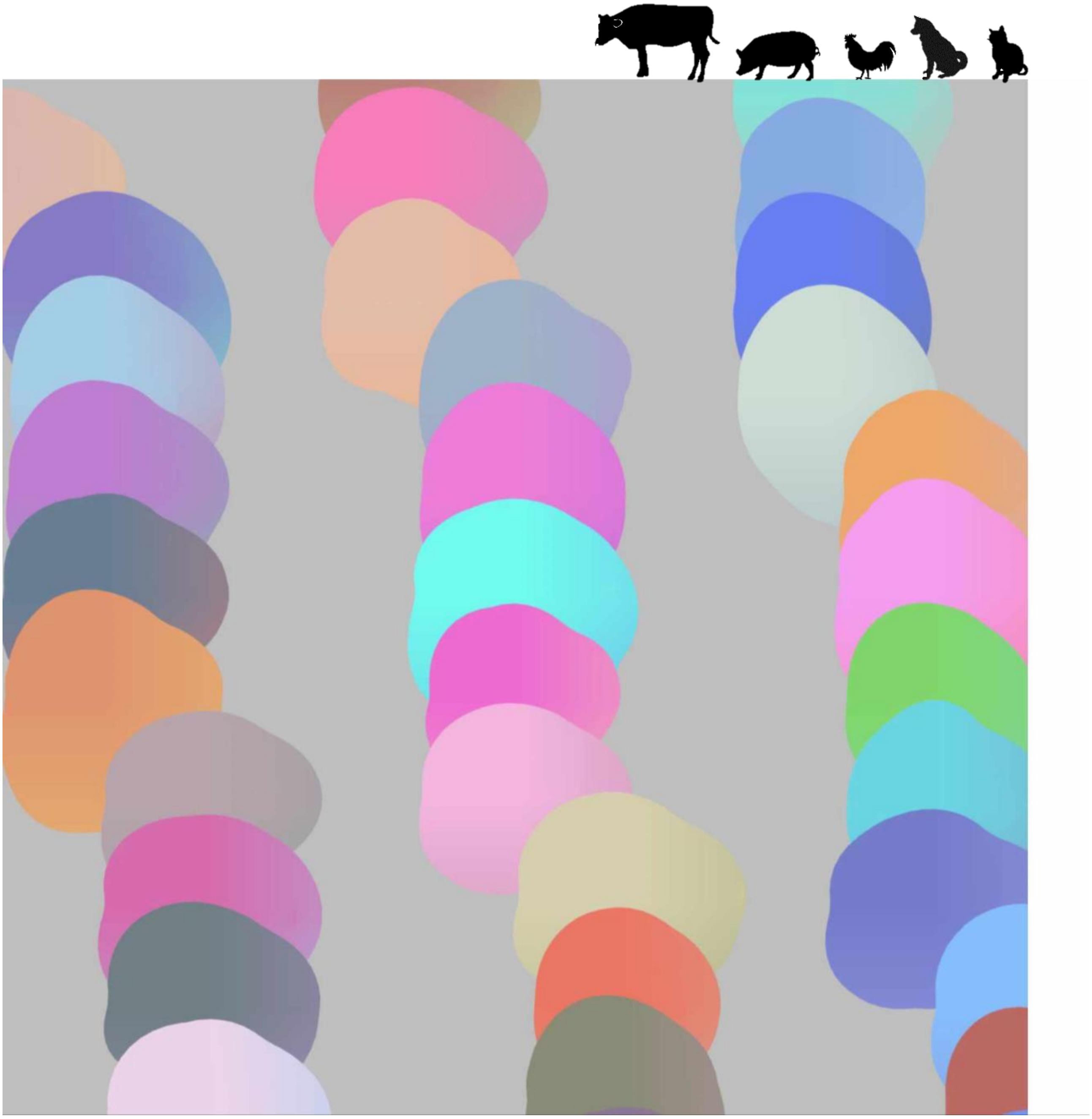
JVARM REPORT

2021



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動物分野AMRセンター
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I. 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 the "new AMR Action Plan (2023-2027)" (hereinafter referred to as the 'New Action Plan') is currently underway from 2023.

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). The MAFF has been carrying out the Japanese Veterinary Antimicrobial Resistance Monitoring (JVARM) since 1999 in conjunction with prefectures and 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 2021.

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. Summary of Monitoring Results 2021

List of bacteria collected in 2021

<table>
<tr><th>Category</th><th>Health / Disease</th><th>Animal species</th><th colspan="6">Species</th></tr>
<tr><td rowspan="6">Livestock</td><td rowspan="3">Health
(3-1)</td><td>Cattle</td><td rowspan="6">Escherichia coli</td><td rowspan="3">Enterococcus spp.</td><td rowspan="3">Campylobacter jejuni / coli</td><td></td><td></td><td></td></tr>
<tr><td>Pigs</td><td></td><td></td><td></td></tr>
<tr><td>Chicken</td><td>Salmonella spp.</td><td></td><td></td></tr>
<tr><td rowspan="3">Disease
(clinical isolates)
(3-2)</td><td>Cattle</td><td rowspan="3">Salmonella spp.</td><td rowspan="3">Staphylococcus aureus</td><td>Mannheimia haemolytica</td><td></td><td></td></tr>
<tr><td>Pigs</td><td>Streptococcus suis</td><td></td><td></td></tr>
<tr><td>Chicken</td><td></td><td></td><td></td></tr>
<tr><td rowspan="2">Companion animal</td><td>Health
(3-3)</td><td>Dogs
Cats</td><td rowspan="2">Escherichia coli</td><td rowspan="2">Enterococcus spp.</td><td></td><td></td><td></td><td></td></tr>
<tr><td>Disease
(3-4)</td><td>Dogs
Cats</td><td>Klebsiella spp.</td><td>Pseudomonas aeruginosa</td><td>Acinetobacter spp.</td><td>Coagulase positive Staphylococcus spp.</td></tr>
</table>

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)

Healthy livestock

- The resistance rates of *E. coli* from healthy livestock animals to third-generation cephalosporins and fluoroquinolone, which are critically important antimicrobials in human medicine, as the outcome indices specified in the New Action Plan, are considered to remain 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, for tetracycline, the most frequently used antimicrobials in the animal sector, although there has been a decrease in sales volume for pigs, no significant change was observed in the resistance rate.
- In chickens(broilers), 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*. It is necessary to continue to pay attention to changes in sales of these antimicrobials.
- 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 isolates

- In *E. coli*, the resistance rate to TC has leveled off, while the resistance rate to CL has decreased. The resistance rate to CPFX tended to decrease in cattle, while it tended to increase in pigs and chickens. The resistance rates to CTX have increased slightly in pigs, decreased in cattle and leveled off in chickens.
- *M. haemolytica* showed the resistance rates of more than 30% for DSM (36.4%) and ABPC (32.5%). Resistance rates were low for the second-line drugs CQN (1.3%) and CTF (0.0%).
- In *S. suis*, the susceptibility to penicillins and CTF approved for veterinary drugs in streptococci has been maintained.
- *Salmonella* spp. has a high resistance rate of more than 40% in TC approved for cattle, pigs and chickens. Dublin isolated from cattle showed high resistance rate to CL and multi-drug resistance.
- In *S. aureus*, the susceptibility was generally maintained in cattle and chickens, but the resistance rate was more than 80% to PCG and more than 50% to TC in pigs.

- Overall, bacterial resistance to approved antimicrobial drugs were detected in diseased livestock. Although resistance rates to second-line drugs were generally low, they exhibited concerning upwards trends. It is important to continue perform antimicrobial susceptibility testing and to use appropriate antimicrobial drugs only when necessary.

Healthy companion animals (dogs and cats)

In *E. coli* and *Enterococcus* spp. derived from healthy dogs and cats, the resistance rates were 20% or less in many of the tested drugs including the second-line drugs. Resistance rates to carbapenem in *E. coli* and vancomycin in *Enterococcus* spp. remained at 0.0%. It was confirmed that the resistance rates of bacteria from healthy companion animals were lower than that from diseased companion animals for many tested drugs.

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 like the previous results. In *Pseudomonas aeruginosa* and *Acinetobacter* spp., the susceptibility was generally maintained for the tested drugs. Three strains of *P. aeruginosa* and one strain of *Acinetobacter* spp. were resistance to carbapenems. In *Enterococcus* spp., the resistance rate to VCM was 0.0%. For second-line drugs, resistance rate to CTX were high among *Klebsiella* spp. and *S. aureus* from cats, but less than 30% in other bacteria. It showed a broad resistance rate of 15.2 – 91.8% 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 2021.

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 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 2021 was 800.9 tonnes, declining by about 20% from 2001, but has remained around 800 tonnes in recent years. Tetracyclines are the most common, but the sales volume of tetracyclines has been declining and have been below 40% since 2018. 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), which is the second largest use after pigs, macrolides (EM) increased since 2015 with the occurrence and treatment of infections caused by the type II α-hemolytic Streptococcosis 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 distribution volume of antimicrobial feed additives from 2007 to 2021 ranged from 157.2 to 214.8 tonnes and has remained almost unchanged since 2010. However, a comparison of the distribution volume by class shows that the number of polyethers (ionophores, not used in humans), which account for most of the distribution volume, is on the rise. The ratio of polyether to the total feed additives was 59.7% in 2007 but 87.5% in 2021.

Sales of human antimicrobials for companion animal clinics in 2021 were 4.8 tonnes, almost the same as in 2020, but were the lowest since the survey began in 2016. There was no significant change in the situation of each class of human antibiotics and the ratio of each drug to the total number of human antimicrobials. The most common antimicrobials were first-generation cephalosporins and penicillins. Drugs not approved or marketed for veterinary use and classified as "only for use in humans" on the WHO list of medically important antimicrobials accounted for 2% of the total. The use of these antimicrobials should be avoided.

3. Results of resistance rates

3-1 Healthy livestock

This report provides an overview of the results of antimicrobial resistance monitoring for indicator bacteria, such as *Escherichia coli* and *Enterococcus,* isolated from healthy livestock (cattle, pigs, and chickens) at slaughterhouses and poultry slaughterhouses in fiscal year 2021, as well as foodborne pathogens that pose a public health risk, such as *Campylobacter* and *Salmonella.* The data for *Enterococcus* is based on information provided by the Food and Agricultural Materials Inspection Center (FAMIC).

3-1-1 Outcome indices of the Action Plan for Antimicrobial Resistance (AMR)

Based on the achievements of the antimicrobial resistance (AMR) Action Plan (2016-2020), the New Action Plan sets the resistance rates of *E. coli* to tetracycline, third-generation cephalosporins, and fluoroquinolones as outcome indices in the animal sector, with a focus on continuity. Since resistance patterns and hygiene management differ by livestock species, specific target values for each livestock species were set to be achieved by 2027, serving as indices for addressing issues specific to each species (Table 3-1-1). According to the outcome indices of the New Action Plan, the resistance rates of *E. coli* from healthy livestock to tetracycline were 23.8% in cattle, 52.0% in pigs, and 46.2% in chickens in 2021. The resistance rates to third-generation cephalosporins were 0.0% in cattle, 2.0% in pigs, and 2.1% in chickens. The resistance rates to fluoroquinolones were 0.0% in cattle, 2.0% in pigs, and 14.5% in chickens. No significant increases or decreases were observed in any species or drug (Figures 3-1-1 to 3)

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

Indices (resistance rate of *E. coli*)	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

Fig. 3-1-1 Tetracycline resistance rates

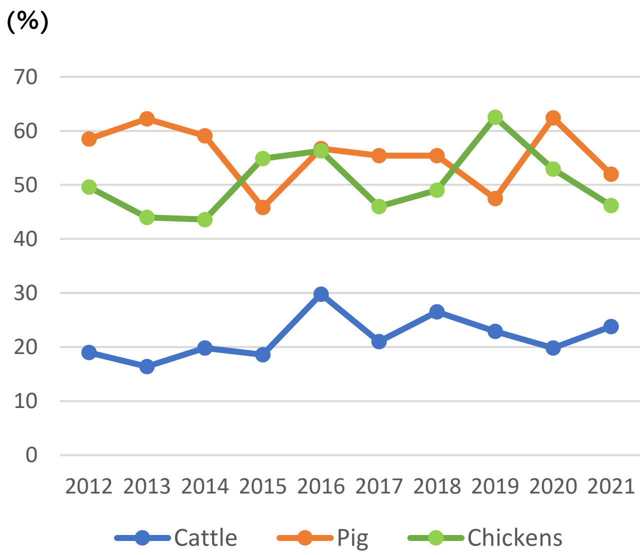


Fig. 3-1-2 Fluoroquinolones resistance rates

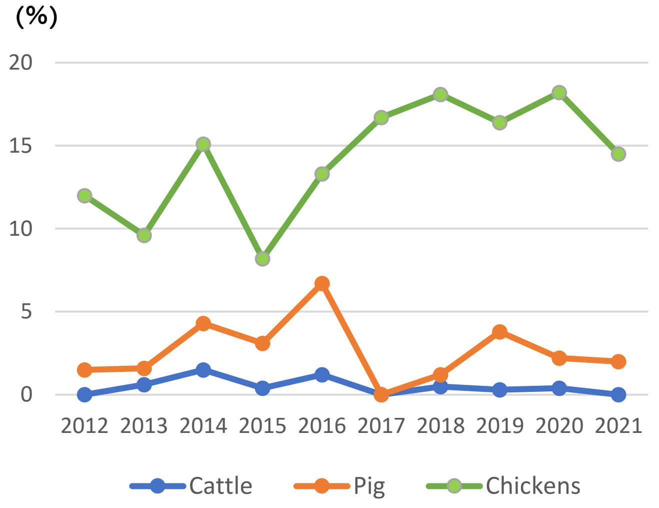
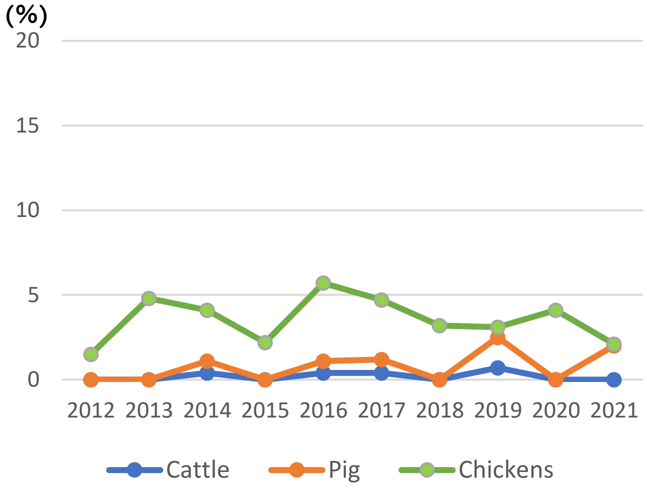


Fig. 3-1-3 Third-generation cephalosporins resistance rates



3-1-2 Resistance rates by species

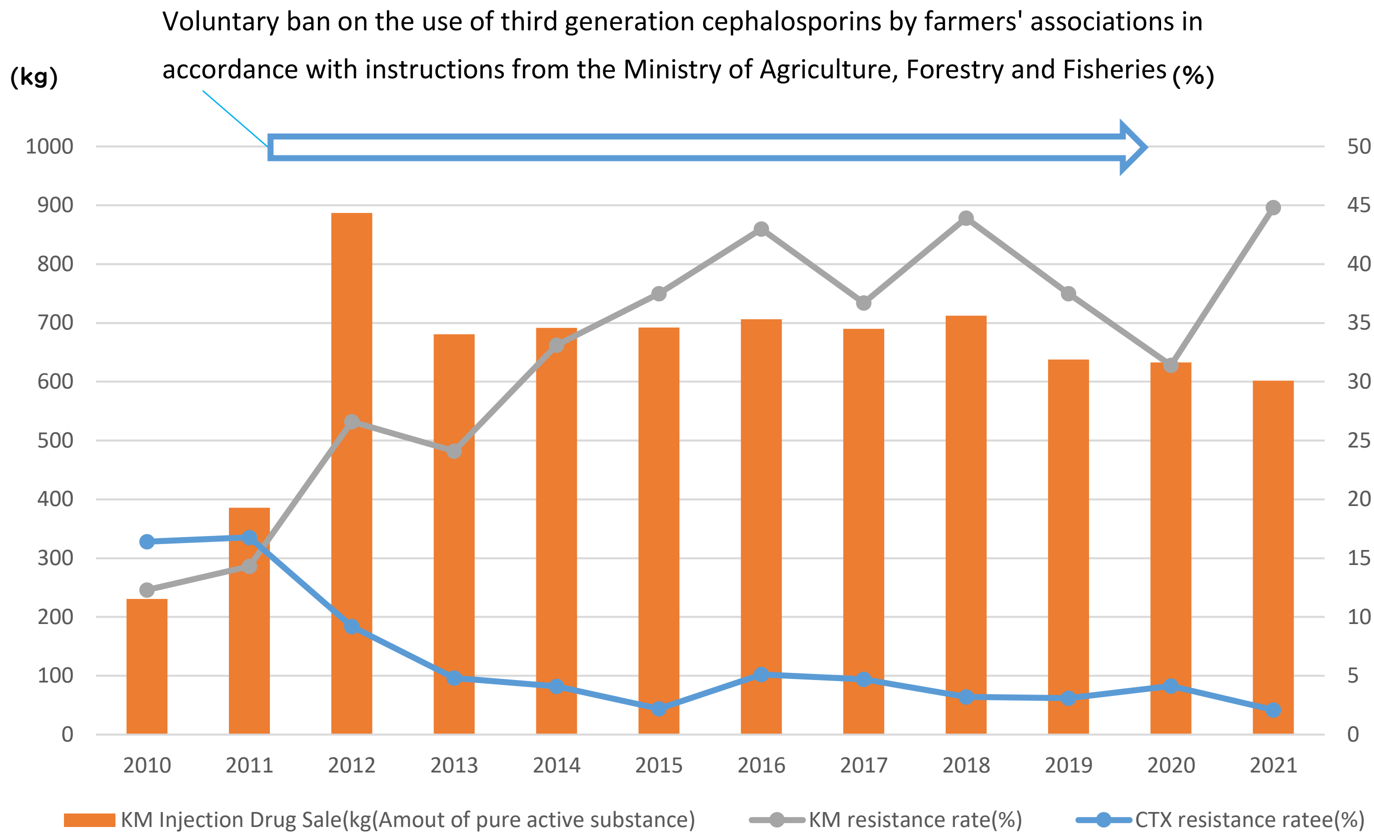
The main changes in antimicrobial resistance in each species other than the outcome indices of National Action Plan on AMR are described below. For specific numbers and trends in resistance rates, please refer to the following URL (https://www.maff.go.jp/nval/yakuzai/yakuzai_AMR_2.html).

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

Based on the risk assessment conducted by the Food Safety Commission, CL was reclassified as a second-line drug for veterinary use and its designation as a feed additive was revoked in 2018. In 2021, 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. 3-1-4 Resistance rates of KM and CTX 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. 3-1-4). 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. (cattle, pigs, broilers)

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. The sales volume of macrolide antibiotics in chickens has decreased since 2012, and the resistance rate of TS in chickens has shown a decreasing trend, with significantly lower results in 2021 compared to 2012, 2014, 2016, and 2017 (Figures 3-1-5, 6). Although the designation of feed additive tylosin phosphate, used in pigs, was revoked in 2019 based on the risk assessment by the Food Safety Commission. However, the sales volume of macrolide antibiotics for animals in pigs increased from 2015 to 2016, and then remained stable. The resistance rate of TS in pigs remained stable until 2020 and showed a decrease in 2021, but future trends need to be monitored. Although the sales volume of macrolide antibiotics in cattle is lower than in chickens and pigs, the resistance rate has been increasing, with the resistance rate to TS in 2021 being 2% higher than in 2020. ※It is not approved for veterinary use.

Fig. 3-1-5 Resistance rates of TS

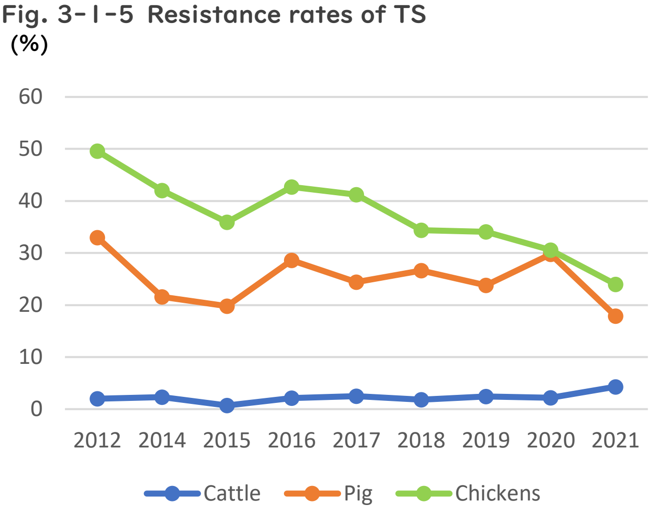


Fig. 3-1-6 sales volumes of macrolides

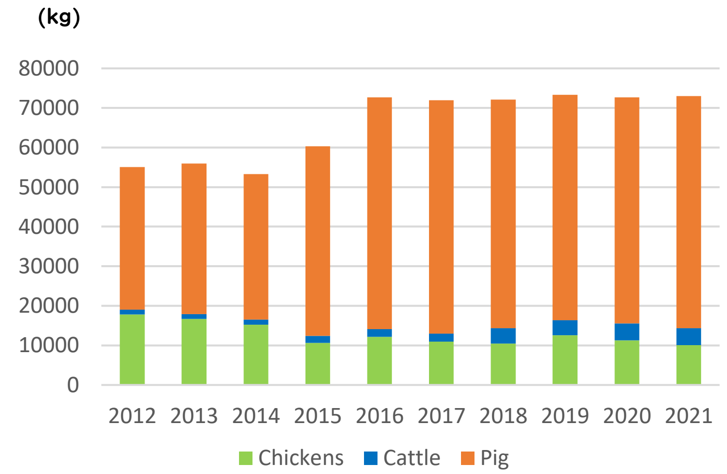


Fig. 3-1-7 Proportion of *Enterococcus* spp. in Chickens

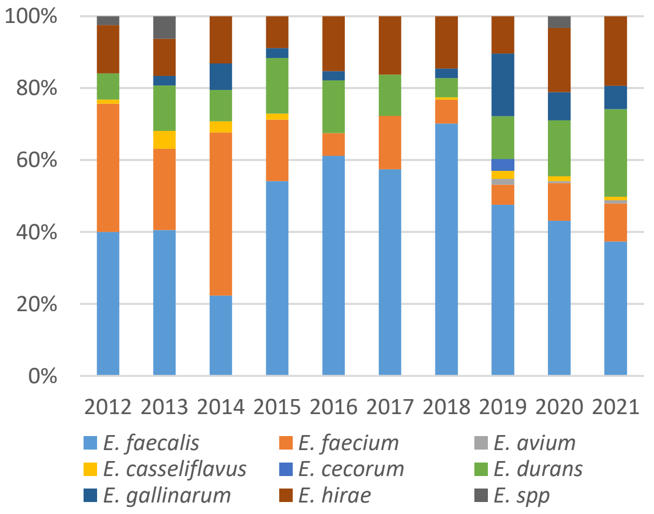
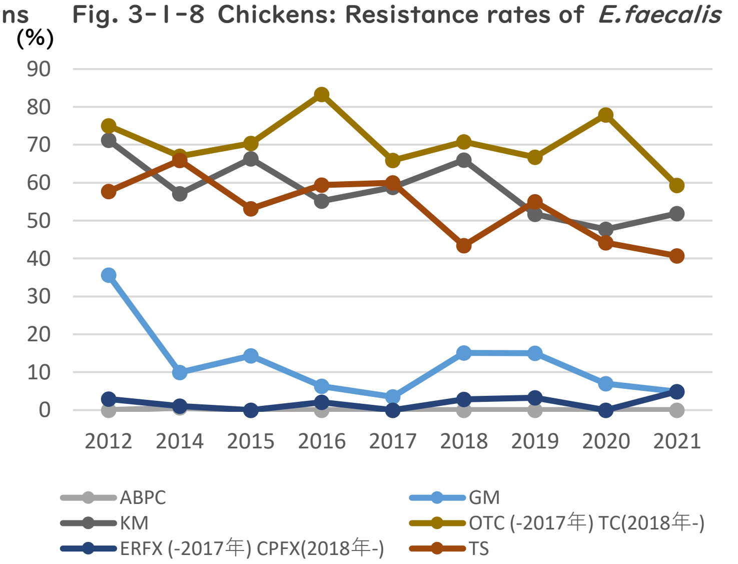


Fig. 3-1-8 Chickens: Resistance rates of *E.faecalis*



Enterococcus includes many species, and since resistance trends may differ depending on the species, it is important to monitor changes in the proportion of species being isolated. For human medicine, *E. faecalis* and *E. faecium* are considered opportunistic pathogens. In chickens, *E. faecalis* is the predominant species (Fig. 3-1-7). In 2021, the resistance rate to ABPC, an antibiotic used in human treatment, was 0.0%, and the resistance rates to CPFX and GM were also low at 5%. On the other hand, the resistance rates to KM, TS, and tetracyclines (OTC, TC) have remained above 40% since 2012 (Fig. 3-1-8). In cattle and pigs, the proportion of *E. hirae*, which is not considered a human medical issue, was high (2021: cattle 88%, pigs 59%)

3−1−2−3 *Campylobacter jejuni* (*cattle, broilers*), *C. coli* (pigs)

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, the resistance rates of *C. jejuni* to TC, NA, and CPFX were high, exceeding 30%, with an upward trend observed (Figure 3-1-9). The resistance rate to TC in 2021 was significantly higher than from 2012 to 2015, while the resistance rates to NA and CPFX were significantly higher than from 2012 to 2018 (excluding 2017) (Fig. 3-1-9).

In pigs, *C. coli* showed higher resistance rates to all drugs except for NA and CPFX compared to *C. jejuni* from cattle and chickens. The resistance rates of *C. coli* from pigs and *C. jejuni* from chickens fluctuated, either staying level or showing periodic increases and decreases, with no clear increasing or decreasing trend observed (Figures 3-1-10, 11).

Fig. 3-1-9 Cattle: resistance rate of *C. jejuni*

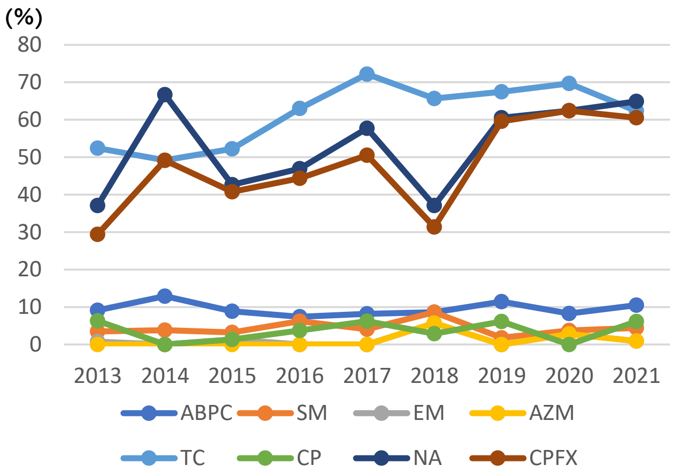


Fig. 3-1-10 Pig: Resistance rates of *C. coli*

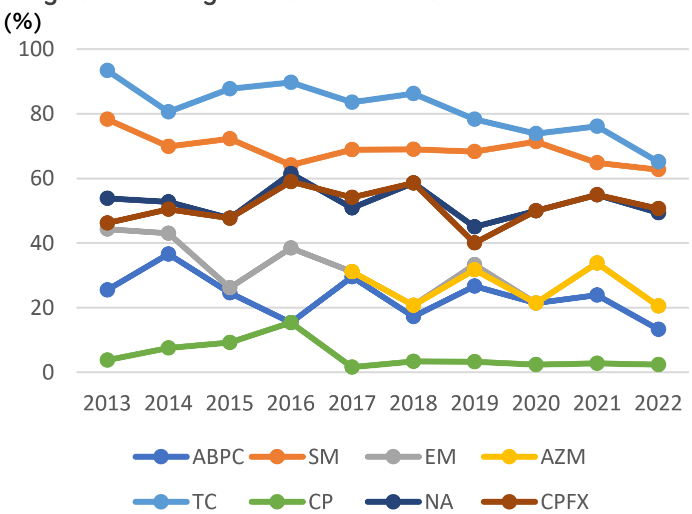
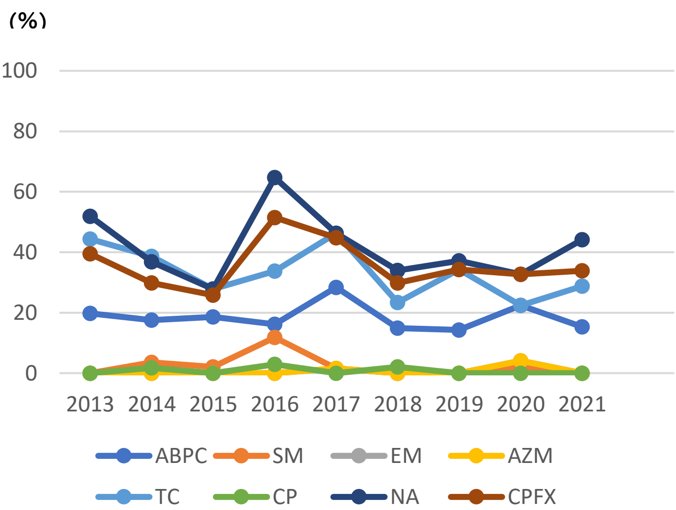


Fig. 3-1-11 Chicken: Resistance rates of *C. jejuni*



3-1-2-4 *Salmonella* spp. (broilers)

Salmonella spp. is 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. Additionally, since 2020, Manhattan has been isolated, and it will be necessary to monitor its increase or decrease in the future (Fig. 3-1-12). 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 mentioned above may have had an impact (Fig. 3-1-13).

Fig. 3-1-12 *Salmonella* serotypes

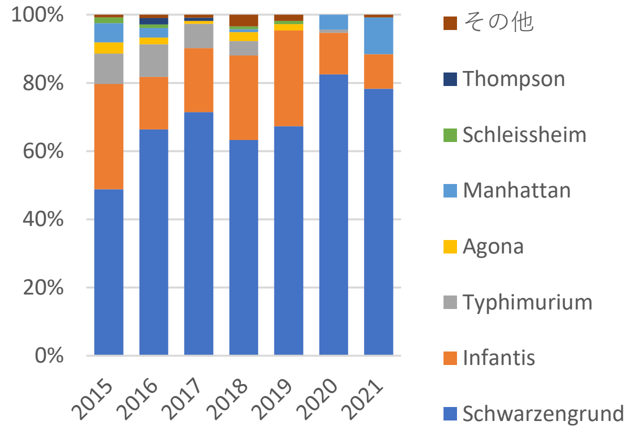
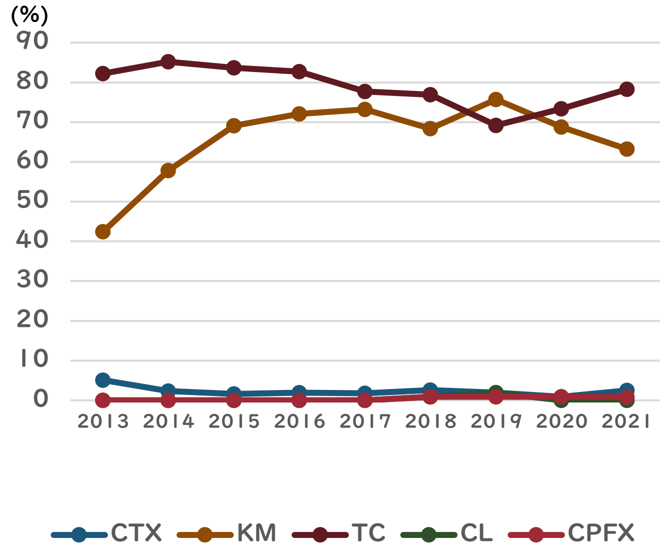


Fig. 3-1-13 Resistance rate of each antimicrobial agent



3-1-3 Summary

Under the New Action Plan, the resistance rates of *Escherichia coli* to fluoroquinolones and third-generation cephalosporins, which are critically important antimicrobial agents in human medicine and have been set as outcome indices, were generally maintained at low levels based on the resistance rates to CPFX and CTX. Additionally, the resistance rate to CL, which is also critically important antimicrobial agents in human medicine, was kept low. This was considered to be the result of prudent use as a second-line drug by stakeholders such as livestock farmers and veterinarians. Furthermore, although MEPM and VCM are not approved as veterinary drugs and considered last-resort treatments for multidrug-resistant bacteria in human medicine, were both 0.0% across all animal species. On the other hand, tetracycline (TC), which is the most widely used in the animal sector and set as an outcome index in the Action Plan, showed a decrease in sales volume for pigs, but the resistance rate remained stable without any decrease.

Additionally, when examining the resistance rates for each bacterial species and livestock species, it was noted that the sales volume of KM for chickens has increased since 2012, and correspondingly, the resistance rates of *E. coli* and *Salmonella* to KM have increased and remained at elevated levels.

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. 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 directly 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 slaughterhouses, poultry slaughterhouses, 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 2021, *Escherichia coli*, *Manheimia 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.

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

A total of 256 isolates, 108 from cattle, 88 from pigs and 60 from chickens, were collected from 37 prefectures.

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

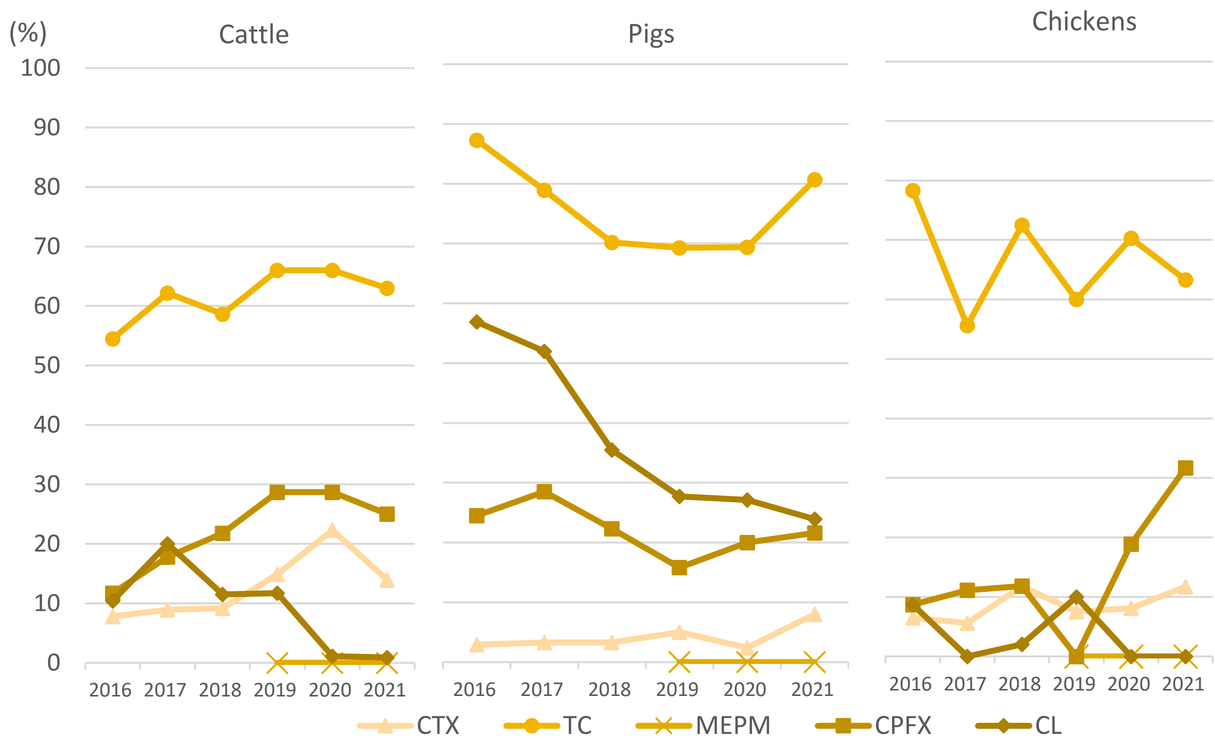


Fig.3-2-1-1 shows the resistance rates in each species.

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

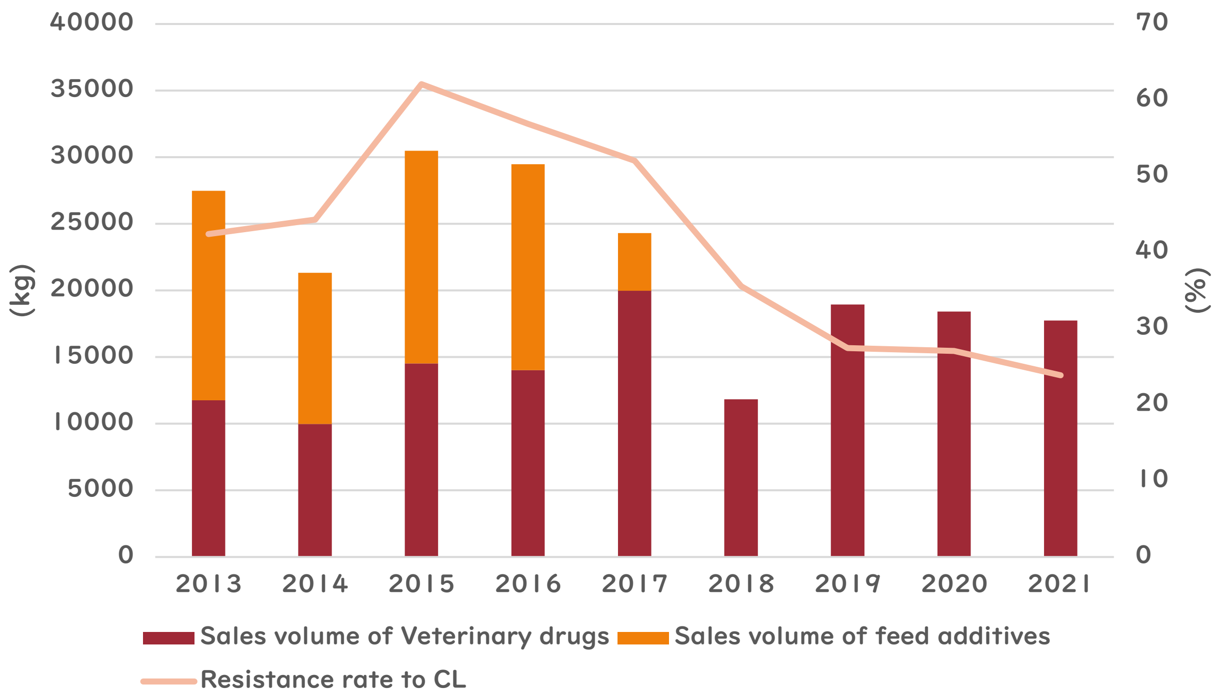
Resistance rates above 50% for ABPC and 40% for SM were observed, but not for TC. These drugs are used as veterinary drugs for cattle and pigs' bacterial diarrhea caused by *E. coli* and colibacillosis in chickens. The results of this survey suggest that confirmation of antimicrobial susceptibility is essential for administration in clinical practice.

The resistance rates to CPFX and CTX were less than 35% in all species from 2013 to 2021, and in cattle decreased in 2021. On the other hand, the resistance rate to CPFX in chicken increased from 18.9% to 31.7% from 2020 to 2021.Therefore, it is necessary to continue to monitor the resistance rate.

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. The volume of CL sold to pigs and the CL resistance rate of pigs-derived *E. coli* are shown in Fig. 3-2-1-2. Since the rescindment of its feed additive designation in 2018, total CL sales have declined, and resistance rate has also decreased. Sales of veterinary medicines as second-line drugs have ceased to fall, but a vaccine for edema disease, one of the indications, has been developed and is now available for sale, so a further fall in sales volume is expected in the future.

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

Fig. 3−2−1−2 Sales of CL to pigs and the rate of resistance in *E. coli* from diseased pigs



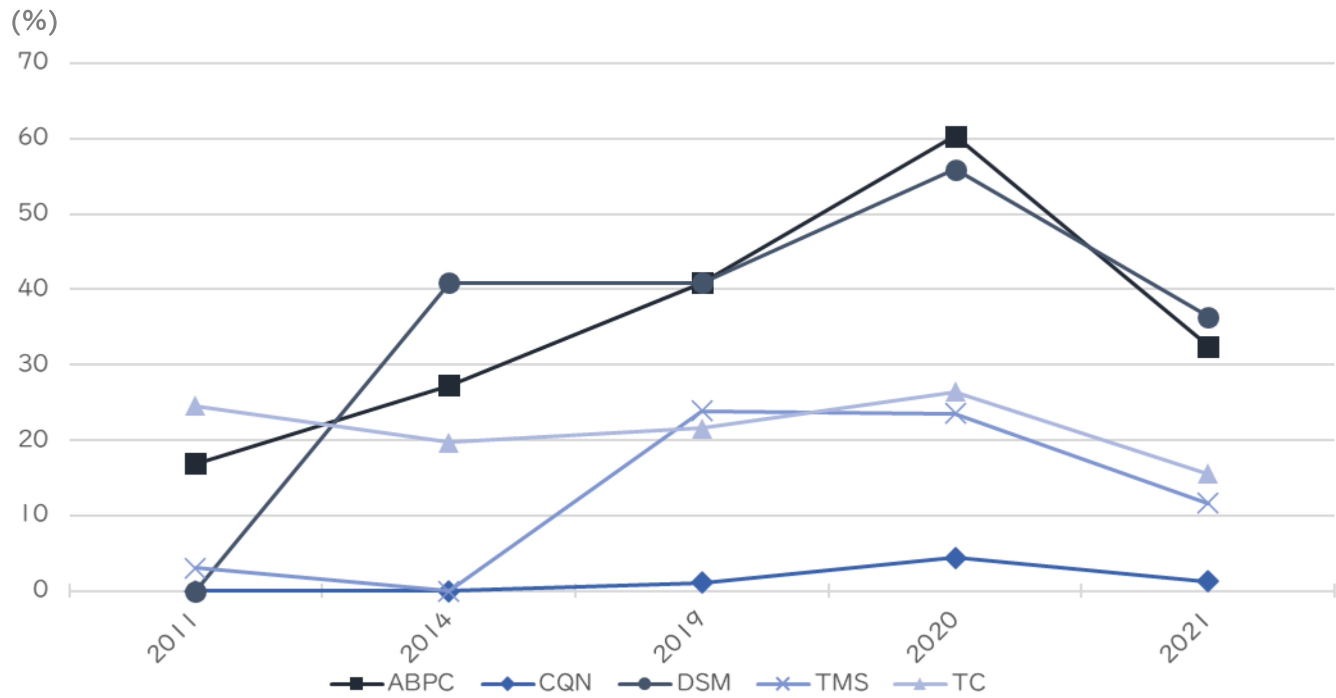
3-2-2 *Mannheimia haemolytica* (cattle)

From 25 prefectures, 77 isolates from cattle were collected.

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. MIC values for 2019-2021 were determined for ABPC, CEZ, CTF, CQN, DSM, KM, TUM, TMS, ERFX, TC and FFC among drugs approved for bovine pneumoniae or *M. haemolytica.* However, no breakpoints have been set in CLSI for CEZ, so no resistance rates are shown. (Fig.3-2-2-1)

The resistance rates to ABPC and DSM exceeded 40% in 2019 and 2020 but decreased to the 30% range in 2021. However, compared to other drugs, resistance rates were higher in all years from 2011. For CTF, CQN, TUM and FFC, the resistance rate was below 5% in all years.

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



The MIC distribution of CEZ showed that MIC_{50} and MIC_{90} were distributed at low concentrations of 1 μg/mL and 2 μg/mL, respectively. (Table 3-2-2-1)

Table 3-2-2-1 MIC distribution of drugs with no breakpoints set in the CLSI

Drugs	MIC (μg/mL)											MIC range	MIC_{50}	MIC_{90}
	≦0.06	0.12	0.25	0.5	1	2	4	8	16	32	>64			
CEZ			32	55	99	39	7	1	0	0	0	≦0.25-8	1	2

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

From 16 prefectures, 34 isolates from cattle were collected.

The antimicrobial resistance rates from 2019 to 2021 are shown in Fig. 3-2-2-1

Streptococcus suis causes pneumonia and arthritis in pigs. Of the drugs approved for *S. suis* or Streptococcus spp. in pigs, ABPC, PCG and CTF showed low resistance rates of less than 10%. On the other hand, the resistance rates were as high as 100% in TC and more than 80% in EM. The trend in resistance rates remained unchanged for all drugs.

Fig. 3-2-3-1 Antimicrobial resistance rates in *S.suis*

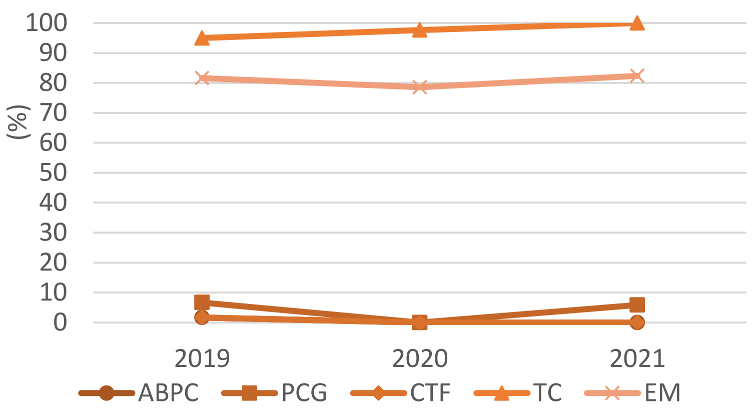
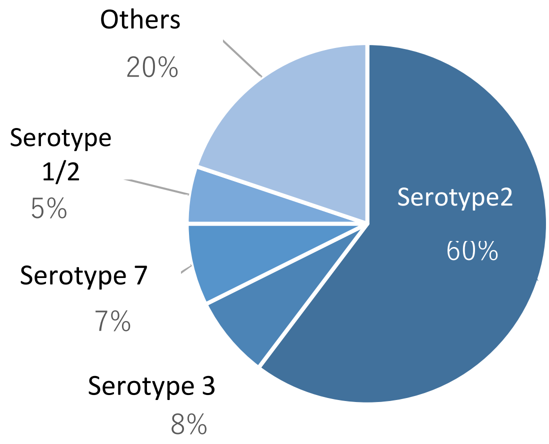


Fig.3-2-3-2 Serotypes of *S.suis* (2019-2021)



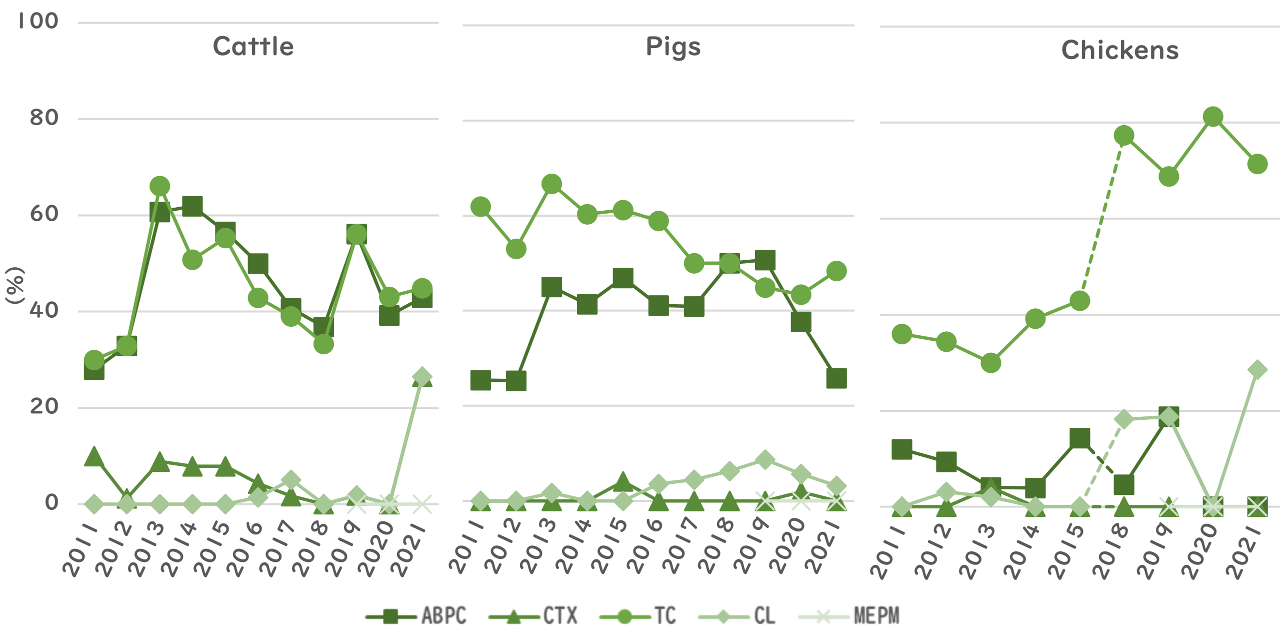
In 2019-2021, serotype 2 accounted for about 60% of isolates, and more than 10 diverse serotypes other than serotype 2 were isolated (Fig. 3-2-3-2). Serotype 2 is the most reported serotype in sick pigs in Japan, and most strains derived from human patients are also typed to serotype 2. In serotype 2 strains the resistance rate to ABPC and PCG in serotype 2 strains remained 0.0% in all years, while the resistant strains were detected in strains other than serotype 2.

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

A total of 87 isolates were collected from 22 prefectures, including 39 isolates from cattle, 31 isolates from pigs and 7 isolates from chickens.

The predominant serovars were 31 strains of Typhimurium, 19 strains of 4:i:-, followed by 7 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 2021; the resistance rate in pigs peaked in 2013 and showed a declining trend, while in chickens it increased to more than 70% between 2020 and 2021.

The resistance rate to ABPC was 42.9% in cattle, while in pigs it decreased after 2019 to 25.8% in 2021. On the other hand, in chicken it was 0% as in 2020. The resistance rates to CTX remained below 15% in cattle, pigs and chickens until 2020, but in cattle it was 26.5% in 2021. The serotypes of CTX-resistant strains were Typhimurium, 4:i:-, Dublin and Minnesota, of which all Dublin strains were resistant.

On the other hand, the CL resistance rate in cattle, pigs and chickens remained low, at less than 20% until 2020, while the resistance rate increased to 26.5% in cattle and 28.6% in chickens in 2021. More than 80% of Dublin was resistant to CL. The serotypes isolated in the future and the resistance rate need to be monitored.

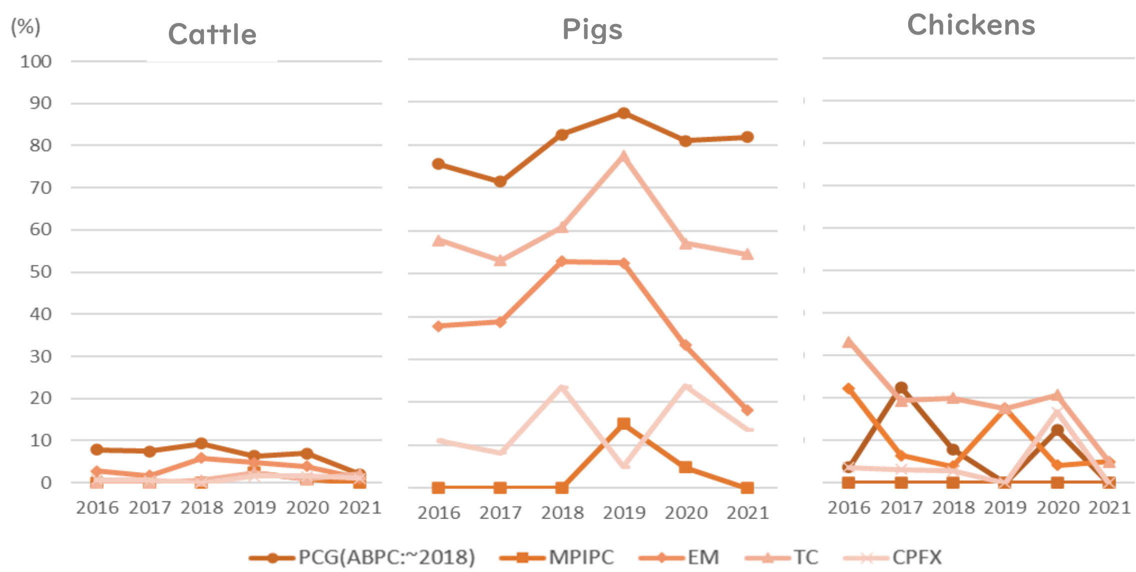
3-2-5 *Staphylococcus aureus* (cattle, pigs, broilers)

A total of 143 isolates were collected from 39 prefectures, including 101 isolates in cattle, 22 isolates in pigs and 20 isolates in chickens.

Resistance rates of *S. aureus* from diseased livestock from 2016 to 2021 are shown (Fig. 3-2-5-1).

The resistance rates of isolates from cattle and chickens were maintained at less than 10% for all drugs in 2021. On the other hand, in pigs, the resistance rates to PCG (ABPC until 2018) and TC were high. The resistance rate to CPFX was 1.0% in cattle and 13.6% in pigs, and in chickens increased to 16.7% in 2020, but decreased again to 0.0% in 2021. No MPIPC-resistant strains were detected in all animal species. Note that the comparison of resistance rates in pigs and chickens should be kept in mind due to the small number of strains.

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



The 5 drugs are either approved for veterinary use or with similar resistance mechanisms to approved drugs and MPIPC for the detection of MRSA, generally remained susceptibility in cattle and chickens. On the other hand, in pigs, the resistance rates to PCG and TC were high, making it important to perform antimicrobial susceptibility testing for administration in clinical practice.

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 rate to CPFX decreased in cattle and tended to increase in pigs and chickens. The resistance rate to CTX increased slightly in pigs, decreased in cattle and remained unchanged in chickens.

In *M. haemolytica,* the resistance rate to ABPC and DSM shows more than 40% from 2019 to 2021. The resistance rate to KM, TMS and TC remained below 30%, and the resistance rate to TUM, ERFX and FFC were below 10%. The resistance rate to CTF was 0.0% throughout the three years.

In *S. suis*, the resistance rates to all drugs have leveled off for three years from 2019 to 2021, and the sensitivity to β-lactam and CTF maintained. On the other hand, the resistance rates to TC and EM were high. Serotype 2 accounted for 60% of the total.

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 rate for CL and CTX, 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 50% for TC in pigs. The resistance rate to CPFX increased to 16.7% in 2020 in chickens, but 0.0% in 2021. Note the small number of strains in pigs and chickens.

Overall, bacteria resistant to approved antimicrobial drugs were detected in diseased livestock. Although resistance rates against second-line drugs were generally low, some of them were exhibited upward trends. Therefore, it is important to perform antimicrobial susceptibility tests prior to treatment and to use effective and appropriate antimicrobial drugs only when truly necessary.

3-2-7 Acknowledgement

We would like to thank the prefectural livestock hygiene service centers 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 2021, samples from 183 dogs and 180 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
Escherichia coli		154		161
Enterococcus spp.	*E. faecalis*	115	*E. faecalis*	73
	E. faecium	5	*E. gallinarum*	4
	E. casseliflavus	3	*E. faecium*	3
	E. gallinarum	2	*E. hirae*	2
	E. durans	2	*E. casseliflavus*	1
	E. hirae	1	*E. avium*	1
	Total	128	Total	84

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

Resistance rate of *E. coli* from dogs and cats from 2018 to 2021 are shown (Fig. 3-3-1-1, 3-3-1-2)

Fig. 3-3-1-1 Resistance rates of *E. coli* from dogs, 2018-2021

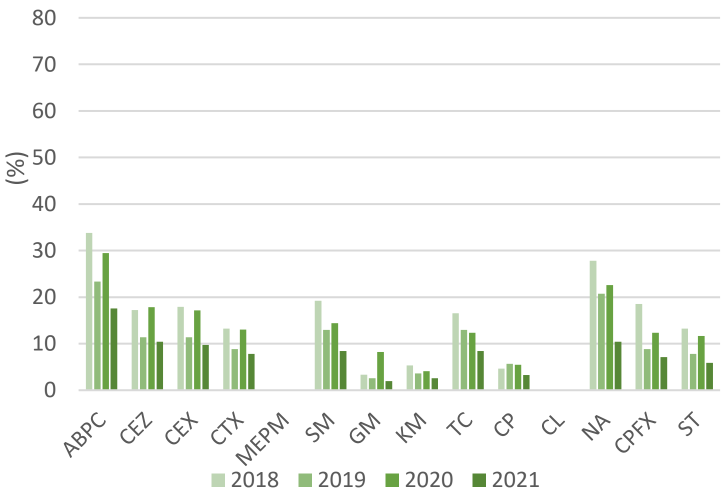
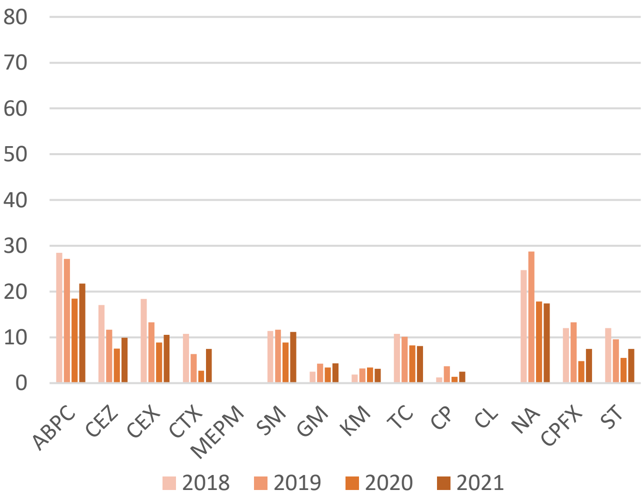


Fig. 3-3-1-2 Resistance rates of *E. coli* from cats, 2018-2021



In 2021, the resistance rate of *E. coli* from dogs was 17.5% for ABPC, which was the highest resistance rate, and less than 20% for all drugs. Resistance rates to CPFX (fluoroquinolones) and CTX (third-generation cephalosporin) were 7.1% and 7.8% respectively while MEPM (carbapenems) and CL (polypeptides) were both 0.0%. The resistance rates of *E. coli* from healthy dogs to ABPC, GM and NA were lower significantly in 2021 than that in 2020 (Fig.3-3-1-1). The resistance rates in healthy dogs were significantly lower than those of *E. coli* in diseased dogs collected in the same year for all drugs except KM (Fig.3-3-1-3).

The resistance rate from cats were less than 20% for all drugs except ABPC, and the highest resistance rate was observed in ABPC (21.7%) followed by NA (17.4%) (Fig.3-3-1-2). The resistance tendency was similar to that of the dog-derived strains, being 7.5% for CPFX and CTX, and 0.0% for MEPM and CL. The resistance rates in 2021 were not significantly different compared to those from healthy cats in 2020. The resistance rates were significantly lower than those of *E. coli* from diseased cats collected in the same year for all drugs except KM (Fig.3-3-1-4).

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

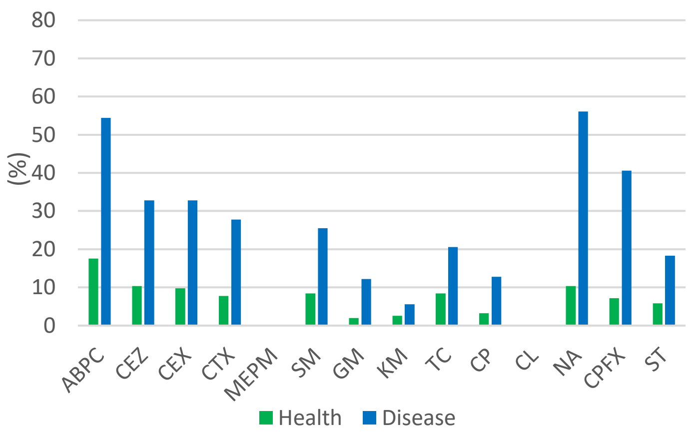
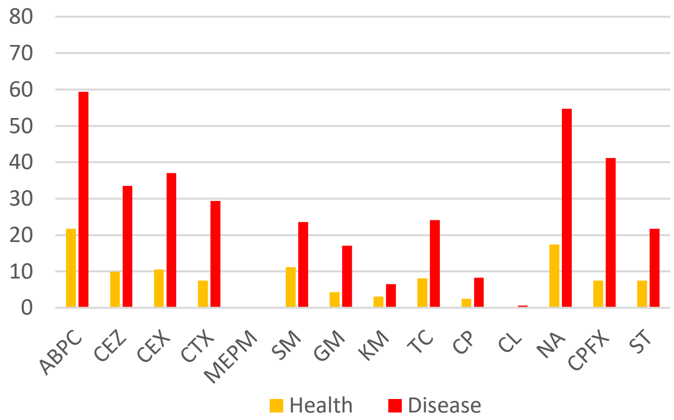


Fig. 3-3-1-4 Resistance rates of *E. coli* from healthy and diseased cats



3-3-2 *Enterococcus* spp. (dogs, cats)

Trends in resistance rates of *Enterococcus* spp. from dogs and cats from 2018 to 2021 are shown (Fig.3-3-2-1, Fig.3-3-2-2).

Fig. 3-3-2-1 Resistance rates of *Enterococcus* spp. from dogs, 2018-2021

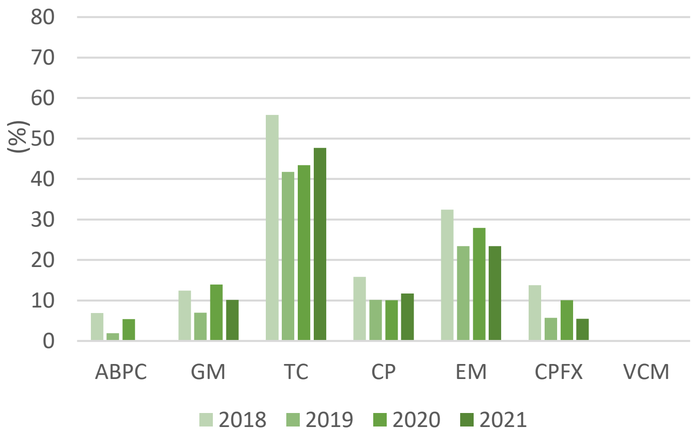
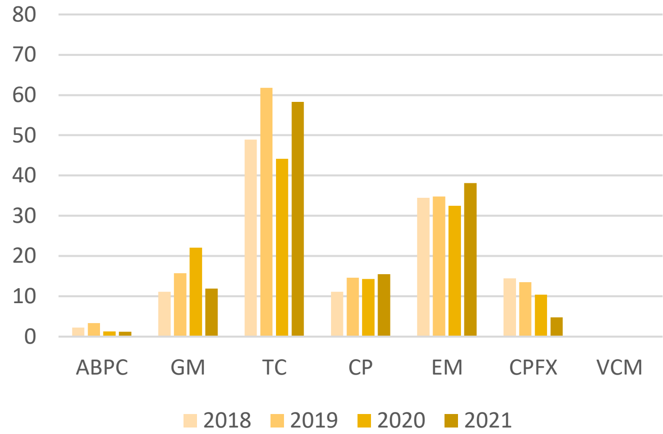


Fig. 3-3-2-2 Resistance rates of *Enterococcus* spp. from cats, 2018-2021



In 2021, the resistance rate 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 5.5%, and to VCM was 0.0%. There were no significant differences in *Enterococcus* spp. from healthy dogs between 2020 and 2021. Resistance rates of all drugs except CP were significantly lower than those of diseased dogs collected in the same year (Fig.3-3-2-3).

The resistance rate of cat-derived strains showed relatively high for TC and EM, and less than 20% of all other drugs (Fig.3-3-2-2). The resistance tendency was similar to that of dog-derived strains. The resistance rate to CPFX was 4.8%, and to VCM was 0.0%. There was little difference between 2020 and 2021. Resistance rates were significantly lower than those of diseased cats collected in the same year except TC, EM and CP (Fig.3-3-2-4).

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

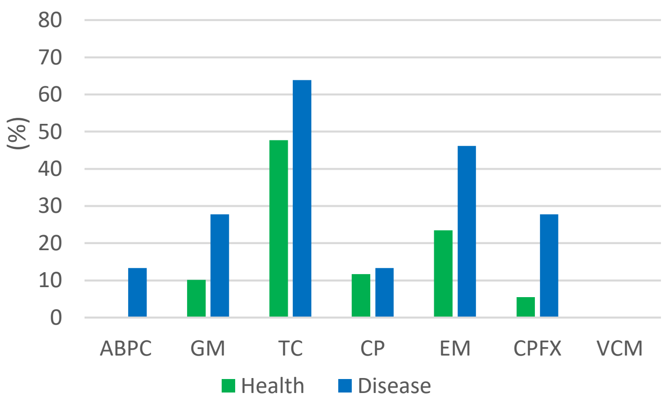
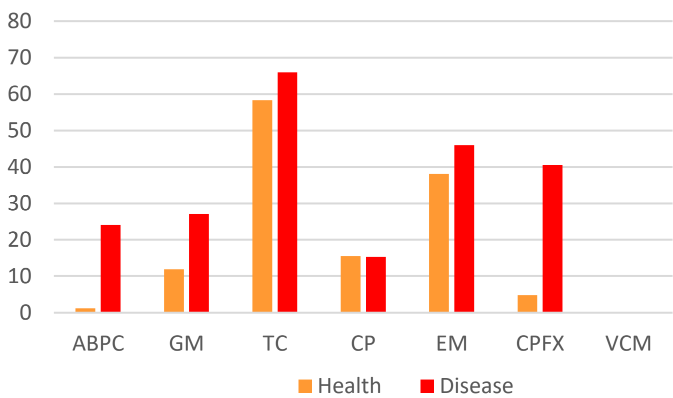


Fig.3-3-2-4
Resistance rates of *Enterococcus* spp. from healthy and diseased cats



3-3-3 Questionnaire results

A questionnaire was administered to owners of 183 dogs and 180 cats sampled at animal clinics in 2021, and the results are shown on the following pages.

In terms of sex, about 55% of dogs were females, and about 55% of cats were males. In terms of age, the younger age group of 6-year-old or less in both dogs and cats occupied about 60%. About 50% 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 purpose 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 about 80% of dogs and cats, followed by "canine prescription diet" in dogs and "commercial wet food" in cats

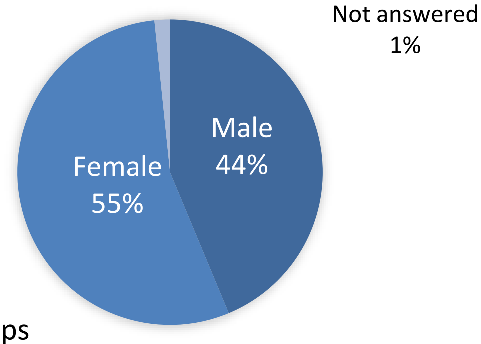
In the “3 month before sample collection” period, the 17% of dogs and 9% of cats had been administered antimicrobials. The most common route of administration was oral in dogs and injection in cats. In addition, more than 90% of both dogs and cats had never been hospitalized and had no contact with a person who had suffered an infection or was hospitalized.

Questionnaire results on dogs sampled in this survey in 2021 (number of responses 183)

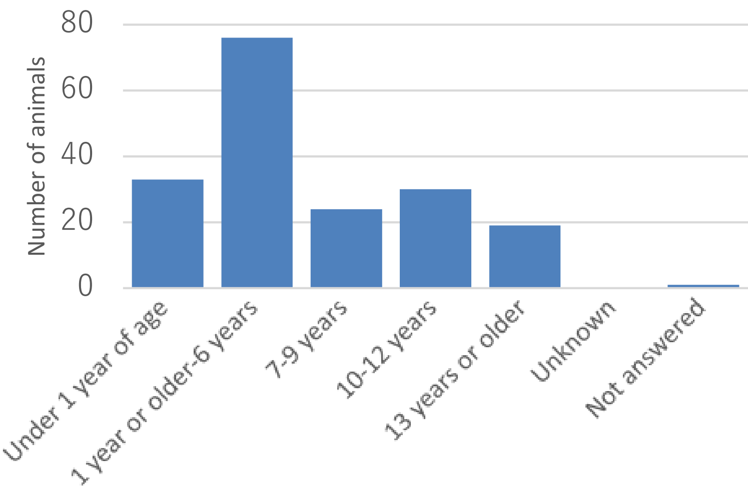
1． Species

Breed	Number of animals
Toy poodle	28
Mongrel	27
Dachshund	20
Shiba dogs	15
Chihuahua	14
Labrador retriever	12
Shih tzu	7
Welsh corgi	6
Golden retriever	6
Miniature schnauzer	5
Maltese	3
Yorkshire terrier	3
Cavalier king charles terrier	3
Other	32
Not answered	2
Total	**183**

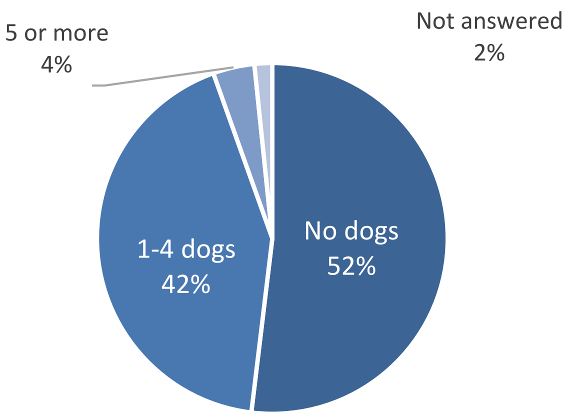
2． Sex



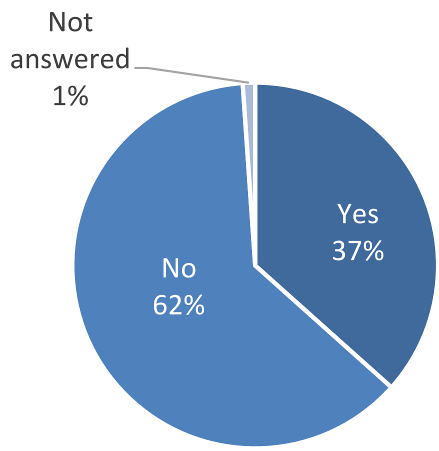
3． Age groups



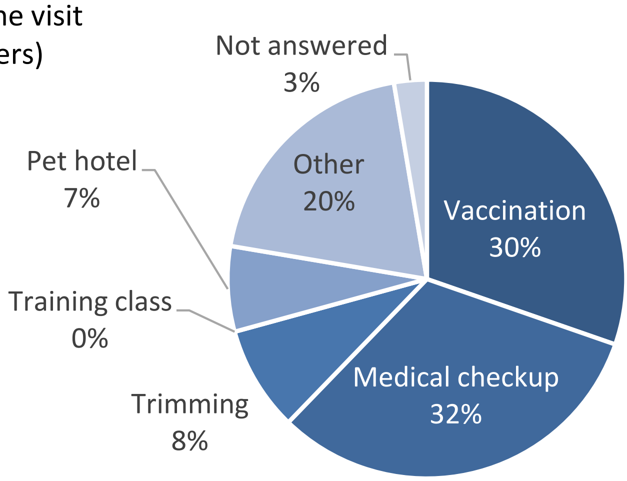
4． Number of dogs living together



5． Cohabitation with animals other than dogs

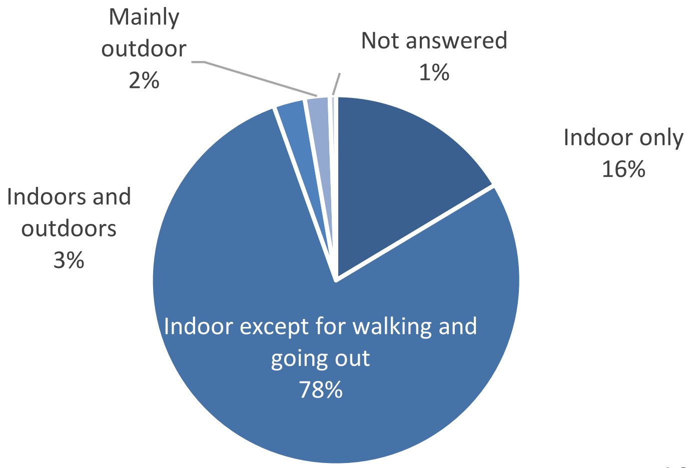


6． Objective of the visit (multiple answers)

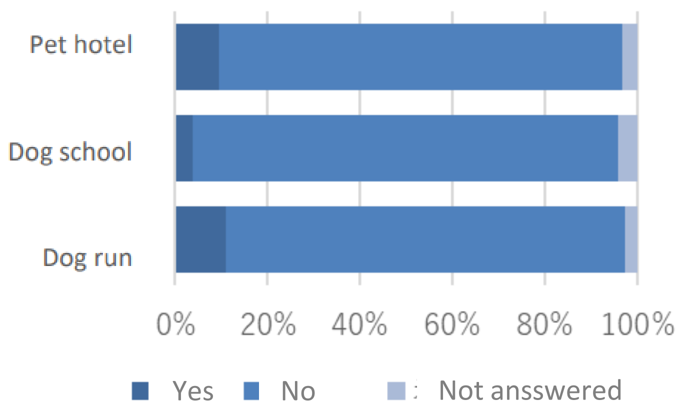


<Questionnaire results: 3 months before sample collection>

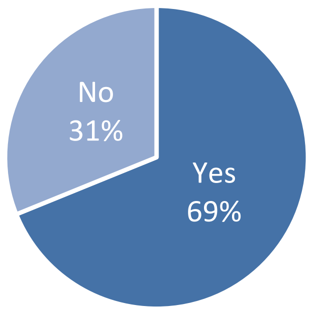
7. Where did you spend most of your time?



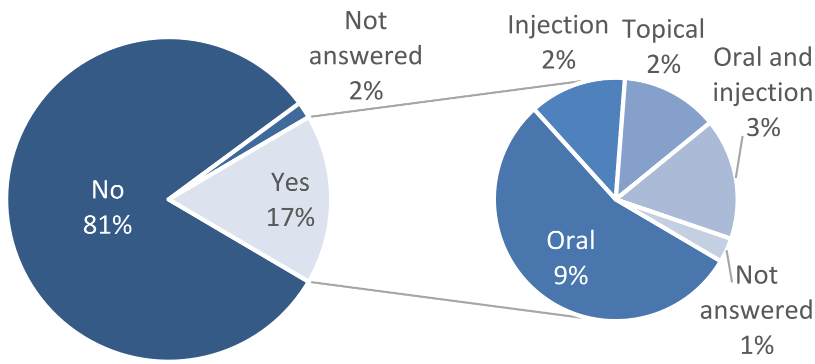
8. Have you used the following services?



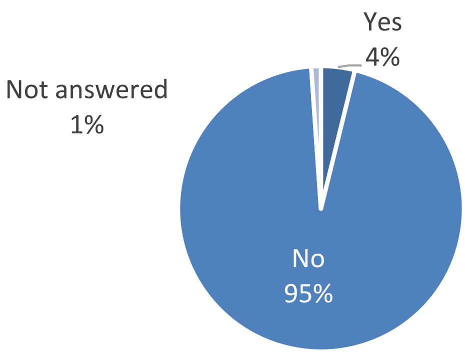
9. Have you visited animal clinic with a dog?
(For any purpose of the visit)



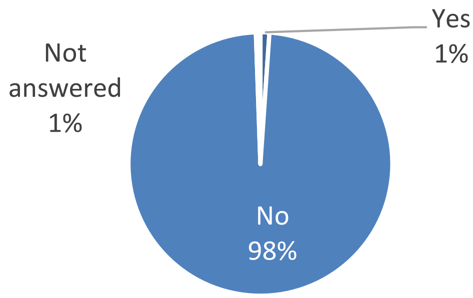
10. Has your dog administrated antimicrobials?
10-1. If yes, which was the route?



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)

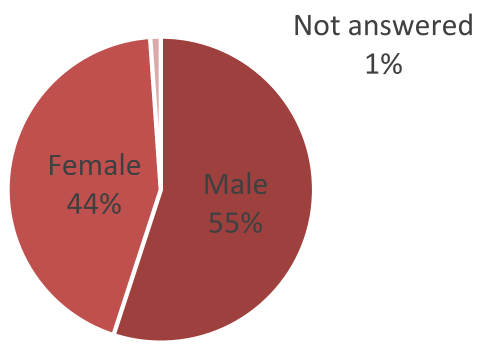
Diet	Number of animals	%
Commercial dry food	145	79.2
Commercial wet food	26	14.2
Commercially semi-dried dog food	9	4.9
Canine prescription diet	36	19.7
The same as a human diet	1	0.5
Cooked for dogs at home	16	8.7
Human meals leftover	3	1.6
Raw vegetables	8	4.4
Raw meat, bone	0	0.0
Not answered	1	0.5

Questionnaire results on cats sampled in this survey in 2021 (number of responses 183)

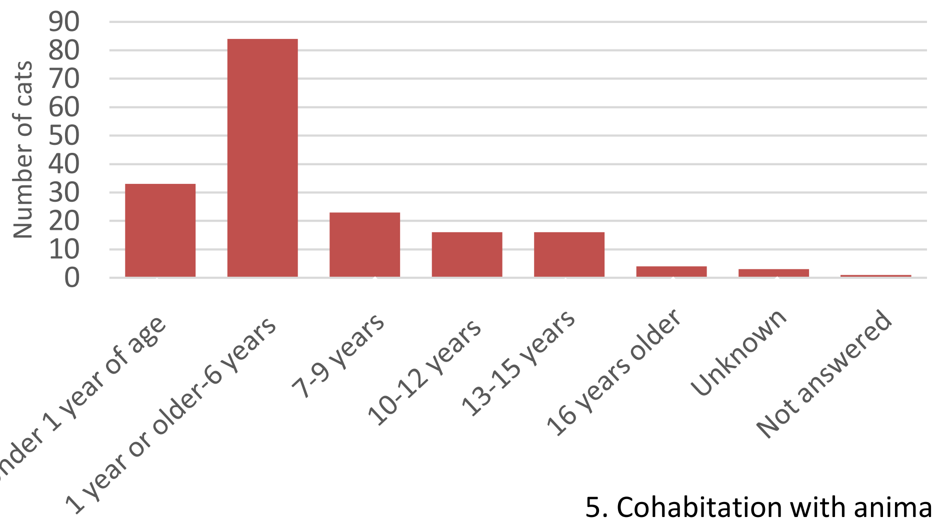
1. Species

Breed	Number of animals
Mongrel (Including those described as Mix, Japanese cats, or cats in the answer)	147
Norwegian Forest Cat	4
Scostish fold	3
American short hair	3
Main coon	2
Minuet	2
British short hair	2
Other	16
Not answered	1
Total	**180**

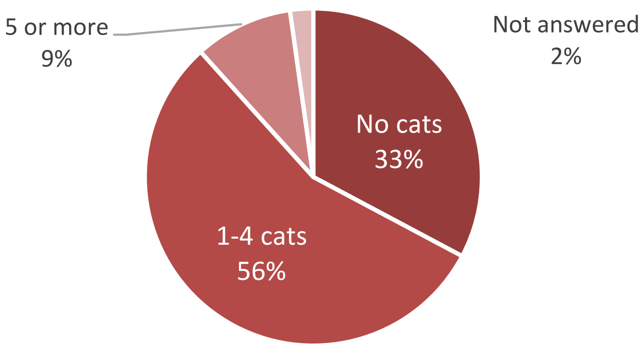
2. Sex



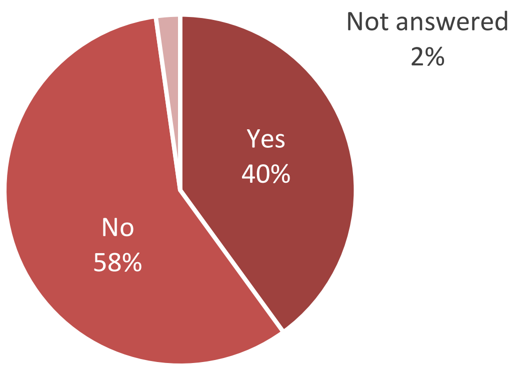
3. Age groups



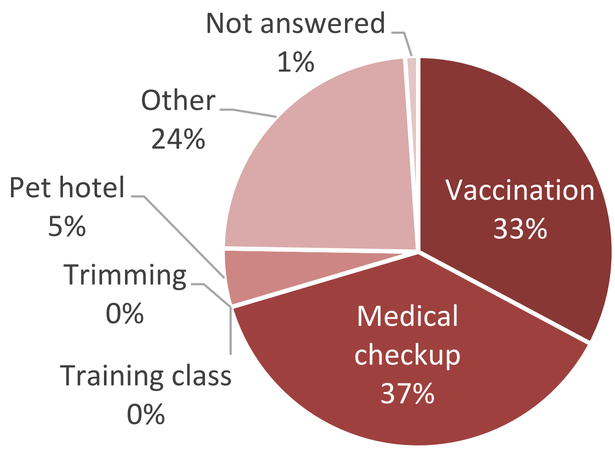
4. Number of cats living together



5. Cohabitation with animals other than cats

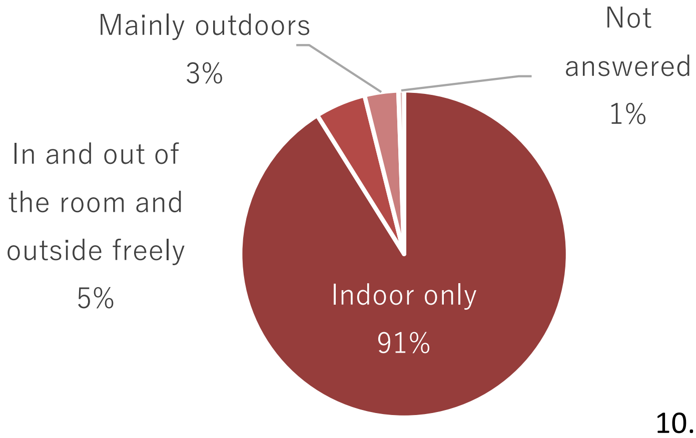


6. Objective of visit (multiple answers)

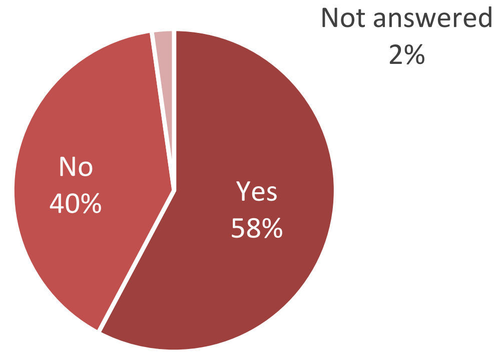


<Questionnaire results: 3 months before sample collection>

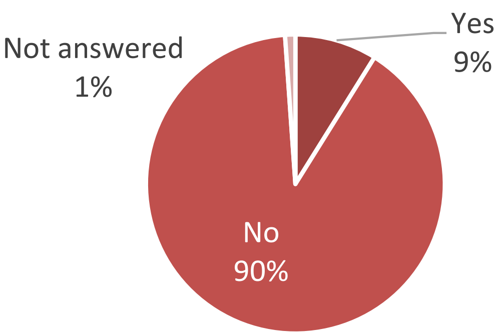
7. Where did you spend most of your time?



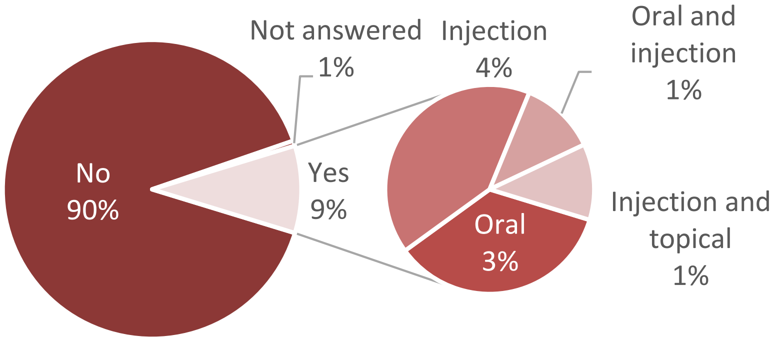
8. Have you visited a veterinary hospital with a cat?
(For any purpose of the visit)



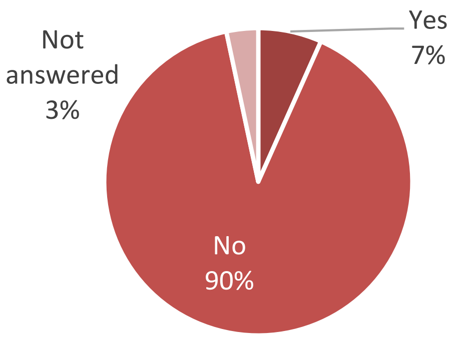
9. Did you use a pet hotel?



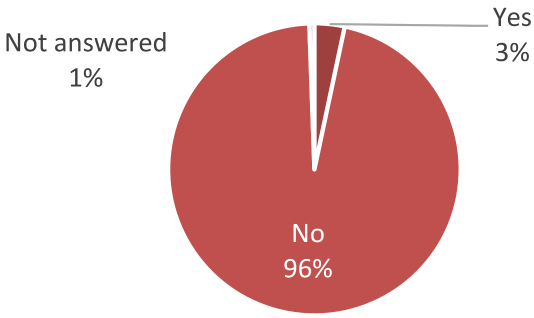
10. Has your cat administrated antimicrobials?
10-1. If yes, which was the route?



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	140	77.8
Commercial wet food	55	30.6
Commercial semi-dried type cat food	2	1.1
Cat prescription diet	53	29.4
The same as a human diet	0	0
Cooked at home for cats	6	3.3
Human meals leftover	0	0
Raw vegetables	1	0.6
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 0.0%. 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 at 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 2021 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 2021

Species	Sampling site
Escherichia coli, *Klebsiella* spp.	urine, reproductive tract
Acinetobacter spp., Coagulase-positive *Staphylococcus* spp.	urine, skin
Enterococcus spp., *Pseudomonas aeruginosa*	urine, ear

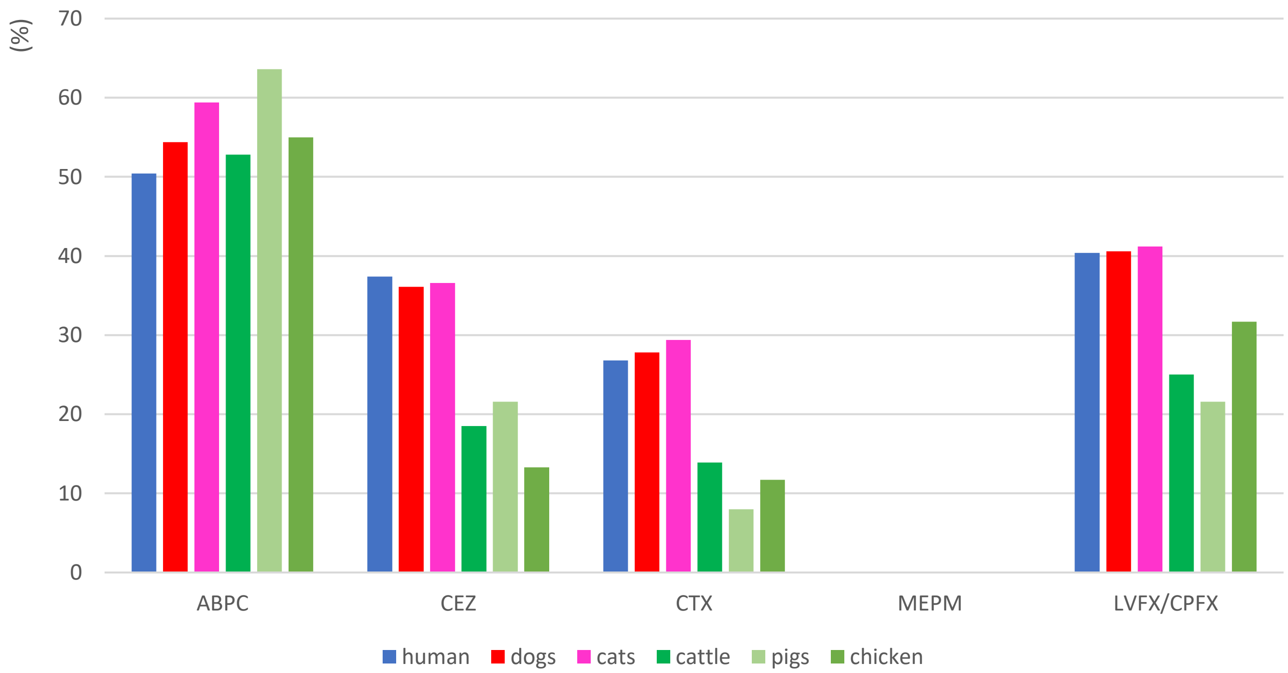
Table 3-4-2 Number of isolates

Species	Dogs	Number of strains	Cats	Number of strains
E. coli		180		170
Klebsiella spp.	*K. pneumoniae*	79	*K. pneumoniae*	59
	K. oxytoca	10	*K. oxytoca*	15
	K. aerogenes	2	*K. aerogenes*	1
	Total	91	Total	75
P. aeruginosa		94		79
Acinetobacter spp.	*A. baumannii*	10	*A. baumannii*	7
	A. radioresistens	4	*A. pitti*	5
	A. lwoffii	4	*A. radioresistens*	4
	A. pitti	3	*A. guillouiae*	2
	A. johnsonii	3	*A. junii*	2
	A. junii	2	*A. berenziniae*	1
	A. nosocominalis	2	*A. ursingil*	1
	A. berenziniae	1	*A. dispersus*	1
	A. lactucae	1	*A. lactucae*	1
	A. piscicola	1	*A. proteolyticus*	1
	A. schindleri	1	*A. johnsonii*	1
	A. soli	1		
	Total	33	Total	26
Coagulase-positive *Staphylococcus* spp.	*S. pseudintermedius*	76	*S. pseudintermedius*	61
			S. aureus	27
	Total	76	Total	88
Enterococcus spp.	*E. faecalis*	141	*E. faecalis*	110

E. faecium	26	*E. faecium*	44
E. avium	5	*E. gallinarum*	10
E. gallinarum	4	*E. casselifravus*	3
E. casselifravus	2	*E. hirae*	1
E. raffinosus	1	*E. raffinosus*	1
E. canintestini	1	*E. canintestini*	1
Total	180	Total	170

The resistance rates in *E. coli* derived from diseased human and animals in 2021 in Japan are shown in Fig. 3-4. The resistance rates in dogs and cats showed similar trends to those in human, and higher than those in cattle, pigs and chickens for third-generation cephalosporins and fluoroquinolones (LVFX in human), which are the most important antimicrobial agents in human medicine.

Fig. 3-4 Resistance rates in *E. coli* from diseased human* and animals in Japan



*Nippon AMR One Health Report (NAOR) 2023 (https://www.mhlw.go.jp/content/10900000/001268944.pdf)

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

In 2021, the resistance rates of *E. coli* were similar trends between dogs and cats. Similar to the results in 2020, resistance rates to ABPC, NA, CPFX, CEX and CEZ tended to be higher (Fig.3-4-1-1, 3-4-1-2). The resistance rate to GM in cats was significantly higher than that in 2020.

The resistance rates in dogs and cats to fluoroquinolones (CPFX) were 40.6% and 41.2%, while the third-generation cephalosporins (CTX) were 27.8% and 29.4%, respectively. One strain from cats was resistant to CL of polypeptides, but no *mcr* gene was detected in this strain. The resistance rate for MEPM of carbapenems was 0.0% in both dogs and cats.

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

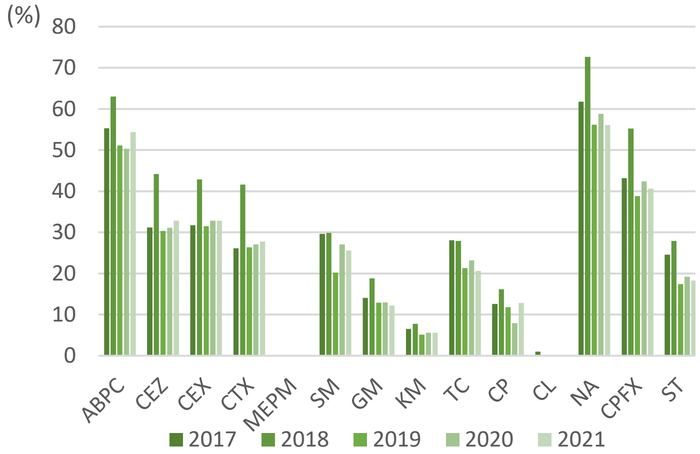
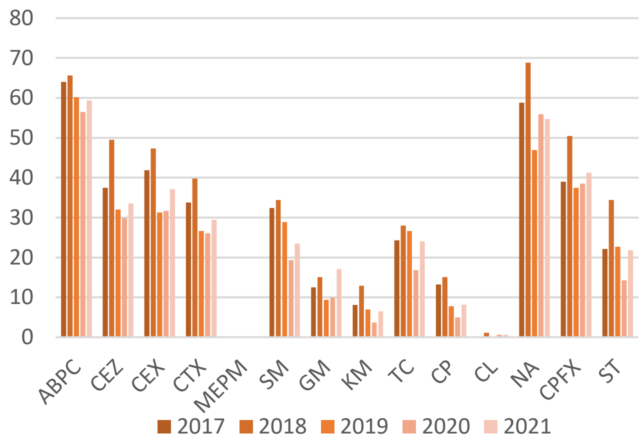


Fig. 3-4-1-2 Resistance rates of *E. coli* from cats



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

Klebsiella spp. isolated from dogs and cats were mostly *K. pneumoniae*, followed by *K. oxytoca, K. aerogenes* (Table3-4-2).

Resistance rates of dog-derived strains against 7 reagents including NA, CPFX, CEZ, CEX, ST, CTX and SM tended to be high. This trend was similar to that observed in 2020 (Fig. 3-4-2-1). In cats, the resistance rates against 9 reagents including NA, CPFX, CEZ, CEX, CTX, TC, ST, SM and GM tended to be high. The resistance rates to NA, CPFX and TC were significantly higher than those in 2020 (Fig.3-4-2-2).

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

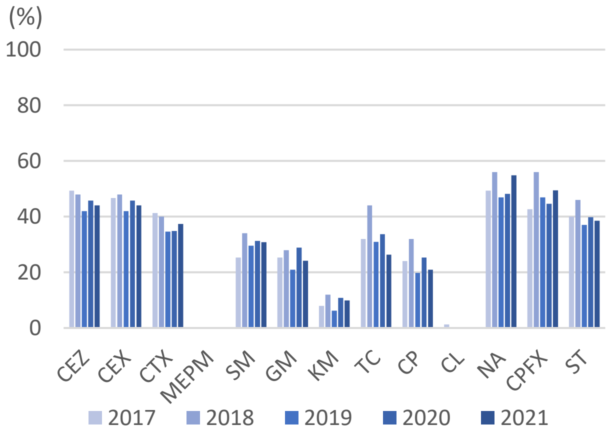
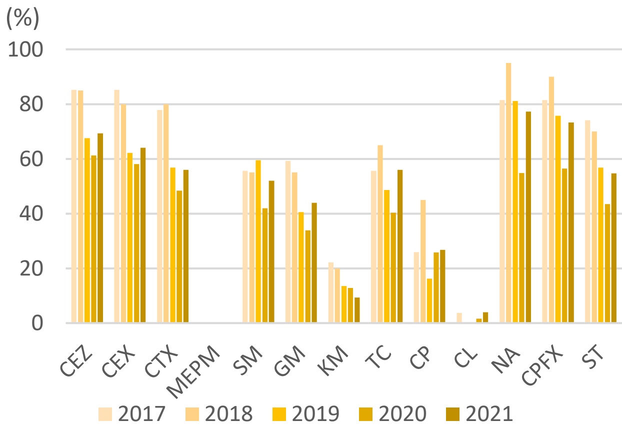


Fig. 3-4-2-2 Resistance rates of *Klebsiella* spp. from cats



3-4-3 *Pseudomonas aeruginosa* (dogs, cats)

Resistance rates of *P. aeruginosa* from dogs and cats in 2021 are shown in Fig.3-4-3-1 and Fig. 3-4-3-2. The resistance rates to CPFX were 22.3% in dogs and 15.2% in cats. The MEPM resistant strains were detected in one strain from dogs and two strains from cats. All these strains were susceptible to GM and CPFX. Two resistant strains to CL were found in dog-derived strains, but they didn't have *mcr* gene. Note that the number of strains of cats in 2018 was less than 20 (18 strains).

Fig. 3-4-3-1 Resistance rates of *P. aeruginosa* from dogs (2018, 2021)

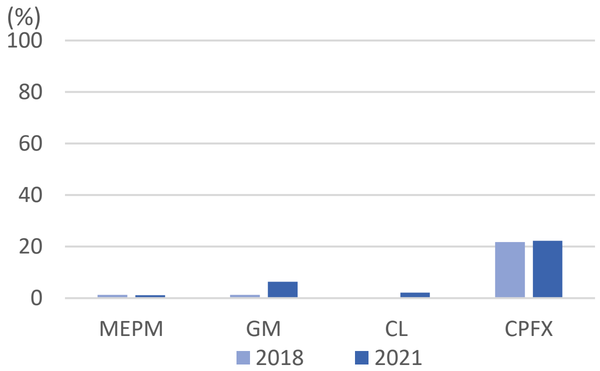
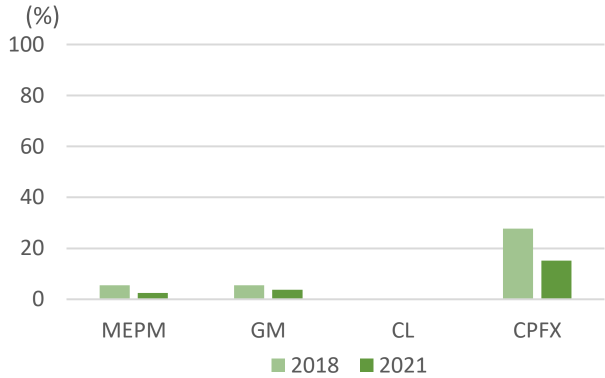


Fig. 3-4-3-1 Resistance rates of *P. aeruginosa* from dogs (2018, 2021)



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

In 2021, 12 species of *Acinetobacter* spp. were isolated from dog-derived strains, the most common being *A. baumannii*, followed by *A. radioresistens* and *A. lwoffii*. 11 species were isolated from cat-derived strains, the most common being *A. baumannii*, followed by *A. pitti* and *A. radioresistens* (Table 3-4-2).

Resistance rates in dogs were less than 20% (Fig.3-4-4-1). On the other hand, the resistance rates in cats tended to be higher in CPFX (57.7%), ST(46.2%), TC(34.6%) and GM(30.8%) (Fig.3-4-4-2). It should be noted that the number of strains of cats is only 26. The number of isolates from cats in 2020 was small (11 strains) and the resistance rate is not shown.

One of the cat-derived strains was resistant to MEPM, but susceptible to GM. In addition, one of the cat-derived strains was resistant to CL, but it had no *mcr* gene.

Fig. 3-4-3-1 Resistance rates of *Acinetobacter* spp. from dogs (2018, 2021)

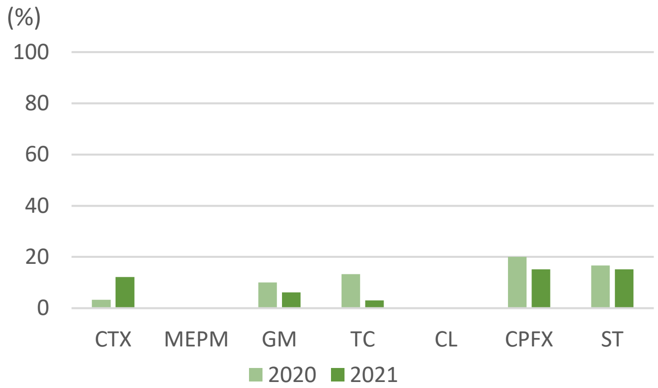
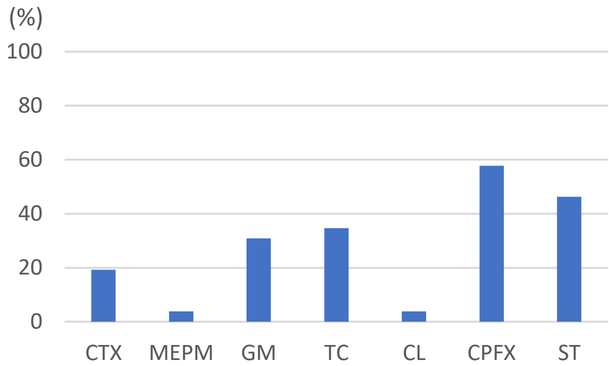


Fig. 3-4-3-1 Resistance rates of *Acinetobacter* spp. from cats (2021)



3-4-5 Coagulase positive Staphylococci (dogs, cats)

In 2021, all strains of coagulase-positive *Staphylococcus* spp. isolated from dogs were *Staphylococcus pseudintermedius.* In cats, *S. pseudintermedius* and *S. aureus* were isolated (Fig.3-4-2). As breakpoints by CLSI and EUCAST varies among staphylococci depending on species, the resistance rates were calculated by species. The results are shown for *S. pseudintermedius* from dogs and cats and *S. aureus* from cats in Fig. 3-4-5-1 and Fig. 3-4-5-2.

In 2021, the resistance rates of *S. pseudintermedius* derived from dogs were highest in the following order: PCG, CPFX, TC, EM, AZM, MPIPC, CP and GM. The resistance rate to GM was significantly higher than that in 2020 (Fig. 3-4-5-1). The resistance rates of *S. pseudintermedius* derived from cats were highest in the following order: PCG, EM, AZM, CPFX, TC, CP, MPIPC and GM (Fig. 3-4-5-2).

The resistance rates of *S. aureus* derived from cats were highest for PCG followed by EM, AZM, CPFX, CEX, CTX, MPIPC, CFX and CEZ. The resistance rates to GM and TC were significantly lower than those in 2020 (Fig.3-4-5-3). Caution is necessary when comparing the resistance rates as the number of strains in 2018 was less than 20.

Resistance rates of cat-derived strains in *S. pseudintermedius* were significantly higher than those of dog-derived strains. Resistance rates in dogs and cats were 73.7% and 91.8% for CPFX, and 71.1% and 91.8% for AZM of 15-membered ring macrolides, respectively. Resistance rates to MPIPC were 56.6% and 78.7% in *S. pseudintermedius* derived from dogs and cats, and 51.9% in *S. aureus* derived from cats.

Fig. 3-4-5-1 Resistance rate in *S. pseudintermedius* from dogs

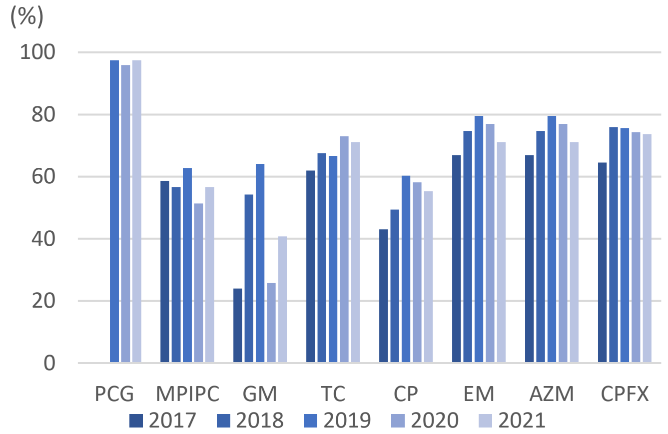


Fig. 3-4-5-2 Resistance rate in *S. pseudintermedius* from cats

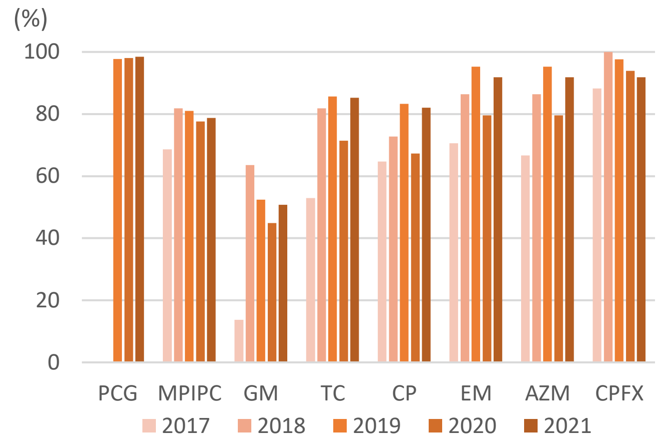
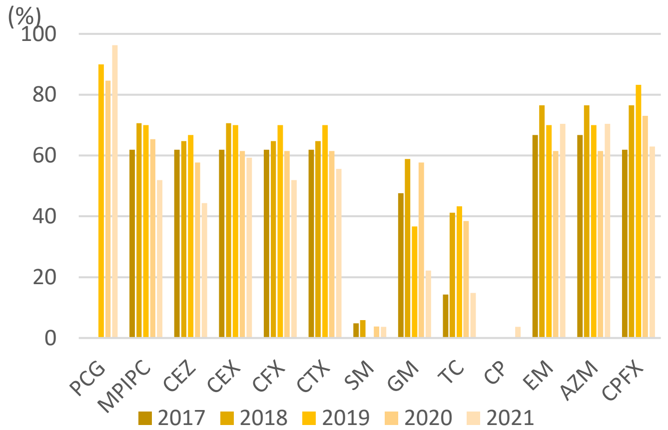


Fig. 3-4-5-3 Resistance rate in *S. aureus* from dogs



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

In 2021, *Enterococcus faecalis* was the most commonly isolated *Enterococcus* spp. from dogs and cats, followed by *E. faecium*. Additionally, *E. avium*, *E. gallinarum*, *E. casseliflavus*, *E. raffinosus* and *E. canintestini* were isolated from dogs while *E. gallinarum*, *E. casseliflavus*, *E. hirae*, *E. raffinosus* and *E. canintestini* were isolated from cats (Table 3-4-2).

Resistance rates of *Enterococcus* spp. in both dogs and cats were highest in the following order: TC, EM, CPFX and GM. These rates were similar to those of 2020 (Fig.3-4-6-1, Fig.3-4-6-2).

The resistance rates to ABPC and CPFX in cats were significantly higher than those in dogs. The resistance rates to CPFX were 27.8% in dogs and 40.6% in cats. The resistance rates to VCM, where nosocomial infections caused by the resistant bacteria are a problem in human, were 0.0 % in both dogs and cats.

Fig. 3-4-6-1 Resistance rate in *Enterococcus* spp. from dogs

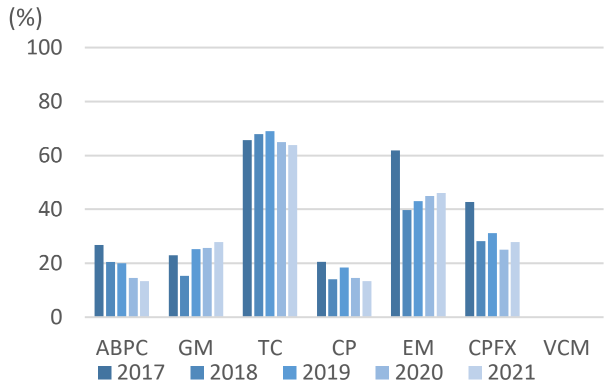
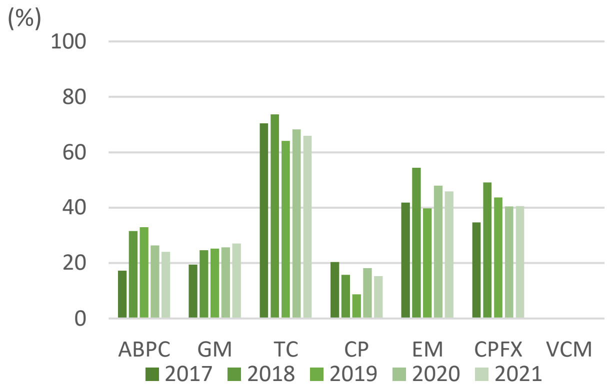


Fig. 3-4-6-2 Resistance rate in *Enterococcus* spp. from cats



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 number of strains and resistance rates are shown (Fig. 3-4-6-3 ~ Fig. 3-4-6-6)

Resistance rates of *E. faecalis* were found to be the highest for TC, followed by EM. The resistance rate to ABPC was 0.0% and the resistance rate in dogs to CPFX was significantly higher than that in 2020.

Resistance rates of *E. faecium* were highest for CPFX, ABPC, EM, TC and GM. As there are only 26 strains of *E. faecium* from dogs, the resistance rates are shown as a reference.

Fig. 3-4-6-3 Resistance rates in *E.* faecalis from dogs

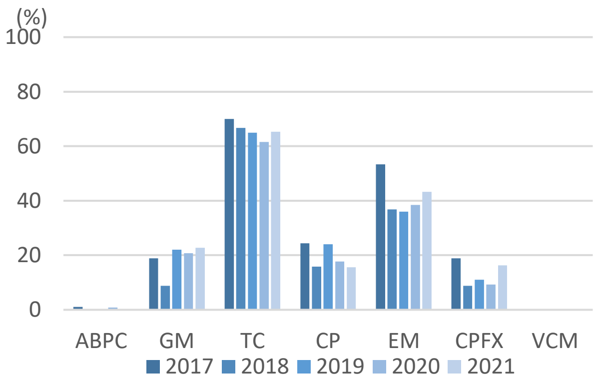


Fig. 3-4-6-4 Resistance rates in *E.* faecalis from cats

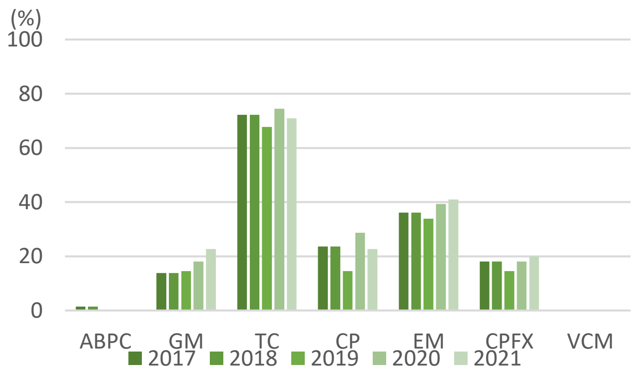


Fig. 3-4-6-3 Resistance rates in *E. faecium* from dogs

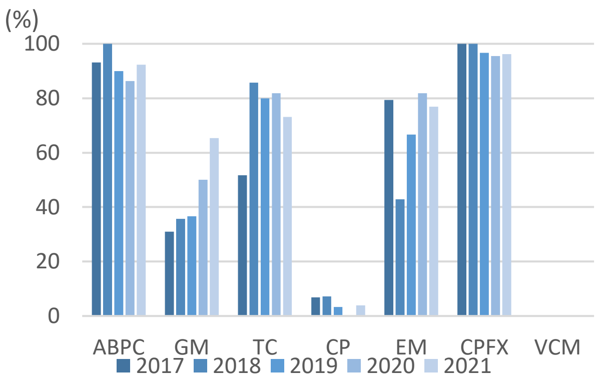
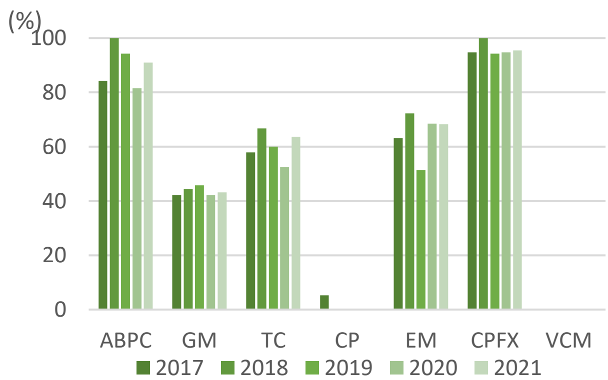


Fig. 3-4-6-4 Resistance rates in *E. faecium* from cats



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 2021, the susceptibility of *P. aeruginosa* was generally maintained. Resistance rates of *Acinetobacter* spp. derived from dogs were less than 20%.

Three strains of *P. aeruginosa* and one strain of *Acinetobacter* spp. were resistant to MEPM, a carbapenem antibacterial agent that is one of the most important antimicrobial agents in human medicine, although not approved for veterinary use. Resistance rate to VCM, which is a concern in human nosocomial infections, was 0.0% in *Enterococcus* spp.

The third-generation cephalosporins, fluoroquinolones, 15-membered ring macrolides and colistin 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 of *E. coli* from diseased dogs and cats were closer to human strains than to those from livestock, and higher than those from livestock for second-line drugs. Resistance rates to CTX were high in *Klebsiella* spp. derived from dogs and cats, and *S. aureus* from cats additionally, but less than 30% in other species. Resistance rate to CPFX was broad, ranging from 15.2% to 91.8%, and more than 70% for AZM in 15-membered ring macrolides (only coagulase-positive staphylococci). For CL, only a few strains showed resistance, and no mcr gene was detected in all CL-resistant strains isolated in 2021.

This survey was about bacteria from diseased dogs and cats, and the results are influenced by treatment with antimicrobials. There are many drugs with high resistance in some species. It is important to ensure the prudent use of antimicrobials, such as selecting effective antimicrobials by carrying out susceptibility testing before treatment and considering measures other than administration of antimicrobials, such as washing and disinfection for dermatitis, so that antimicrobials can continue to be used effectively in the treatment of bacterial infections.

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 Overview of veterinary antimicrobials sales volume

The sales volume of antimicrobials for animals is estimated to understand trends in their usage. Overall antimicrobial sales have decreased by about 20% since 2001 but have remained relatively stable at around 800 tonnes in recent years [Fig 4-1-1]. Tetracyclines account for the largest proportion by class, though their sales have been declining, falling below 40% of the total since 2018.

Fig. 4-1-1 Trends in Sales Volume of Veterinary Antimicrobials (2001-2021)

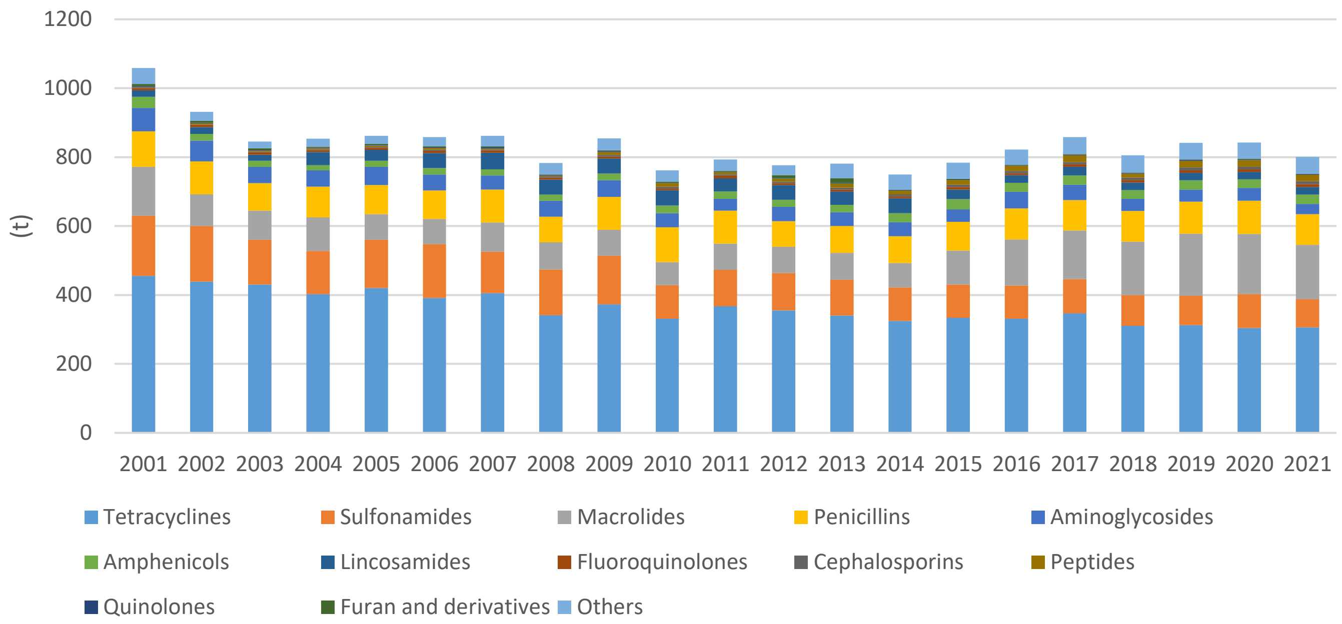


Figure 4-1-2 shows the trends in sales volume of antimicrobial agents for animals among G7 countries. Following the WHO global action plan, each country has been developing their own national action plans and various measures have been taken in each country. Japan has been experiencing a decrease in sales volume since the implementation of the action plan. However, the extent of the decline is smaller than that of other countries.

Fig. 4-1-2　Trends in Sales Volume of Veterinary Antimicrobials in G7 countries

Pigs account for the largest estimated proportion of sales by animal species, followed by fisheries (seawater) and broiler chickens (Fig 4-1-3).

Trends in Sale volume of Antibiotics across animal species are shown below (Fig. 4-1-4).

Fig. 4-1-3 Percentage of veterinary antimicrobial sales by animal species (2021)

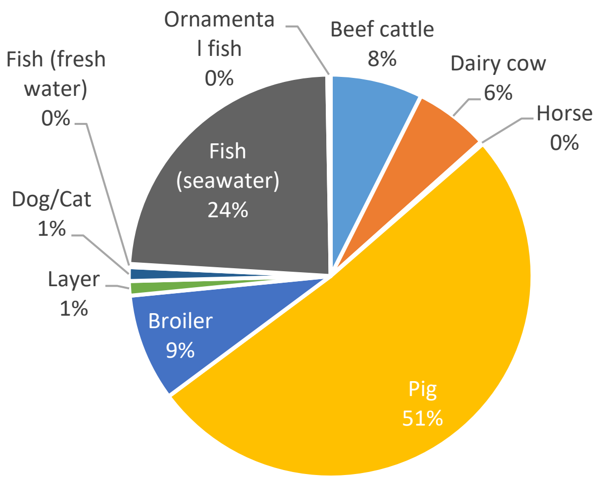
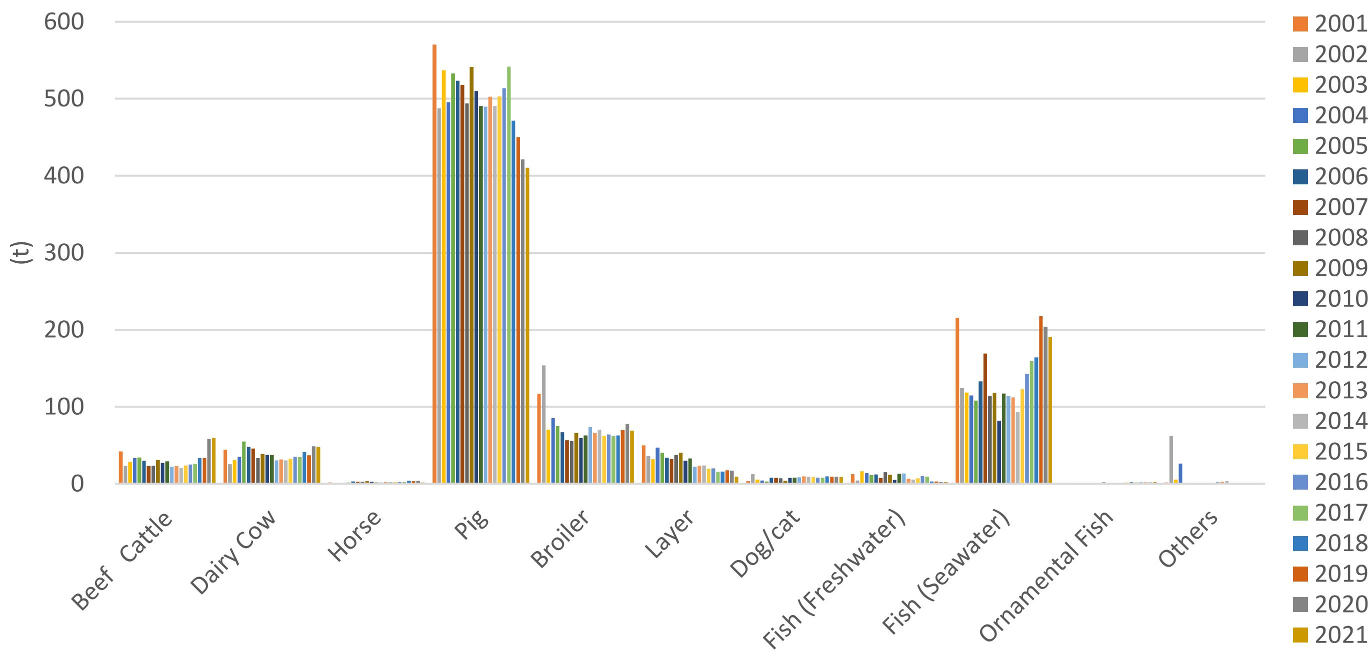
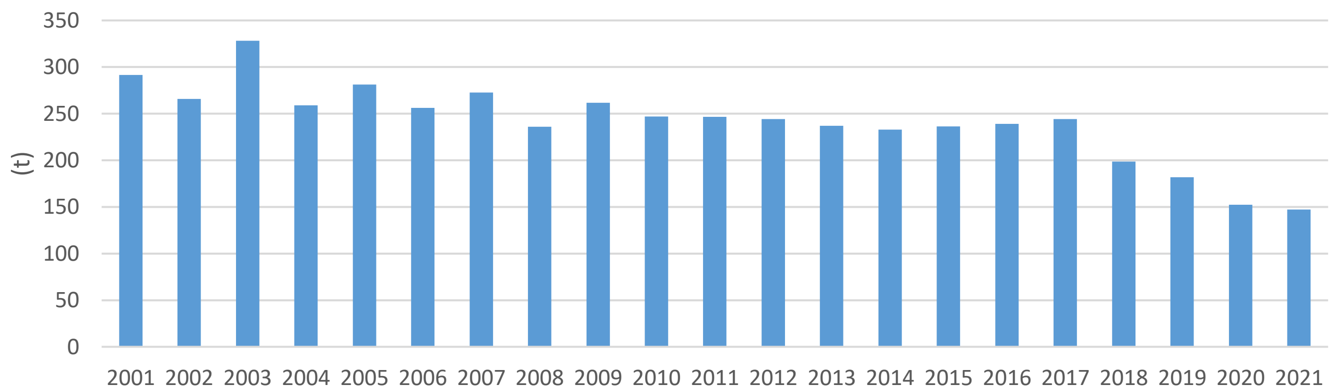


Figure 4-1-4 Trends in Sales Volume of Antibiotics by Animal Type (2001-2021)



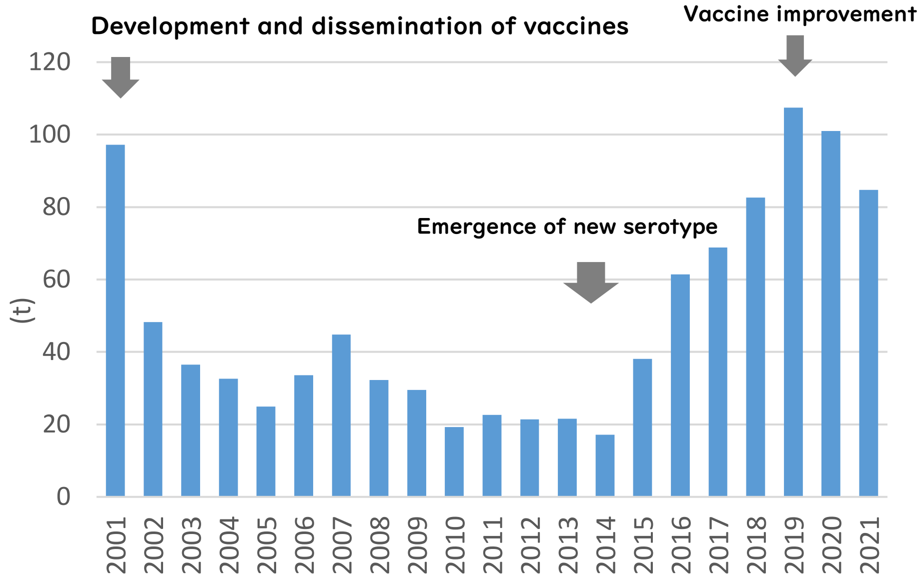
Sales for pigs, the largest category, have been decreasing in recent years, mainly due to declining tetracyclines sales. Sales in 2021 were half of the sales they had in 2001 (Fig.4-1-5).

Fig. 4-1-5 Trends of Tetracycline Sales Volume in Pigs



Fisheries (seawater), the second largest category after pigs, has seen increased sales since 2015, mainly due to increased use of macrolides (EM). The outbreak and treatment of the new serotypes of *Lactococcus garvieae* which is cause of type II α-hemolytic streptococcal disease was thought to be one of the main causes of that rise. However, the increasing trend reversed in 2020, possibly due to improvements in vaccine (Fig. 4-1-6).

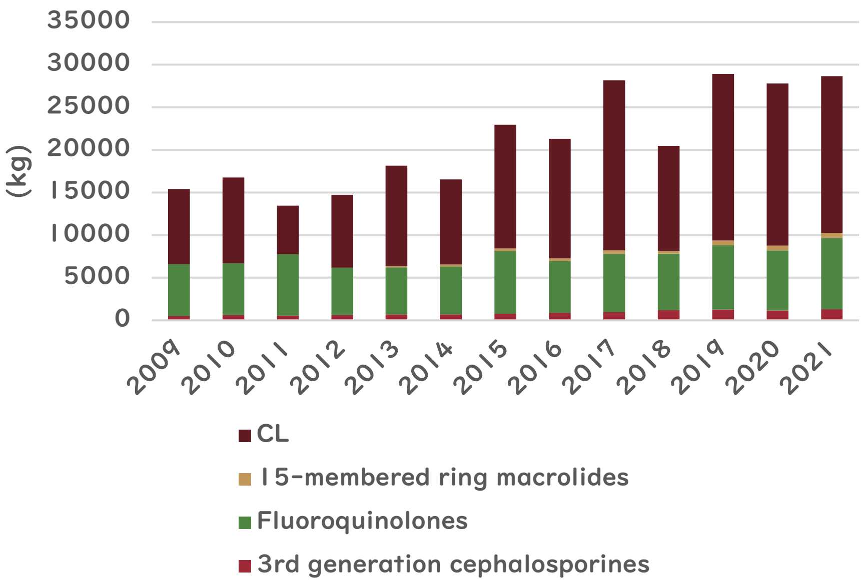
Fig. 4-1-6 Trends in EM sales volume for fisheries (Seawater) and Lactococcus garvieae infection-related matters



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

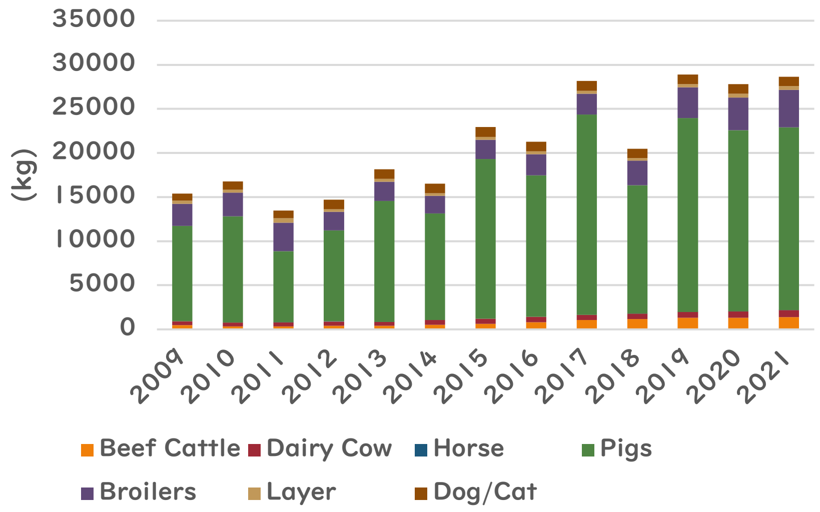
Second-line drugs are antimicrobials that are important for human medicine and are reserved for use only when first-line antimicrobials prove ineffective. Second-line drugs include fluoroquinolones, third-generation cephalosporins, 15-membered ring macrolides, and CL. The trends in sales volume of second-line drugs by class are shown in Figure 4-1-7. CL was sold the most, followed by fluoroquinolones.

Fig. 4-1-7 Trends in second-line drug sales by drug (2009-2021)



Pigs account for a large proportion of sales by animal species (Fig. 4-1-8). Approximately 80% of sales for pigs are CL, which increased after the withdrawal of colistin as a feed additive in 2018. The vaccine for edema disease, that is treated with CL, was developed and it, is expected to lead to a decrease of CL hereafter.

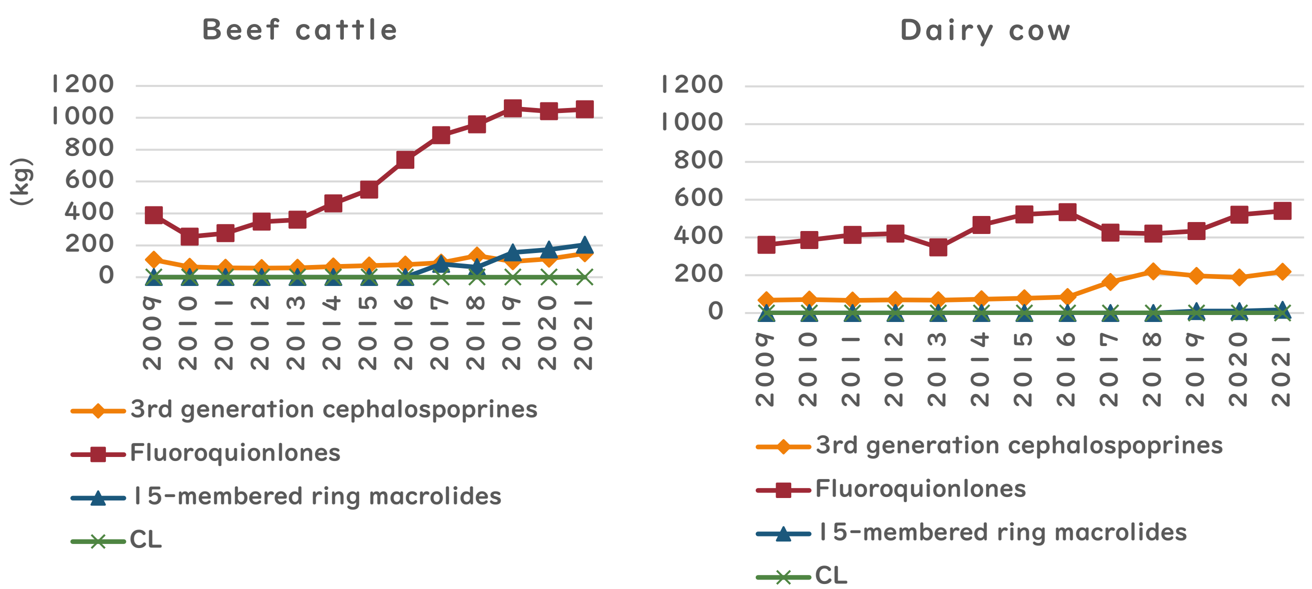
Fig. 4-1-8 Trends in second-line drug sales by animal species (2009-2021)



Excluding CL, broilers account for the largest sales volume of second-line drugs, with most being fluoroquinolones. The amount of fluoroquinolones sold to broilers has also been increasing in recent years (Fig. 3-1-6), and it is necessary to monitor resistance rates as well.

Sales of second-line drugs for cattle (beef and dairy) are lower than for pigs and broiler, but sales of third-generation cephalosporins, fluoroquinolones, and 15-membered ring macrolides for beef cattle, and third-generation cephalosporins and fluoroquinolones for dairy cattle, have been increasing in recent years (Fig. 4-1-9). This also requires continued monitoring along with resistance rates.

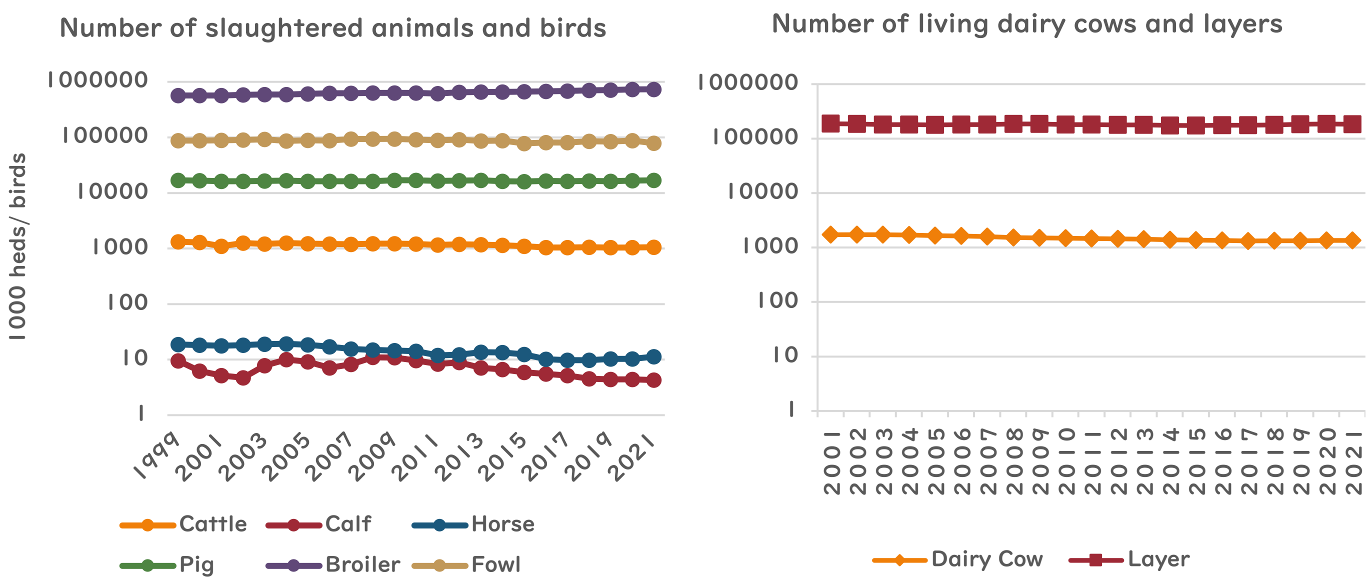
Fig. 4-1-9 Trends in second-line drugs sold to beef and dairy cattle (2009-2021)



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

The number of slaughtered livestock and the number of living dairy cattle and layer chickens in Japan are shown in Figure 4-1-10. It is observable that no major changes occurred over the past 20 years. Therefore, trends in sales volume are not greatly affected by increases or decreases in the number of animals.

Figure 4-1-10 Number of slaughtered animals and birds, and dairy cattle and layers number in farms (2001-2021)



4-1-4 Summary

In 2021, sales of veterinary antimicrobials remained at around 800 tonnes but decreased by approximately 42 tonnes from 2020. Tetracyclines increased by 1.3 tonnes, while macrolides decreased by 16.0 tonnes and sulfonamides decreased by 16.6 tonnes compared to the previous year.

In 2021, the decreases in antimicrobial sales volume by animal species were seen in fisheries (seawater) (13.6 tonnes), pigs (10.8 tonnes), broiler (8.4 tonnes), and layer (7.8 tonnes). On the other hand, sales for beef cattle increased by 0.9 tonnes, with tetracyclines increasing by 2.0 tonnes. The rapid increase in macrolides for fisheries (seawater) has reversed since 2020, which is thought to be due to the improvement of vaccines for the disease that was causing the increase.

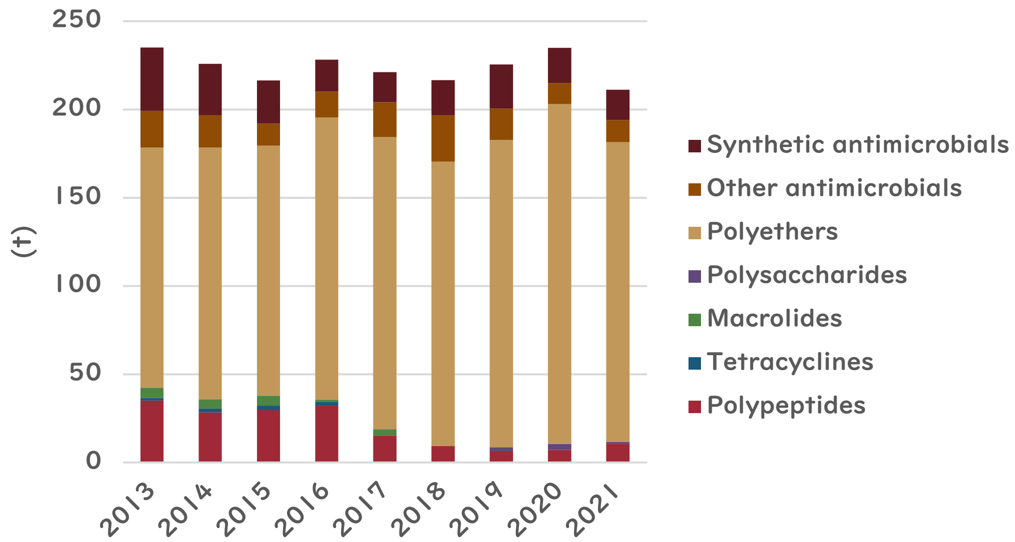
Regarding second-line drugs, CL accounts for the largest volume, and since CL is almost exclusively used for pigs, sales for pigs are high. Although it slightly decreased from the last year, further reduction is expected with the spread of vaccines. Broiler account for the largest sales volume of fluoroquinolones among all animal species, and this has been increasing in recent years. Third-generation cephalosporins, fluoroquinolones, and 15-membered ring macrolides are increased in beef cattle. Third-generation cephalosporins and fluoroquinolones are also increasing in dairy cattle in recent years. These trends should be closely monitored in accordance 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. In 2021 it was 211.1 t, that is the lowest amount since 2013. The polyethers (not used in humans) showed an increasing trend, as its proportion of the toral increased 57.8% in 2013 to 80.4% in 2021. It should be noticed that the designation as feed additives of CL (polypeptides), TS (macrolides) and two substances (tetracyclines) was revoked in July 2018, in May 2019 and in December 2019 respectively, and they have not been in circulation since their revocation.

Data source: Food and Agricultural Materials inspection Center (FAMIC) and the Japan Scientific Feeds Association (Nippon Antimicrobial Resistance One Health Report 2023)

Fig. 4-2 Distribution amount of antimicrobial feed additives (2013-2021)



4-3 Antimicrobials for human use sold to animal clinics

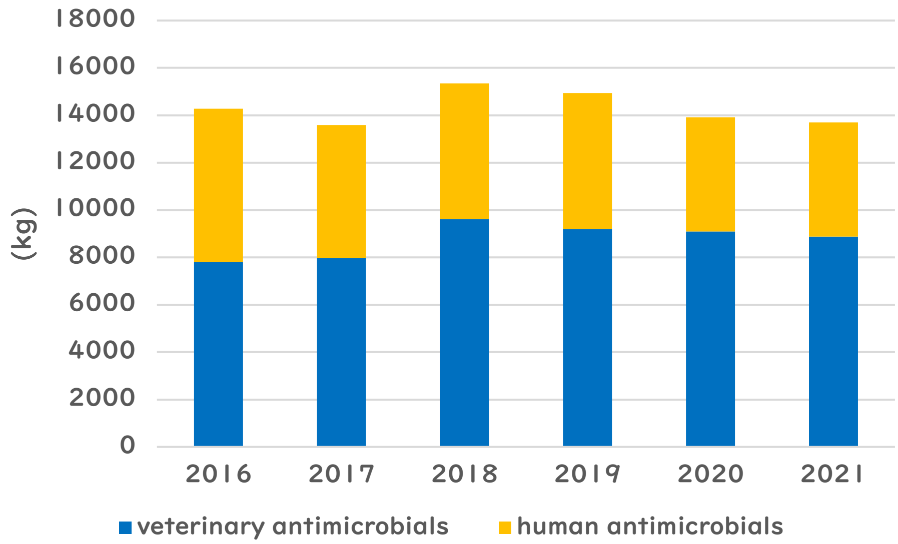
The sales volume of antimicrobial agents approved as veterinary drugs (hereinafter referred to as "veterinary antimicrobials") has been surveyed by JVARM since 2001. However, since antimicrobial agents approved as human drugs (hereinafter referred to as "human antimicrobials") are also used in veterinary clinics according to veterinarians' decisions, a survey of the sales volume of human antimicrobials sold to animal clinics is being conducted.

4-3-1 Survey results

The total annual volume of human antimicrobials sold to veterinary clinics in 2021 was 5464.7 kg in active ingredient, 88.2% (4819.8 kg) of which were for companion animal clinics. The remaining 11.8% were sold to animal clinics including horses and exhibition facilities such as zoos etc.

The sales volume for companion animal clinics in 2021 was slightly lower than in 2020, which was the lowest since the start of the survey in 2016. The total amount combined with the amount of antimicrobials for small animals was 13691.79 kg, and human antimicrobials accounted for 35.2%. In the five years of surveys since 2016, there have been no major changes in the proportions of each class and human antimicrobials sold to companion animal clinics.

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

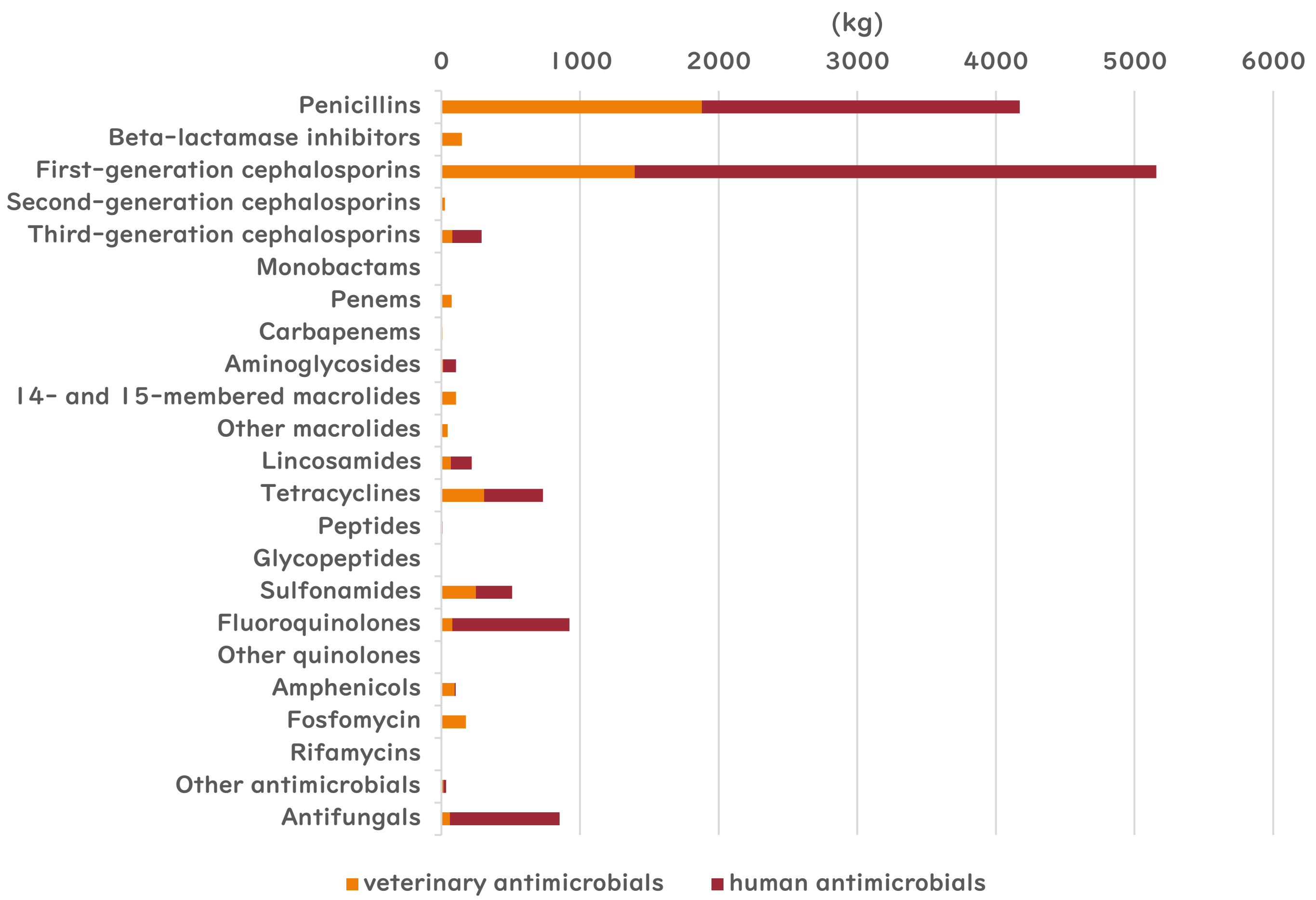


In 2021, human antimicrobials that were most sold to small animal clinics were first-generation cephalosporins and penicillins, and these accounted for 67.9% of the total. Next were tetracyclines, sulfonamides, and fosfomycin. These top five classes accounted for 83.1% of the total. In terms of active ingredients, amoxicillin (AMPC), a penicillin, and CEX, a first-generation cephalosporin, were the top two, accounting for 62.5% of the sales volume of human antimicrobials.

On the other hand, the total amount of fluoroquinolones, cephalosporins (from the third generation onward), macrolides, penems, carbapenems, peptides and glycopeptides was low. These are critically important antimicrobials in human medicine. Fluoroquinolones accounted for 1.7%, third generation onward cephalosporins for 1.7%, macrolides for 3.1%, penems for 1.5%, carbapenems for 0.1%, 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 35.2% and 27.0% in the first-generation cephalosporins and 45.0% in the penicillins, which were lower proportions than in the 2020 survey. For antimicrobials important in human medicine, fluoroquinolones accounted for 8.6% and cephalosporins from the third generation and onwards accounted for 27.4%, indicating a tendency for veterinary antimicrobials to be used more (Fig. 4-3-2).

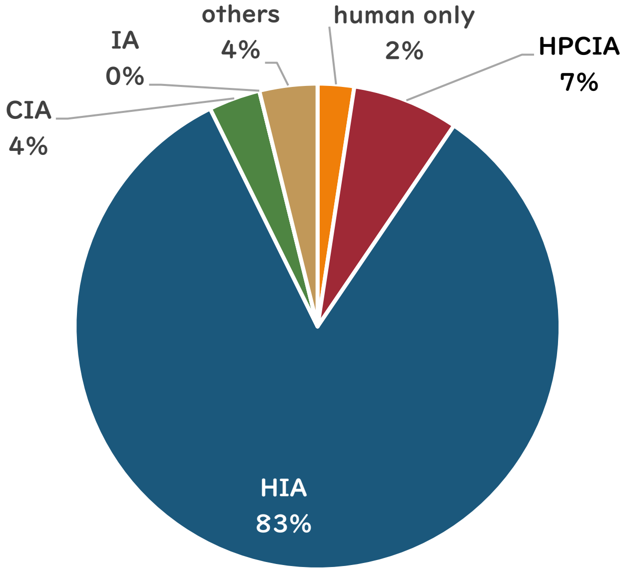
Fig. 4-3-2. Sales volume of human and animal antimicrobials for companion animals by antimicrobial class and the proportion of human antimicrobials



In this survey, it was discovered that antimicrobials not approved for animals are also used, including drugs that the WHO restricts to human use. The WHO publishes a "WHO List of Medically Important Antimicrobials*", and the latest version updated in 2024 has been subtitled "A risk management tool for mitigating antimicrobial resistance due to non-human use". The list has previously classified antimicrobial agents into HPCIA (Highest Priority Critically Important Antimicrobials), CIA (Critically Important Antimicrobials), HIA (Highly Important Antimicrobials), and IA (Important Antimicrobials), but in the updated version categories of drugs authorized for human use only and not medically important (not authorization in humans) such as ionophores, etc. were added. Among human antimicrobials sold for dogs and cats, HIA drugs, first-generation cephalosporins and penicillins accounted for the largest proportion, followed by HPCIA (Fig. 4-3-3). Incidentally, salazosulfapyridine, which is classified as a sulfonamide by structure but is used as an antirheumatic drug and for ulcerative colitis, is not included in the list and is therefore classified as "other". Drugs authorized for use in human only include carbapenems and vancomycin, which are used to treat multidrug-resistant bacteria infection, and drugs authorized for use in human only in the companion animal sector accounted for 2% of sales volume. In the active ingredient, faropenem and piperacillin-tazobactam were sold relatively frequently. The use of these drugs should be avoided, and it is important to thoroughly implement of prudent use so as not to fall into a state where treatment is impossible without using them.

*:https://cdn.who.int/media/docs/default-source/gcp/who-mia-list-2024-lv.pdf?sfvrsn=3320dd3d_2

Fig. 4-3-3 Proportion of human antimicrobials sold for dogs and cats classified by WHO List of Medically Important Antimicrobials



4-3-2 Summary

The volume of human antimicrobials sold for animals has continued from the first survey in 2016, with 90% of these sales going to companion animal clinics, accounting for around 40% of the total companion animal sector for veterinary and human antimicrobials combined. However, the proportion of human antimicrobials was decreasing. The lack of approval for veterinary medicines is not considered to be the main reason for the use of human antimicrobials because the human antimicrobials such as AMPC are widely used in dogs and cats while there are equivalent animal antimicrobials that are approved for dog and cats.

In addition, it was confirmed that drugs that are not approved as veterinary drugs and categorized as "authorized for use in human only" in the WHO List of Medically Important Antimicrobials are sold in companion animals by approximately 2%. The use of such drugs requires extreme caution, and their use should be avoided.

In small animal practice, it is important to select veterinary drugs approved and marketed for companion animals over human drugs because the efficacy and safety of these drugs have been established for animals. In addition, we recommend using the "Guidebook for the Prudent Use of Antimicrobials in Companion animals-2020-[1]" to enforce prudent antimicrobial use.

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

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 specimen and target species

5-1-1 Healthy livestock: strains from slaughterhouses and poultry 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 2021".

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 (farm-derived strains)

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	*Escherichia coli*	Cattle, Pigs, Chickens	Various
	Mannheimia haemolytica	Cattle	
	Salmonella spp.	Cattle, Pigs, Chickens	
Gram-positive bacteria	*Streptococcus suis*	Pigs	
	Staphylococcus aureus	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	Isolation site
Gram-negative bacteria	*Escherichia coli*	Dogs, Cats	Rectal swab
Gram-positive bacteria	*Enterococcus* 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	*Escherichia coli*	Urine, genital tract
	Klebsiella spp.	
	Pseudomonas aeruginosa	Urine, ear
	Acinetobacter spp.	Urine, skin
Gram-positive bacteria	Coagulase-positive *Staphylococcus* spp.	Urine, skin
	Enterococcus 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 Preston enrichment broth followed by Modified Cefoperazone Charcoal Deoxycholate 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 chromagar salmonella medium, respectively, for isolation and culture. Identification was carried out by morphological, biochemical properties inspection, and specific antiserum testing.

5-2-5 *Mannheimia haemolytica*

Conducted following the Pathogenicity Diagnostic Manual (cited ＊).

5-2-6 *Streptococcus suis*

Conducted following the Pathogenicity Diagnostic Manual (cited ＊).

5-2-7 *Klebsiella* spp.

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

5-2-8 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa strains sent by clinical laboratories were re-identified by API20NE (Biomerieux Japan).

5-2-9 *Acinetobacter* spp.

Acinetobacter strains sent by clinical laboratories were re-identified by API20NE (Biomerieux 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 Tested 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, 12th ed. CLSI, Wayne, PA, USA. 2024.
*CLSI. Performance Standards for Antimicrobial Susceptibility Testing M100, 34th ed. CLSI, Wayne, PA, USA. 2024.

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 authorization 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 Laboratory[1] 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 Report[2] and the Antimicrobial Resistance (AMR) Platform[3].

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

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 for use mainly by veterinarians 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.

Table: List of target bacteria and tested antimicrobials

Animal species		Species	ABPC	PCG	CEZ	CTX	CTF	CFX	CEX	CQN	GM	KM	SM	DSM	AZM	EM	CLDM	TC	OTC	NA	CPFX	ERFX	CL	BC	CP	TMP	ST	MEPM	MPIPC	FFC	TP	TMS	TS	LCM	VCM	SNM
Livestock	health	*Escherichia coli*	○		○	○					○	○	○					○		○	○		○		○		○	○								
		Enterococcus spp	○								○	○	○		○	○		○		○	○			○	○								○	○	○	○
		Campylobacter spp.	○								○		○		○	○		○		○	○				○											
		Salmonella spp.	○		○	○					○	○	○					○		○	○		○		○		○	○								
	disease	*Salmonella* spp.	○		○	○					○	○	○					○		○	○		○		○		○	○								
		Escherichia coli	○		○	○					○	○	○					○		○	○		○		○		○	○								
		Staphylococcus aureus		○	○						○		○		○	○	○	○			○				○				○							
		Mannheimia haemolytica	○		○		○			○		○		○				○				○								○		○				
		Streptococcus suis	○	○	○		○				○		○		○	○	○	○			○	○			○		○		○							
Companion animals	health	*Escherichia coli*	○		○	○			○		○	○	○					○		○	○		○		○		○	○								
		Enterococcus spp.	○								○					○		○			○				○										○	
	disease	*Escherichia coli*	○		○	○			○		○	○	○					○		○	○		○		○		○	○								
		Enterococcus spp.	○								○					○		○			○				○										○	
		Pseudomonas aeruginosa									○										○		○					○								
		Acinetobacter spp.				○					○							○			○		○				○	○								
		Coagulase positive *Staphylococcus* spp		○							○				○	○		○			○				○				○							
		Klebsiella spp.			○	○			○		○	○	○					○		○	○		○		○		○	○								

Veterinary AMR Center

National Veterinary Assay Laboratory,
Ministry of Agriculture, Forestry and Fisheries

April 2025