1
	Total	171	Total	148

3-4-1 *Escherichia coli* (dogs, cats)

Fig. 3-4-1-1 Resistance rates of *E. coli* from dogs, 2017-2020

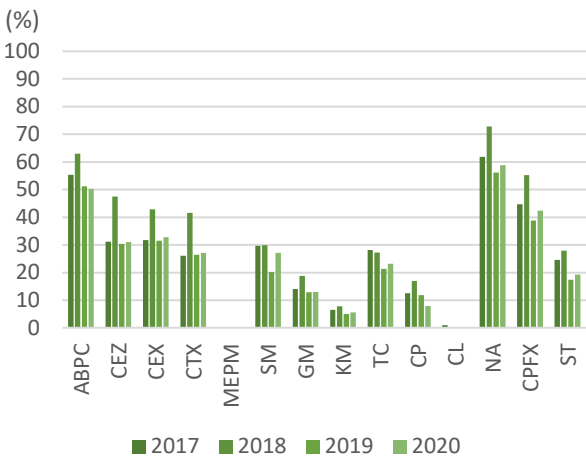
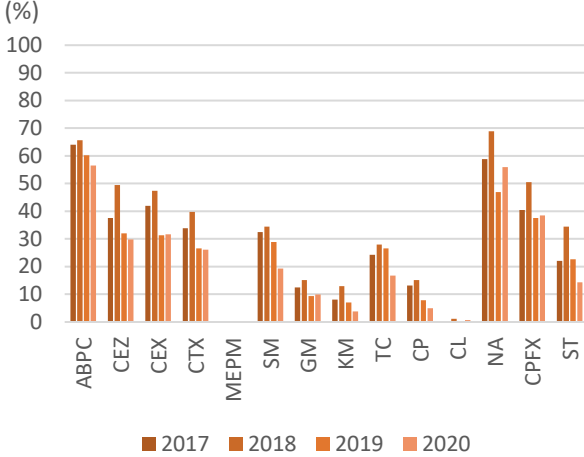


Fig. 3-4-1-2 Resistance rates of *E. coli* from cats, 2017-2020



In 2020, the resistance rates of *E. coli* isolated from dogs and cats showed similar trends to previous results. Resistance rates to ABPC, NA and CPFX, CEX and CEZ tended to be higher (Fig. 3-4-1-1, Fig.3-4-1-2).

The resistance rates in dogs and cats to fluoroquinolones (CPFX) were 42.4% and 38.5%, while the third-generation cephalosporins (CTX) were 27.1% and 26.1% respectively. One strain from cats was resistant to CL of polypeptide but no *mcr1* –*mcr10* gene was detected in this strain. The resistance rates for MEPM in carbapenems was 0.0% in both dogs and cats.

3-4-2 *Klebsiella* spp. (dogs, cats)

Klebsiella spp. isolated from dogs and cats were mostly *K. pneumoniae*, followed by *K. oxytoca*, *K. aerogenes* (Table 3-4-2).

Fig. 3-4-2-1 Resistance rates of *Klebsiella* spp. from dogs, 2017-2020

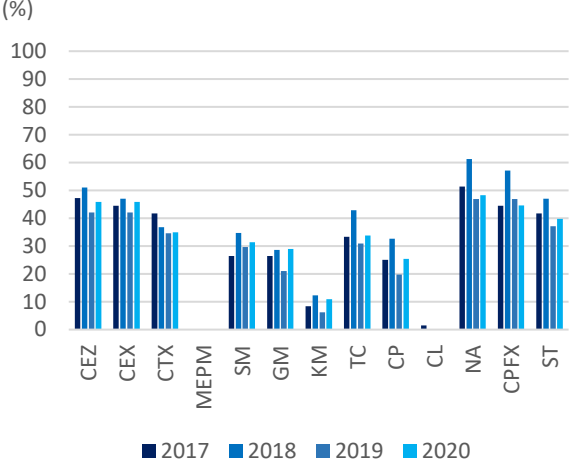
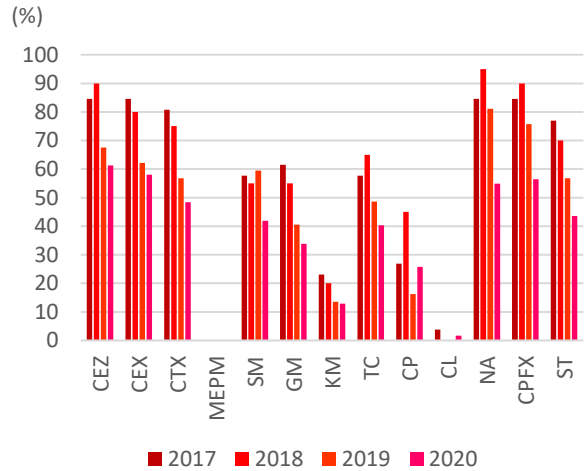


Fig. 3-4-2-2 Resistance rates of *Klebsiella* spp. from cats, 2017-2020



3-4 Diseased companion animals

The resistance rates of dog-derived strains against the tested drugs were highest in the following order: NA, CEZ, CEX, CPFX, ST, CTX, TC and SM. This trend was similar to the results observed in 2019 (Fig. 3-4-2-1).

The resistance rates of cat-derived strains against the tested drugs were highest in the following order: CEZ, CEX, CPFX, NA, CTX, ST, SM, TC and GM, and the resistance rate to NA was significantly lower than that in 2019 (Fig. 3-4-2-2).

The resistance rates of isolates from dogs and cats to CPFX were 44.6% and 56.5%, and that to CTX was 34.9% and 48.4%, respectively. The resistance rates to MEPM were both 0.0%. Additionally, one strain of cat-derived strains was resistant to CL.

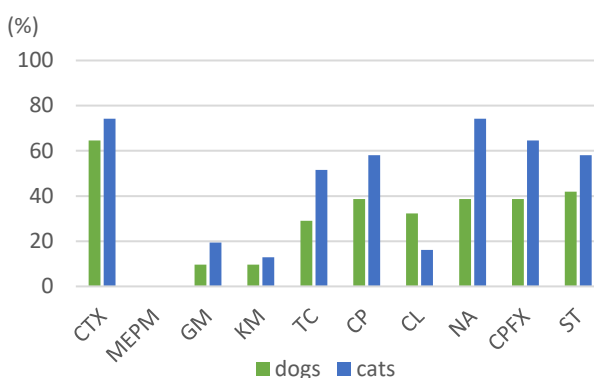
3-4-3 *Enterobacter* spp. (dogs, cats)

Enterobacter spp. isolated in 2020 were almost all *E. cloacae*, accompanied by 1 isolate of *E. asburiae* from dogs and *E. bugandensis* from cats (Table 3-4-2).

The resistance rates to the tested antimicrobials were the highest for CTX followed by ST, CP, NA, CPFX and CL in dog-derived strains, while the highest rates in cat-derived strains were for CTX and NA followed by CPFX, CP, ST and TC (Fig. 3-4-3).

The resistance rates of CTX in dogs and cats were 64.5% and 74.2%, respectively. For CPFX, the resistance rate were 38.7% and 64.5% in dogs and cats, respectively. However, the resistance rates to MEPM in strains derived from dogs and cats were 0.0%. Although the *mcr* gene is the cause of CL resistance and is known to spread through plasmids, there are known strains of *Enterobacter* spp. that express CL resistance without the *mcr* gene. These isolates express CL resistance while having no *mcr* gene detectable by PCR.

Fig. 3-4-3 Resistance rates of *Enterobacter* spp. from dogs and cats, 2020



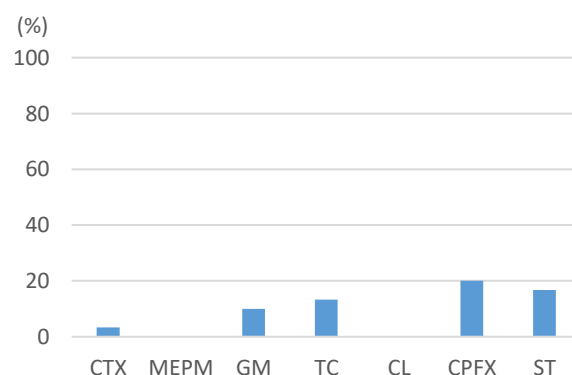
3-4-4 *Acinetobacter* spp. (dogs)

30 *Acinetobacter* spp. Isolates were obtained from dogs, with the most common species being *A. pittii*, followed by *A. radioresistens* and *A. baumannii* in 2020 (Table 3-4-2). The number of isolates from cats was small (11 isolates) and the resistance rate is not shown.

The resistance rate of CPFX was higher than the other antimicrobials in dog-derived strains while still being less than 20%.

The resistance rate of CTX in dog-derived strains was 3.3% and that of CPFX was 20.0%. The resistance rate of MEPM was 0.0%.

Fig. 3-4-4 Resistance rates of *Acinetobacter* spp. from dogs, 2020



3-4-5 Coagulase positive *Staphylococcus* spp. (dog, cats)

In 2020, most of the coagulase-positive *Staphylococcus* spp. isolated from dogs were *Staphylococcus pseudintermedius* with a few *S. aureus* isolates as well. Similarly, both *S. pseudintermedius* and *S. aureus* were isolated from cats, although the distribution of species being varied between dogs and cats (Table 3-4-2). As breakpoint by CLSI and EUCAST varies among staphylococci depending on the species, thus the resistance rate was calculated by species. The results are shown for *S. pseudintermedius* from dogs and cats and *S. aureus* from cats, with more than 20 strains isolated (Fig. 3-4-5-1, Fig. 3-4-5-2).

Fig. 3-4-5-1 Resistance rate of *S. pseudintermedius* from dogs, 2017-2020

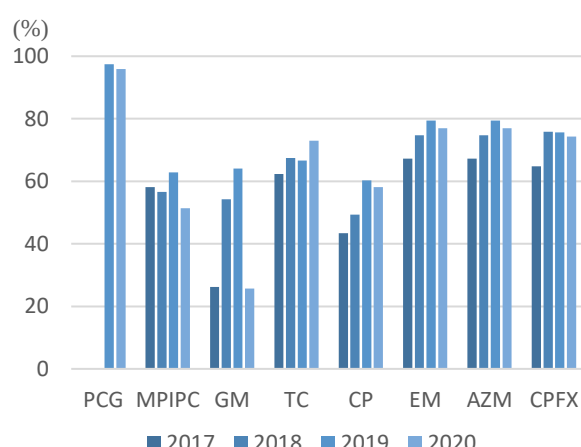
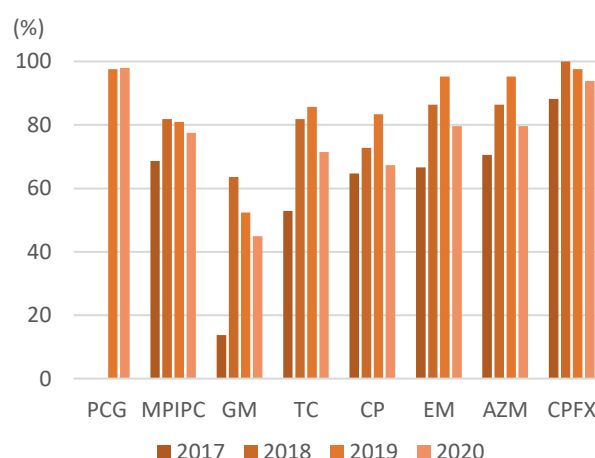


Fig. 3-4-5-2 Resistance rate of *S. pseudintermedius* from cats, 2017-2020

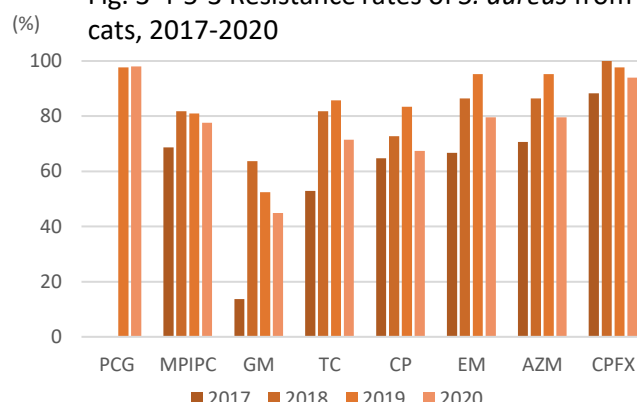


In 2020, the resistance rates of *S. pseudintermedius* derived from dogs against the tested antimicrobials were highest in the following order: PCG, EM, AZM, CPFX, TC, CP and MIPIC. The resistance rate of GM was significantly lower than that in 2019 (Fig. 3-4-5-1). The resistance rates of *S. pseudintermedius* derived from cats to the tested drugs were highest in the following order: PCG, CPFX, EM, AZM, MIPIC, TC, CP and GM. The resistance rate of EM and AZM were significantly lower than that in 2019 (Fig. 3-4-5-2).

The Antimicrobial resistance rates of *S. aureus* derived from cats against the tested drugs were highest in the following order: PCG, CPFX, MIPIC, CEX, CFX, CTX, EM, AZM, CEZ, GM and TC (Fig. 3-4-5-3). This trend is similar to the results observed in 2019.

Caution is necessary when comparing the resistance rates as the number of strains in 2018 was less than 20.

Fig. 3-4-5-3 Resistance rates of *S. aureus* from cats, 2017-2020



The resistance rates of cat-derived strains in *S. pseudintermedius* were significantly higher than those of dog-derived strains in CPFX, MIPIC and GM. In dogs and cats, the resistance rates to CPFX were 74.3% and 93.9%, and 77.0% and 79.6% for AZM of 15-membered ring macrolides, respectively. The resistance rates to MIPIC were 51.4% in *S. pseudintermedius* from dogs, 77.6% in *S. pseudintermedius* from cats, and 65.4% in *S. aureus* from cats.

3-4-6 *Enterococcus* spp. (dogs, cats)

In 2020, *Enterococcus faecalis* was the most commonly isolated enterococci from dogs and cats, followed by *E. faecium*. Additionally, *E. gallinarum*, *E. avium*, *E. casseliflavus*, *E. durans* and *E. hirae* were isolated from dogs while *E. gallinarum*, *E. casseliflavus*, *E. hirae*, *E. raffinosus*, *E. avium* and *E. durans* were isolated from cats (Table 3-4-2).

Fig. 3-4-6-1 Resistance rates of *Enterococcus* spp. from dogs, 2017-2020

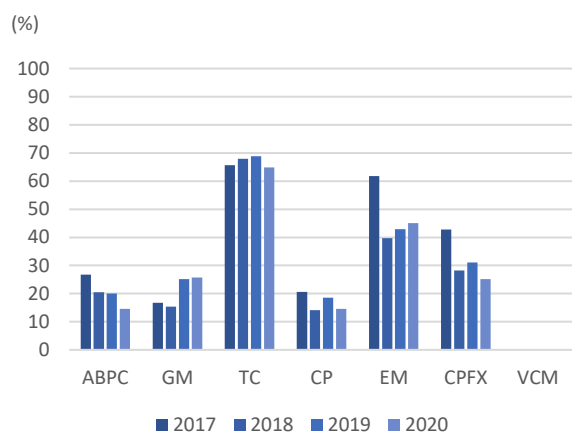
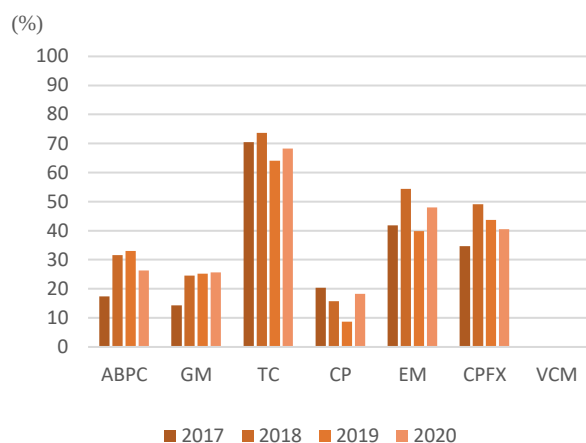


Fig. 3-4-6-2 Resistance rates of *Enterococcus* spp. from cats, 2017-2020



In 2020, the resistance rates against tested antimicrobials in both dogs and cats were the highest in the following order: TC, EM and CPFX. These rates were similar to those of 2019, but the resistance rate to CP was significantly higher compared to cat-derived strains in 2019 (Fig. 3-4-6-1, Fig. 3-4-6-2).

Additionally, the resistance rates for ABPC and CPFX in cat-derived strains were significantly higher than in dog-derived strains. The resistance rate to CPFX was 25.1% in dogs and 40.5% in cats. The resistance rates of VCM, which cause nosocomial infections in the human medicine, were 0.0% in both dog-derived strains and cat-derived strains.

As enterococci differ in their resistance profiles between *E. faecalis* and *E. faecium* (e.g., for ABPC, *E. faecalis* is usually susceptible but *E. faecium* is often resistant), the numbers of resistant strains and resistance rates are individually shown (Fig. 3-4-6-3 ~ Fig. 3-4-6-6).

Fig. 3-4-6-3 Resistance rates of *E. faecalis* from dogs, 2017-2020

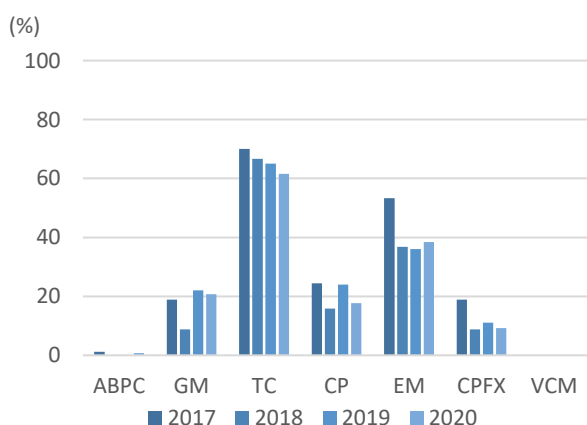
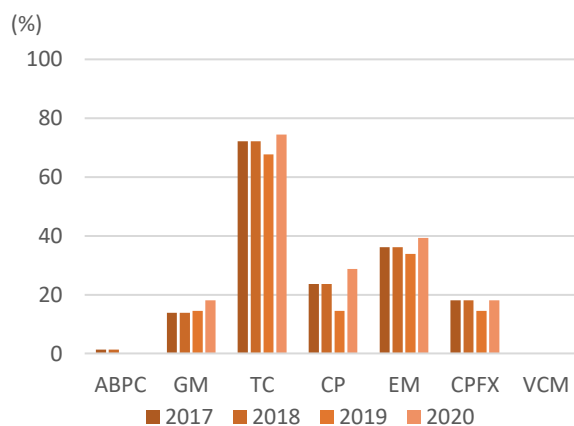


Fig. 3-4-6-4 Resistance rates of *E. faecalis* from cats, 2017-2020



The resistance rates of *E. faecalis* were found to be the highest for TC and EM, which is consistent with the results in 2019. Only one strain showed resistance to ABPC.

The resistance rate of *E. faecium* was the highest for CPFX, followed by ABPC, EM, TC and GM, in decreasing order. As there were only 22 strains of *E. faecium* from dogs, the resistance rate is shown as a reference. The resistance profile of *E. faecalis* and *E. faecium* tended to be similar regardless of whether they were derived from dogs and cats.

Fig. 3-4-6-5 Resistance rates of *E. faecium* from dogs, 2017-2020

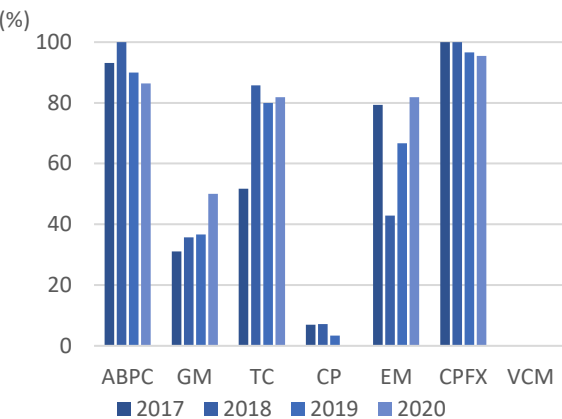
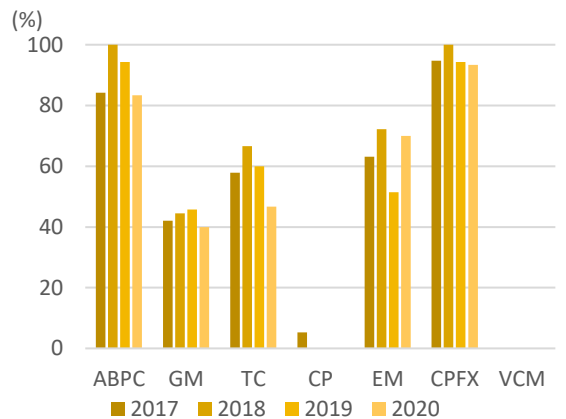


Fig. 3-4-6-6. Resistance rates of *E. faecium* from cats, 2017-2020



3-4-7 Summary

The overall trend was similar to previous years for *E. coli*, *Klebsiella* spp., coagulase-positive *Staphylococcus* spp., and *Enterococcus* spp., which have been collected continuously since the survey began in 2017. In 2020, *Enterobacter* spp. showed higher resistance to third-generation cephalosporins and fluoroquinolones. On the other hand, the dog-derived strains of *Acinetobacter* spp. showed resistance rates of less than 20% to all tested drugs. The resistance rate to MEPM in carbapenems, one of the most important antimicrobial agents in human medicine, was 0.0% in the investigated Gram-negative bacteria. Additionally, the resistance rate to VCM, which is a concern in human nosocomial infections, was also 0.0% in *Enterococcus* spp.

Third-generation cephalosporins and fluoroquinolones, as well as 15-membered ring macrolides and colistin, not approved for dogs and cats, are critically important in human medicine and are therefore second-line drugs used in the veterinary field when other antimicrobial agents are ineffective. Resistance rates to CTX were high in *Klebsiella* spp., *Enterobacter* spp. and *S. aureus* from cats, but not more than 30% in other strains. The resistance rate was broad for CPFX, ranging from 5.9 to 97.6%, and more than 70% for AZM in 15-membered ring macrolides (only coagulase-positive staphylococci). For CL, only a few strains showed resistance, primarily *Enterobacter* spp., and no *mcr* gene was detected in all CL-resistant strains isolated in 2020.

This survey was about bacteria from diseased dogs and cats, and the results are considered to be influenced by treatment with antimicrobials. There are many drugs with high resistance in some bacterial species. It is important to ensure the prudent use of antimicrobial agents such as selecting effective antimicrobial agents by conducting susceptibility testing prior to treatment and considering measures other than administration of antimicrobial agents such as washing and disinfection for dermatitis, so that antimicrobials can continue to be used effectively in the treatment of bacterial infections. The “Guidebook for the Prudent Use of Antimicrobials in Companion animals-2020-*)” promises to be helpful for the purpose.

* https://www.maff.go.jp/j/syouan/tikusui/yakuzi/attach/pdf/240328_7-8.pdf (only Japanese)

3-4-8 Acknowledgement

We would like to thank Sanritsu Zelkova Veterinary Laboratory, FUJIFILM VET Systems Co., Ltd., MIROKU Medical Laboratory, Inc., and IDEXX Laboratories, Inc. for their cooperation in providing strains for this project.

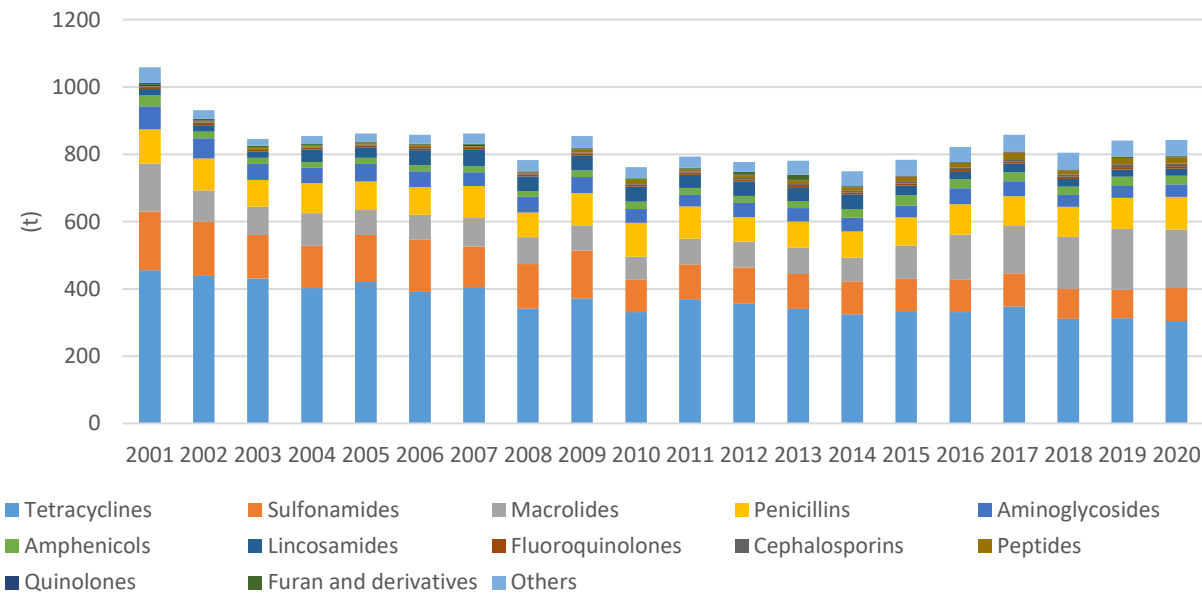
4 Antimicrobial sales volume

4-1 Veterinary antimicrobials

4-1-1 Sales volume of veterinary antimicrobial sales volume

To understand the present status in veterinary antibacterial use, the sales volume of antimicrobials for animals is estimated. Veterinary antibacterial sales volume has declined by around 20% since 2001. However, it has remained around 800t in recent years (Fig. 4-1-1). Tetracyclines are the most common class and accounting for about 40% of the total. But the sales of tetracyclines have been decreasing, reaching the lowest it has ever been in 2020.

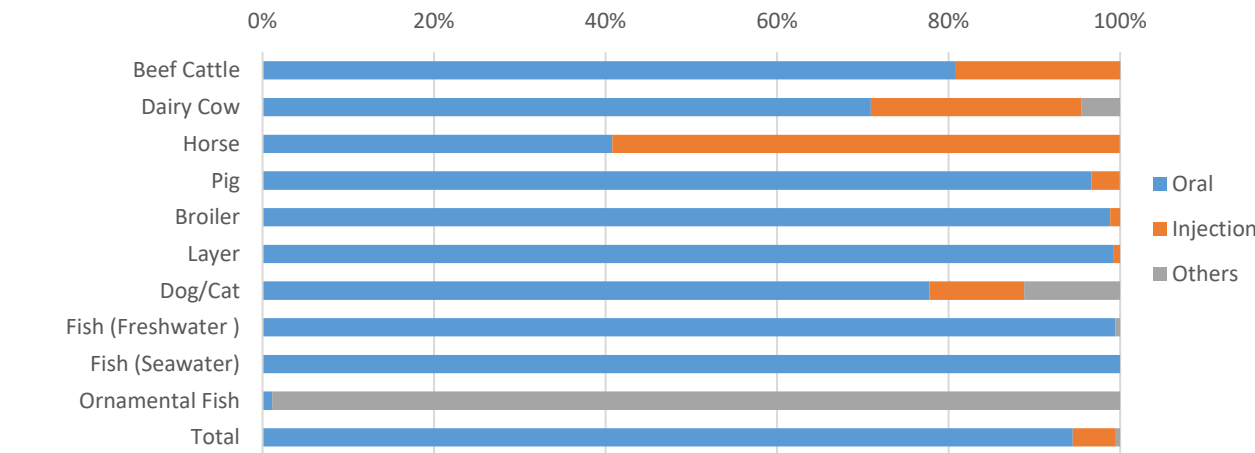
Fig. 4-1-1 Changes in the sales of antibacterials for animals (2001-2020)



Characteristics by administration route

Approximately 95% of antimicrobials are administered orally, followed by injections at less than 1% of others (Figure 4-1-2). “Others” include intramammary, intrauterine, topical etc.

Fig. 4-1-2 Percentage of sales volume of veterinary antimicrobials in each species by route of administration (2020)



4- I Sales volumes of veterinary antimicrobials

Estimated by animal species, the largest sale volume of veterinary antimicrobials was observed in pigs (Figure 4-1-3).

Changes across animal species are shown below (Figure 4-1-4).

Fig. 4-1-3 Percentage of animal antibacterial drug sales by animal species (2020)

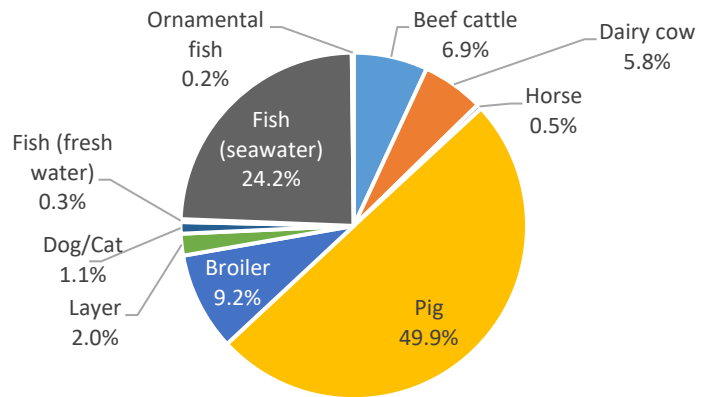
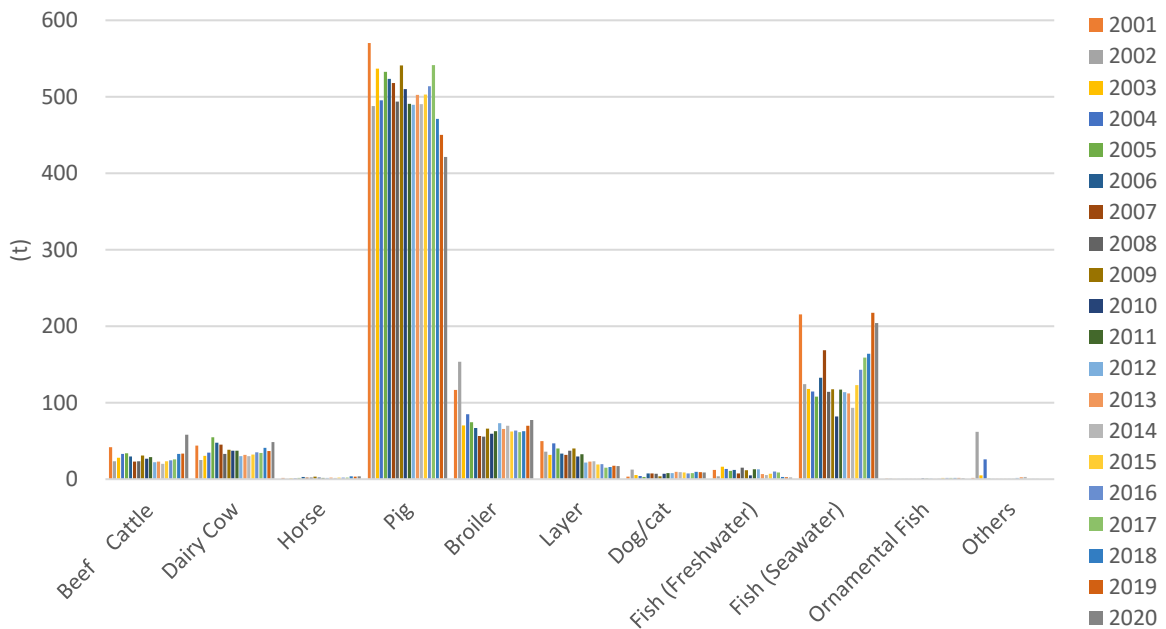
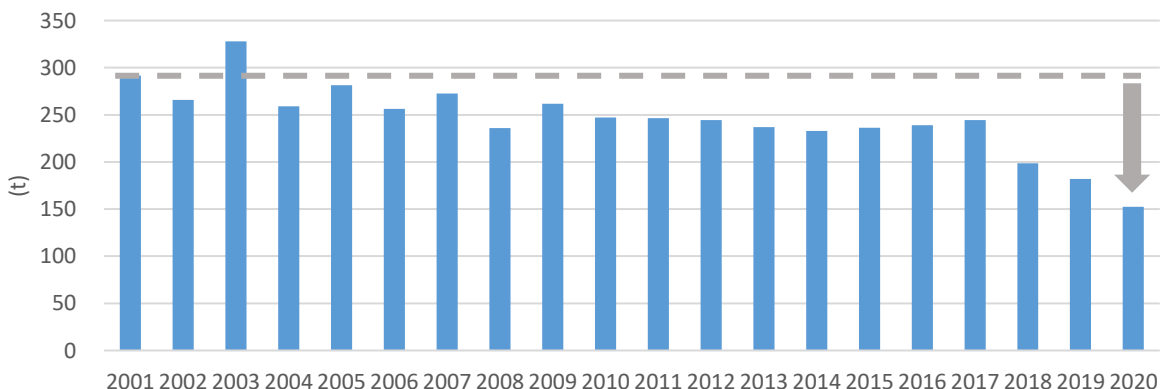


Fig. 4-1-4 Changes in the sales volume of veterinary antibacterials by species (2001-2020)



In recent years, the sales volume of antimicrobials for pigs have been decreasing, mainly due to a decrease in the sales of tetracyclines (Fig. 4-1-5). In 2020, it was almost halved compared to 2001.

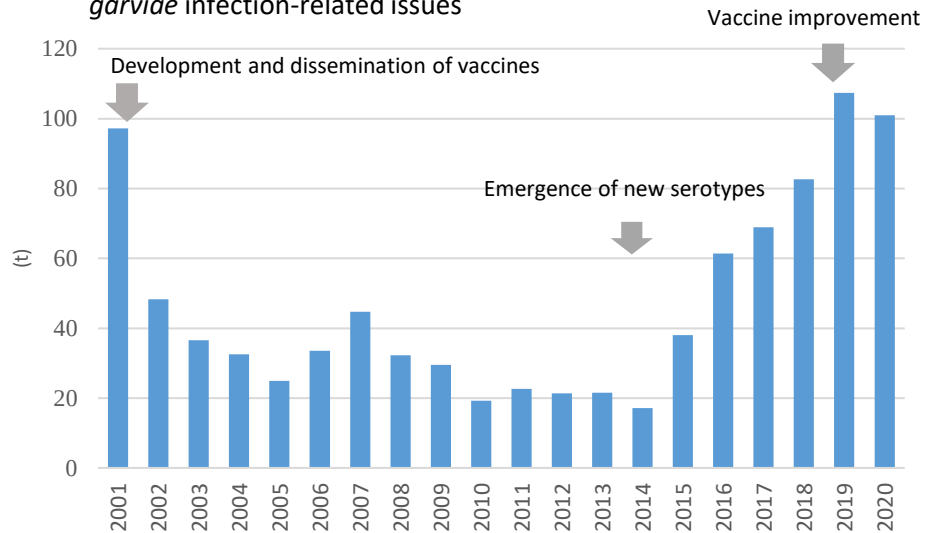
Fig. 4-1-5 Changes in the sales volume of tetracyclines in pigs



4- I Sales volumes of veterinary antimicrobials

The amount of veterinary antimicrobials sold to fish (seawater), which is the second highest after pigs, has increased since 2015, mainly due to an increase in macrolides (EM). The outbreak and treatment of the new serotypes of *Lactococcus garviae* which is cause of type II alpha-hemolytic streptococcosis was thought to be one of the main causes of that rise. In 2020, vaccines were improved and the beginnings at a potential downward trend in sales could be observed (Fig. 4-1-6).

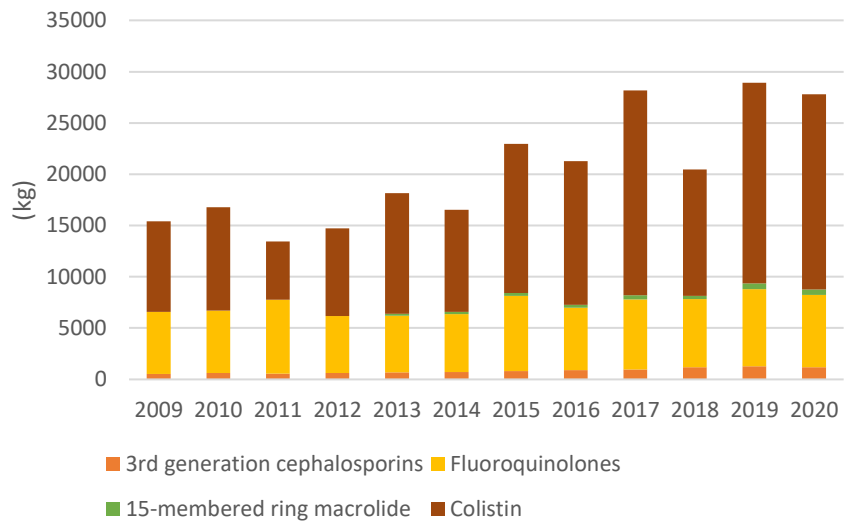
Fig. 4-1-6 Changes in EM sales of fish (seawater) and *Lactococcus garviae* infection-related issues



4-1-2 Sales volume of second-line drugs

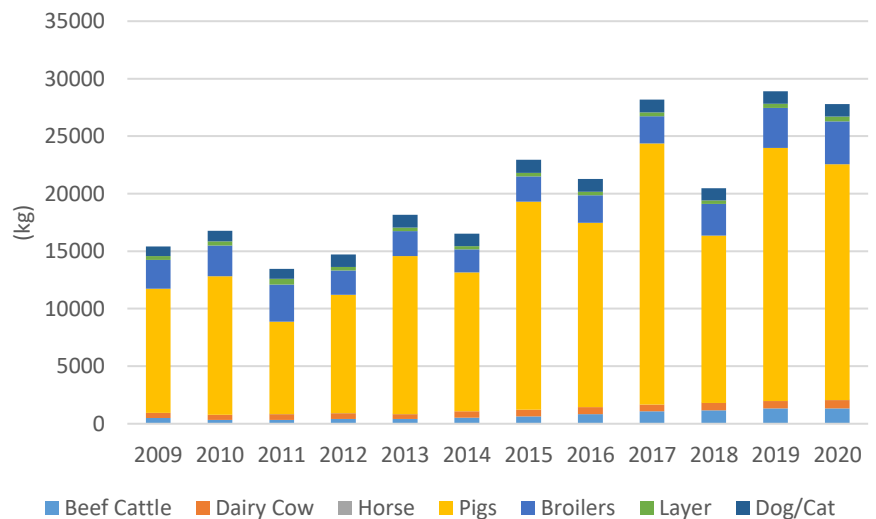
Antimicrobials which are important for human medicine and are limited to be used only when other antimicrobials are ineffective are called the "second-line drugs". Second-line drugs include fluoroquinolones, third-generation cephalosporins, 15-membered ring macrolides, and CL. Fig. 4-1-7 shows the changes in the sales of second-line drugs. The largest volume of sales was for CL, followed by fluoroquinolones.

Fig. 4-1-7 Second-line drug sales by drug (2009-2020)



Pigs accounted for a large proportion of administrated antimicrobials (Fig. 4-1-8). About 80% were CL, which increased after the withdrawal of CL as a feed additive in 2018. It rose again in 2019, but the development of vaccine for edema disease, for which CL is commonly prescribed, has created a downward trend.

Figure 4-1-8 Second-line drug sales by species (2009-2020)

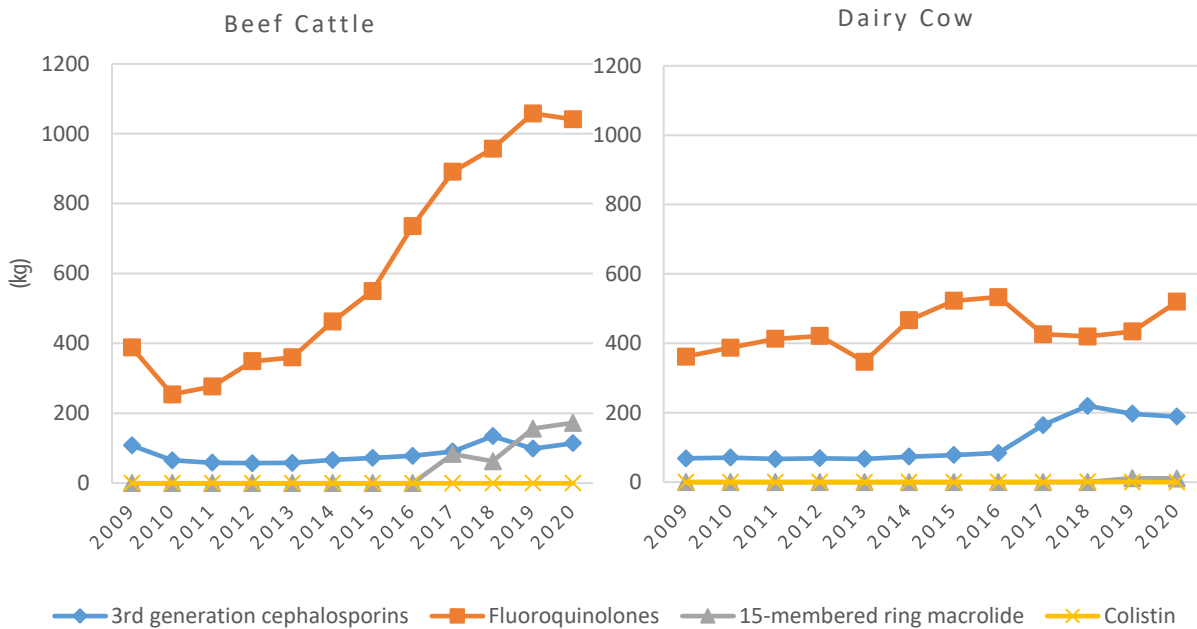


The largest sales amount of second-line drugs, excluding CL, was observed in broiler, and mostly consisted of fluoroquinolones. In recent years there is an observable upward trend in antimicrobial sales (Fig. 3-1-6). This suggests more attention should be paid in the future to change in sales amount and changed in sales.

4- I Sales volumes of veterinary antimicrobials

Although the sales of second-line drugs for cattle (beef cattle and dairy cow) are lower than those for pigs and broiler, fluoroquinolones and 15-membered ring macrolides in beef cattle and third-generation cephalosporins in dairy cow have increased in recent years (Fig. 4-1-9), and the resistance rates should be monitored continuously.

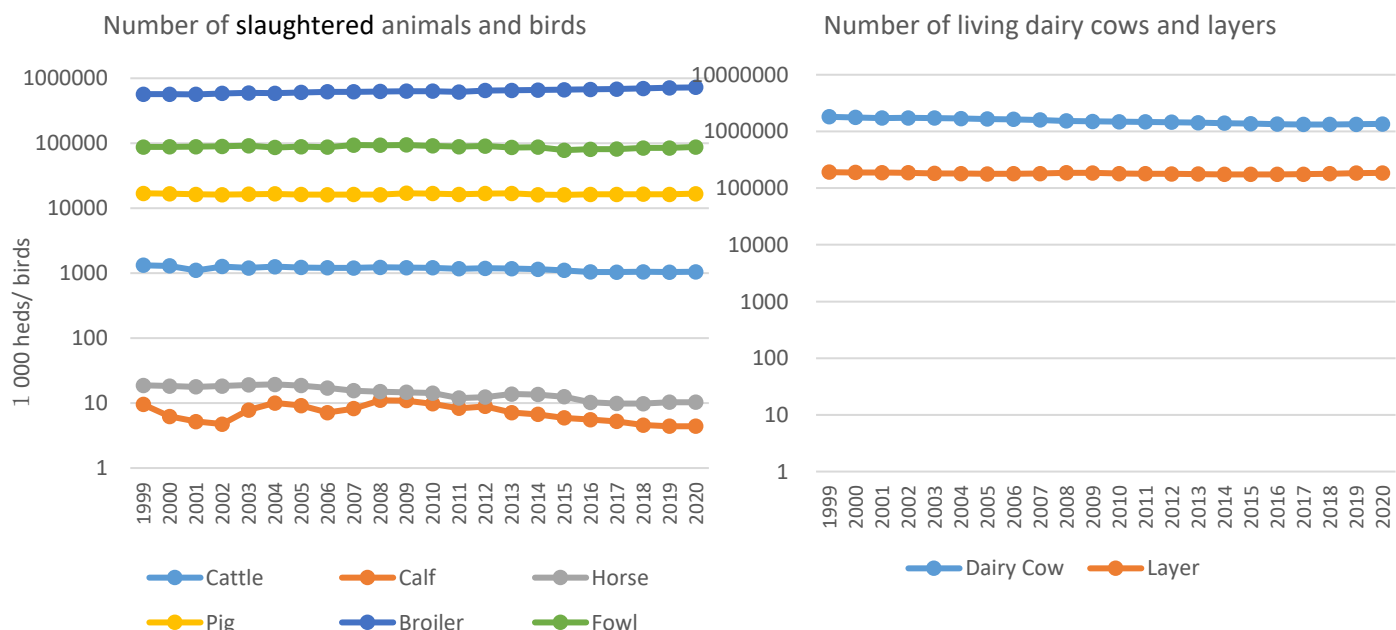
Fig. 4-1-9 Changes in second-line drugs sold to beef cattle and dairy cow (2009-2020)



4-1-3 Changes in the number of domestic animals and poultry in Japan

The change of the number of slaughtered and living number of head/birds for dairy cows and layers is shown (Fig. 4-1-10). It is observable that no major changes occurred in the last two decades. Therefore, the change in sales amount was not affected much by the increase or decrease in the number of animals.

Fig. 4-1-10 The number of slaughtered animals and birds, and dairy cows and layer numbers in farms (2001-2020)



4-1-4 Summary

In 2020, the sales volume of antimicrobials remained almost unchanged (increased by about 1.3t from 2019).

The sales volume of tetracyclines decreased 8.6t and macrolides decreased 7.0t than last year, and sulfonamides increased 13.8t from 2019.

By species, sales volume for pigs continued to decline (29.0t) and fish (seawater) also to decline (13.5t). In pigs, a greater reduction in tetracyclines was observed. Second-line drugs in pigs did not see large increases but the volume sold was higher than other animals. The sharp increase of macrolides in fish (seawater) turned into a decrease trend, and it seemed to be caused by the improvement of the vaccine for the caused disease. In beef cattle, dairy cow, and broilers, sales amounts were increased 25.0t, 11.9t, 7.7t respectively, sulfonamides increased in all of these species, and tetracyclines were also increased in cattle specifically.

Among second-line drugs, CL is the most sold drug, and the market is almost dominated by use in showed a slight decrease in 2020, and is expected to decrease further as a result of the distribution of the new vaccine. Broilers are the most represented in fluoroquinolones sold by species with sales volume increasing in recent years. In beef cattle, third generation cephalosporins, fluoroquinolones, and 15-membered ring macrolides sales showed a steady increase. Fluoroquinolones and third-generation cephalosporins sales volumes in dairy cows have also increased in recent years. These trends should be closely monitored in conjunction with resistance rates, hereafter.

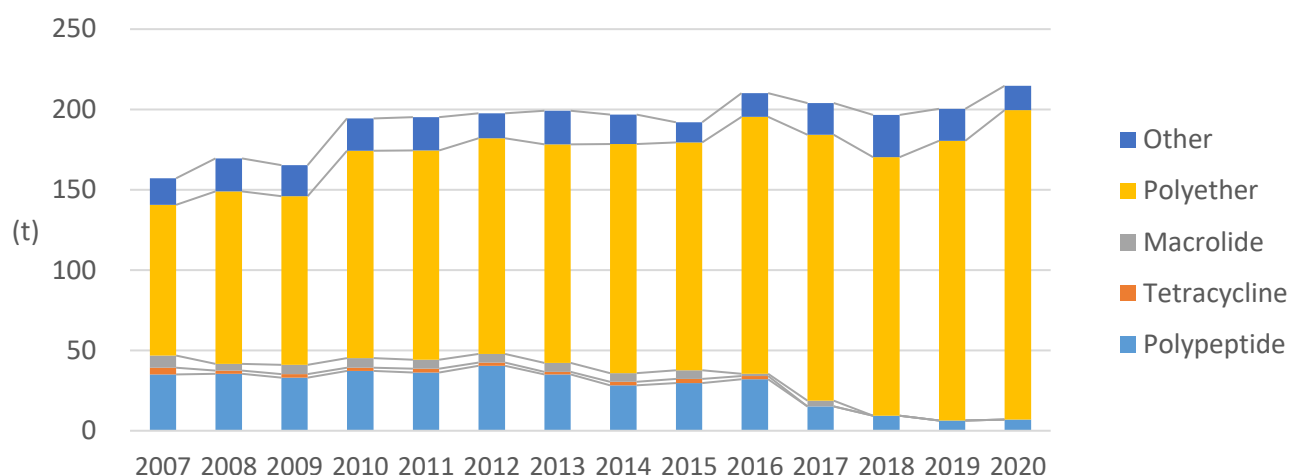
4-2 Antimicrobial feed additives

The graph below (Fig. 4-2) shows the distribution amount of antimicrobial feed additives according to a survey conducted by Food and Agricultural Materials Inspection Center (FAMIC) and Japan Scientific Feeds Association. The distribution amount showed little change over the decade, hovering around 200t.

However, the polyethers (not used in humans) showed an increasing trend, as its proportion of the total increased from 59.7% in 2007 to 89.6% in 2020. It should be noted that CL (polypeptides) in July 2018, TS (macrolides) in May 2019, and two substances (the tetracyclines) in December 2019 were rescinded as feed additives and have not been in circulation since their rescindment.

Data Source: Food and Agricultural Materials Inspection Center (FAMIC) and Japan Scientific Feeds Association

Fig. 4-2 The distribution amount of antibacterial feed additives (2007-2020)



4-3 Antimicrobials for human use sold to animal clinics

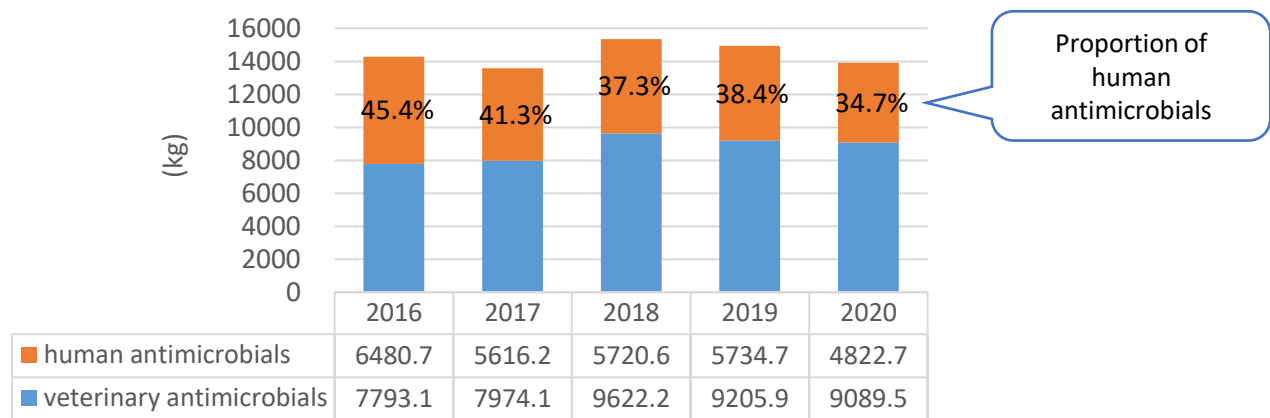
Antimicrobials approved as veterinary drugs (veterinary antimicrobials) have been estimated for their usage based on the sales volume reporting system from veterinary drug manufacturers in JVARM since 2001. However, in veterinary clinics for companion animals, antimicrobials approved as human medicines (human antimicrobials) are also used under veterinarians’ judgement. Since there is no reporting system for their sales volume, we started a survey of the sales volume of human antimicrobials sold to animal clinics since 2016, with the full cooperation of the Japan Animal Drug and Instrument Dealers Association and the Federation of Japan Pharmaceutical Wholesalers Association (the sales volume of pharmaceutical wholesalers belonging to this wholesaler association accounts for 80% of the sales volume of human medicines).

4-3-1 Survey results

The total annual volume of human antimicrobials sold to animal clinics in 2020 was 5446.7 kg (weight of active ingredient), 88.5% (4822.7 kg) of which was for small animal clinics. The remaining 11.5% were sold to animal clinics including horses, exhibition facilities such as zoos, etc.

In 2020, sales volume to small animal clinics was at its lowest since the start of the survey in 2016. The total amount combined with the veterinary antimicrobials was 13910.9 kg, and human antimicrobials accounted for 34.7% of the antimicrobials sold to small animal clinics (Fig. 4-3-1). In the five-year survey from 2016, there was no major change in the situation regarding the proportion of each antimicrobial class and each drug of human antimicrobials in the total human antimicrobials sold to small animal clinics.

Fig. 4-3-1 Changes in the amount of antimicrobial agents sold for companion animals



Human antimicrobials sold to small animal clinics were most commonly first-generation cephalosporins and penicillins, and these accounted for 60% of the total. Next were tetracyclines, sulfonamides, and fosfomycins. These top five classes accounted for 80% of the total.

On the other hand, the total use of fluoroquinolones, cephalosporins (from the third generation onward), macrolides, penems, carbapenems, and peptides and glycopeptides was low. These are critically important antimicrobials in human medicine. Fluoroquinolones accounted for 2.3%, third generation onward cephalosporins for 2.0%, macrolides for 3.8%, penems for 1.8%, carbapenems for 0.2%, and peptides and glycopeptides for less than 0.01% of the total.

The proportion of human antimicrobials in the total quantity of human and veterinary antimicrobials was 28.7% for the first-generation cephalosporins and 50.4% for the penicillins. For antimicrobials important in human medicine, fluoroquinolones accounted for 11.2% and cephalosporins from the third generation and onwards accounted for 31.0% (Fig. 4-3-2). Of the human antimicrobials sold to companion animal clinics, 85.0% belonged to the same classes as those approved for dogs and cats (Figure 4-3-3).

Fig. 4-3-2. Sales volume of human and animal antimicrobials for companion animals by antimicrobial class and the proportion of human antimicrobials

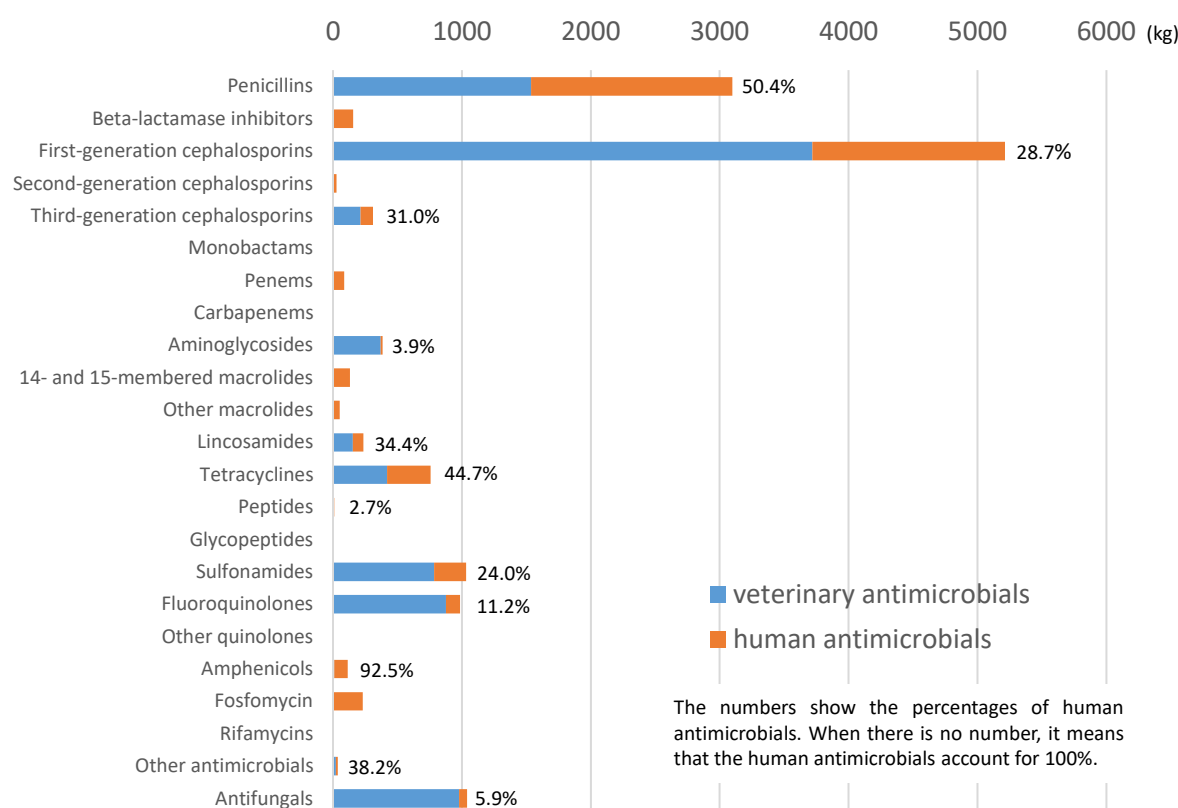
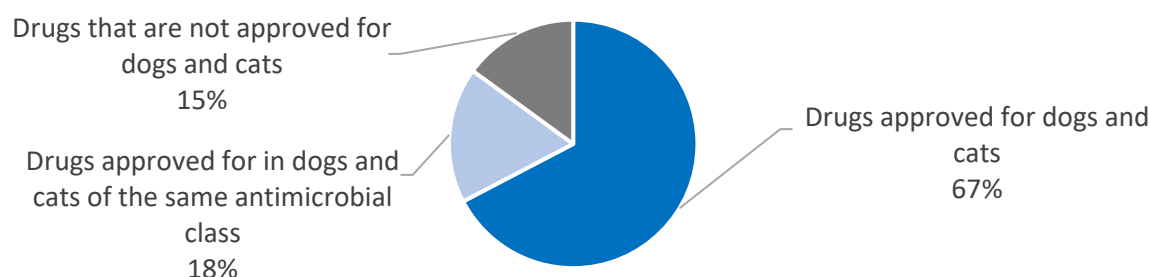


Fig. 4-3-3 The proportion of human antimicrobials sold to small animal clinics whose active ingredients have been approved for dogs and cats.



Ninety percent of the volume of human antimicrobials marketed to animal clinics goes to small animal clinics. In addition, 40% of the total amount of veterinary and human antimicrobials used for companion animals was human antimicrobials. Therefore, in order to promote measures against antimicrobial resistance through the prudent use of antimicrobials in the companion animal medicine, it is important to continue to conduct surveys about human antimicrobial administration.

Throughout the five year survey, first-generation cephalosporins and penicillins were the most common class, and by drug, CEX and AMPC were ranked the first or second; these two drugs accounted for 58.1% to 72.3% of the sales volume of human antimicrobials.

In 2020, CEX, which had the highest sales volume, was only for oral formulation. Since three of the four formulations sold were tablets, and various contents of the formulations (75 mg, 300 mg, 600 mg) exist for veterinary use, it was not considered that the formulations for human use were more suitable. Regarding AMPC, all of the marketed human antimicrobials were also an oral formulation, and three fourths of all AMPC sold was tablets, same as veterinary medicine. Based on this, it was considered that the route of administration and formulation were not the reasons for choosing human antimicrobials.

4-3-2 Summary

It was assumed, before this survey, the main factors contributing to the use of human antimicrobials for animals were lack of veterinary antimicrobials, formulation of the required administration route, and product for companion animals in an easy-prescriptive form. However, since many of human antimicrobial ingredients were also approved for veterinary antimicrobial ingredients, these are not the only reasons, and it is possible that factors such as using it before being approved and sold as veterinary antimicrobials, the low cost, and availability are having an effect.

In addition, it was confirmed that penem, carbapenem and glycopeptide drugs, which are not approved as veterinary antimicrobials and are the last resort for the treatment of multidrug-resistant bacteria in human medicine, are being used for companion animals. At present, in JVARM, the resistant bacteria to these antimicrobial classes have not been identified in companion animals. However, caution should be exercised and the use of these drugs should be discouraged.

Considering the results of this survey, when veterinary drugs for companion animals are approved and marketed, it is important to appropriately use veterinary drugs for which efficacy and safety have been ensured, rather than human drugs. In addition, we recommend to utilize the “Guidebook for the Prudent Use of Antimicrobials in Companion animals-2020-¹⁾” and enforce prudent antimicrobial use in companion animal medicine.

1) https://www.maff.go.jp/j/syouan/tikusui/yakuzi/attach/pdf/240328_7-8.pdf (only Japanese)

4-3-3 Acknowledgement

We would like to express our deep gratitude to the members of the Japan Animal Drug and Instrument Dealers Association and the Federation of Japan Pharmaceutical Wholesalers Association for their great cooperation in conducting this survey.



5. Materials and methods

5-1 Sampling specimens and target species

5-1-1 Healthy livestock: strains from slaughterhouses

The animal species monitored as healthy livestock were cattle, pigs, and chickens (broilers), and the sampling was done at slaughterhouses closest to the final food products to confirm their impact on human health.

Typically, rectal stool samples were collected from 1-3 cattle per farm and 4-8 pigs per farm, and cecal samples were collected from 10 chickens per farm at major slaughterhouses throughout the country. The homogenized samples were used to isolate the target bacterial species.

Bacterial species included the indicator bacteria (*Escherichia coli* and *Enterococcus* spp.), which are the commensal bacteria of livestock, and foodborne pathogenic bacteria (*Campylobacter* spp. and *Salmonella* spp.), which are an important public health concern. Since there is a bias in the isolation status of foodborne pathogenic bacteria in each animal species, the target species were restricted to each animal species as shown in “the list of bacteria collected in 2020 (P.4)”.

If multiple strains of the same target bacteria were isolated from the same farm, the first strain isolated was designated as “the farm representative strain”.

5-1-2 Diseased livestock: Clinical isolates

Bacterial strains isolated and identified from samples for pathological appraisal by livestock hygiene service centers across the country were collected.

Organ of origin and disease name are varied by the target bacteria species, and it is not restricted.

The selection criteria for the number of strains are as follows:

- In principle, when multiple strains of the same species were isolated and identified from the same animal, one strain was selected.
- When multiple strains of the same species are isolated and identified from multiple animals on the same farm, in principle, two strains from different animals are selected (except for cases in which it is judged not to be epidemic due to the same strain, such as when the sampling times for the two bacteria are significantly apart if the same strains are taken from the same farm).

Bacterial species		Animal species	Isolation site
Gram-negative bacteria	<i>Escherichia coli</i>	Cattle, Pigs, Chickens	Various
	<i>Mannhemia haemolytica</i>	Cattle	
	<i>Salmonella</i> spp.	Cattle, Pigs, Chickens	
Gram-positive bacteria	<i>Streptococcus suis</i>	Pigs	
	<i>Staphylococcus aureus</i>	Cattle, Pigs, Chickens	

5-1-3 Healthy companion animals

Rectal swab samples were collected from healthy dogs and cats that visited small animal clinics for medical check, vaccination, trimming and so on, instead of treatment of the disease, and the indicator bacteria were isolated. Sample numbers were allocated to small animal clinics in each prefecture to reduce the bias of the region. The Japan Veterinary Medical Association in full collaboration with this survey and they arranged clinic participation. Before sampling, informed consent was given to the owners, and one sample from each dog and cat was collected per animal clinic. Information regarding species, sex, housing conditions, and so on were also obtained.

Bacterial species		Animal species	Samples
Gram-negative bacteria	<i>Escherichia coli</i>	Dogs, Cats	Rectal swab
Gram-positive bacteria	<i>Enterococcus</i> spp.		

5-1-4 Diseased companion animals

Strains isolated from diseased dogs and cats submitted to clinical laboratories were collected. Based on the review by the Working Group on Companion Animal Antimicrobial Resistance (AMR) Monitoring*, high-priority species, *Escherichia coli*, *Klebsiella* spp., Coagulase-positive *Staphylococcus* spp. and *Enterococcus* spp. were collected every year, while other species were to be carried out every few years. In the collection, the number of animal clinics was considered for six blocks (Hokkaido and Tohoku, Kanto, Chubu, Kinki, Chugoku and Shikoku, and Kyushu and Okinawa), to reduce the bias of the area, it was collected by one clinic, one species, and one strain.

Species collected and sample isolation sites in 2020 are as follows:

Bacterial species		Isolation site
Gram-negative bacteria	<i>Escherichia coli</i>	Urine, genital tract
	<i>Klebsiella</i> spp.	
	<i>Enterobacter</i> spp.	Urine
	<i>Acinetobacter</i> spp.	Urine, skin
Gram-positive bacteria	Coagulase-positive <i>Staphylococcus</i> spp.	Urine, skin
	<i>Enterococcus</i> spp.	Urine, ear

* https://www.maff.go.jp/nval/yakuzai/yakuzai_p3-4.html (only Japanese)

5-2 Isolation and identification of bacteria

In each sample, isolation was performed by the following methods: For diseased livestock, each prefecture carried out the test based on the Disease Diagnosis Manual (*). For diseased companion animals, the strains isolated and identified by each laboratory's method were re-identified by the following methods:

* https://www.naro.affrc.go.jp/org/niah/disease_byosei-kantei2016/index.html (only Japanese)

5-2-1 *Escherichia coli*

Samples were inoculated directly onto desoxycholate-Hydrogen sulfide lactose (DHL) agar medium, and suspect colonies were isolated and identified by morphological and biochemical characterization.

5-2-2 *Enterococcus* spp.

Samples were cultured using a direct method and an enrichment with Azide Citrate (AC) medium, and suspect colonies were isolated using Enterococcosel Agar (ECS medium) and identified by morphological and biochemical characterization.

5-2-3 *Campylobacter* spp.

Samples were cultured using a direct method and prestone enrichment broth followed by Modified Cefaperazone Charcol Desoxycholate Agar (mCCDA). Then identification was carried out by morphological and biochemical characteristic inspection as well as PCR methodology.

5-2-4 *Salmonella* spp.

Samples were cultured using direct and peptone-water followed by secondary enrichment with Rappaport-Vassiliadis medium and, then were inoculated onto novobiocin-containing DHL agar and chromoagar salmonella medium, respectively, for isolation and culture. Identification was carried out by morphological, biochemical properties inspection, and specific antiserum testing.

5-2-5 *Mannhemia haemolytica*

Conducted on the basis of the Pathogenicity Diagnostic Manual (cited *).

5-2-6 *Streptococcus suis*

Conducted on the basis of the Pathogenicity Diagnostic Manual (cited *).

5-2-7 *Klebsiella* spp.

Klebsiella spp. strains sent by clinical laboratories were re-identified by API20E (Biomelue Japan). When it could not be identified by API20E, identification by MALDI-TOF-MS (Bruker Daltonics) was performed.

5-2-8 *Enterobacter* spp.

Enterobacter strains sent by clinical laboratories were re-identified by API20E (Biomelue Japan). When it could not be identified by API20E, identification by MALDI-TOF-MS (Bruker Daltonics) was performed.

5-2-9 *Acinetobacter* spp.

Acinetobacter strains sent by clinical laboratories were re-identified by API20NE (Biomelue Japan). When it could not be identified by API20NE, identification by MALDI-TOF-MS (Bruker Daltonics) was performed.

5-2-10 Coagulase-positive *Staphylococcus* spp.

Coagulase-positive staphylococcal strains sent by the clinical laboratory were re-identified by PCR (Sasaki et al., JCM, 2010 48 765-769). Strains for which bands could not be detected were performed coagulase test and coagulase positive confirmed strains were identified by MALDI-TOF-MS (Bruker Daltonics).

5-3 Antimicrobial susceptibility test

5-3-1 Targeted antimicrobials

In order to understand the trend of antimicrobial resistance of the approved veterinary medicinal products and medically important products (other than diseased food-producing animals), representative drugs were selected for each class and used for the test (see list of target bacteria and tested antimicrobials below).

5-3-2 Antimicrobial susceptibility test

The broth microdilution method* proposed by the Clinical and Laboratory Standards Society (CLSI) to determine the minimal inhibitory concentration (MIC) was performed.

Breakpoints (BP)**: the value provided by CLSI was adopted. If no BP was defined in CLSI, the epidemiological cut-off value (Epidemiological cut-off values, ECOFF) of the European Committee on the Study of Antimicrobial Susceptibility Testing (EUCAST) and the value obtained by JVARM (the midpoint of MIC distribution showing bimodality) were used. Strains with MIC equal to BP or higher were defined as resistant strains, and the percentage of resistant strains in the total number of strains (number of resistant strains/number of strains × 100) was used as the resistance rate.

*CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard M07, 11th ed. CLSI, Wayne, PA, USA. 2018.

**CLSI. Performance Standards for Antimicrobial Susceptibility Testing M100, 30th ed. CLSI, Wayne, PA, USA. 2020.

5-4 Sales volume of antimicrobials

5-4-1 Veterinary antimicrobial sales

The data for veterinary antimicrobial sales volume is collected annually from the marketing authorisation holders in accordance with the Pharmaceutical and Medical Device Act 71-2 (Ministry of Agriculture, Forestry and Fisheries ordinance No. 107, 2004). The results show the amount of each active ingredient by administration route, as well as the estimated percentage of the antimicrobials for each animal species. The results have been published on the websites of the National Veterinary Assay Laboratory¹⁾ as "Annual Report of Sales Amount and Sales Volume of Antibiotics, Synthetic Antibacterials, Anthelmintics and Antiprotozoals". It has also been published in the One Health Report²⁾ and the Antimicrobial Resistance (AMR) Platform³⁾.

1) https://www.maff.go.jp/nval/yakuzai/yakuzai_p3_6.html

2) <https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000120172.html>

3) <https://amr-onehealth-platform.ncgm.go.jp/home>

5-4-2 Antibacterial feed additives

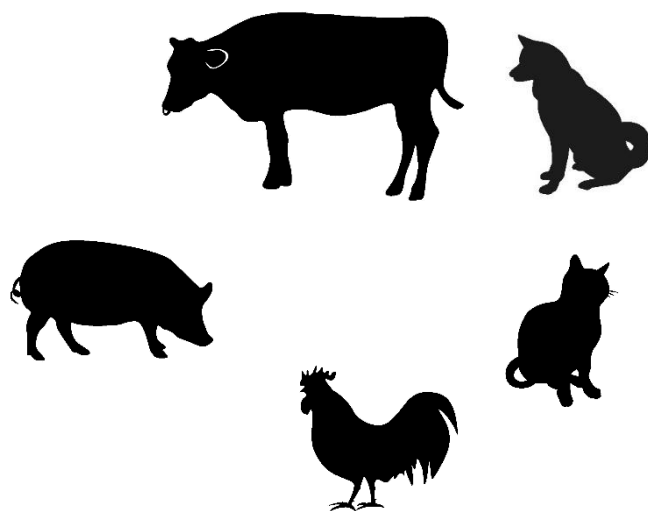
The data for the distribution volumes of antimicrobial feed additives are provided by the Food and Agricultural Materials Inspection Center and by the Japan Scientific Feeds Association.

5-4-3 Antimicrobials for human use sold to animal clinics

In many countries, veterinarians are allowed to prescribe human medicines, including antimicrobials, for treatment of animals under their responsibility. Human medicines are considered to be used by veterinarians mainly in companion animal hospitals. The sales data of antimicrobials for human were provided by members of the Japan Animal Drug and Instrument Dealers Association and the Federation of Japan Pharmaceutical Wholesalers Association.

List of target bacteria and tested antimicrobials

Animal species	Species	ABPC	PCG	CEZ	CTX	CTF	CFX	CEX	CQN	GM	KM	SM	DSM	BCM	AZM	EM	CLDM	TLM	TC	OTC	NA	CPFX	ERFX	CL	BC	CP	TMP	ST	MEPM	MPIPC	FFC	TP	TMS	TML	TS	LCM	VCM	SNM																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																			
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