

Project for “Determining the Outbreak Mechanisms and Development of a Surveillance Model for Multi-Drug Resistant Bacteria.”

Summary of the Project Report (Pharmacology group)

ACTIVITIES PHARMACOLOGY GROUP

1

Study actual situations of antibiotic residues in foods in targeted area of Viet Nam

2

Develop a model of monitoring antibiotic residues in foods

3

Develop research capacity of researchers related to food safety monitoring in Viet Nam

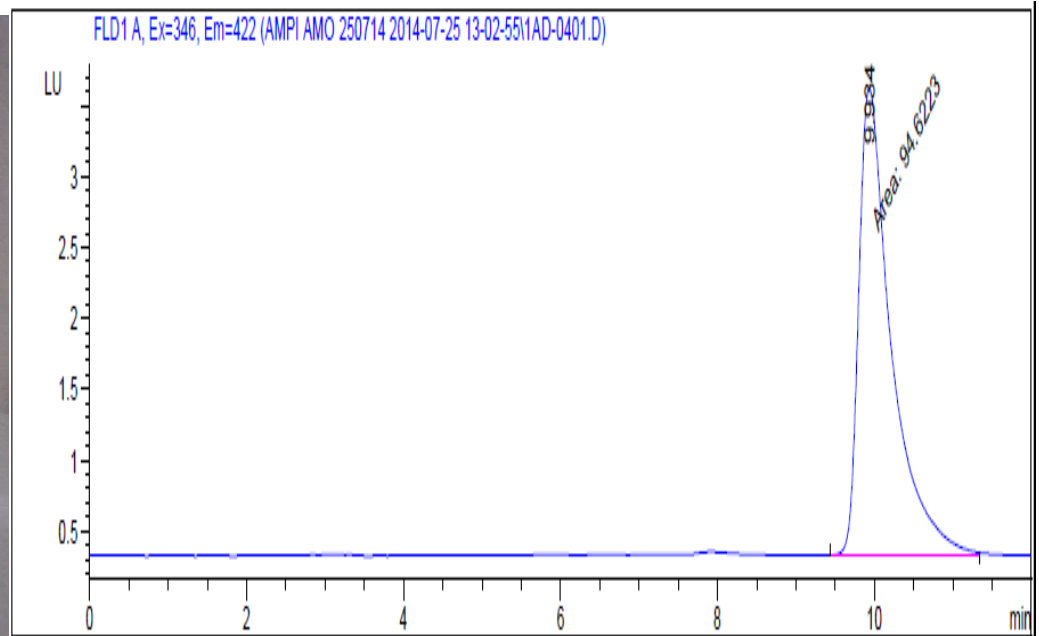
Study on actual situation of antibiotic residue

Pre-mi test validation

- Screening food sample by pre-mi test: pork, chicken, fish, shrimp, for reseach period.

HPLC-FL method validation

Detection of ampicillin in pork(0.7ppb), chicken(1.0ppb), fish(1.2ppb), shrimp(1.4ppb) for monitoring system(144samples/year/institute)
Quarterly sampling (4 times per a year)



Study on actual situation of antibiotic residue

Food

Collect at Wholesale market, Supermarket,
Local market, Slaughterhouse, Farmer



Transport positive samples to Institute of
Public Health, Ho Chi Minh City



QuEChERS sample preparation and
LC/MS/MS analysis

Environmental Water

Collect at river, pond, farm



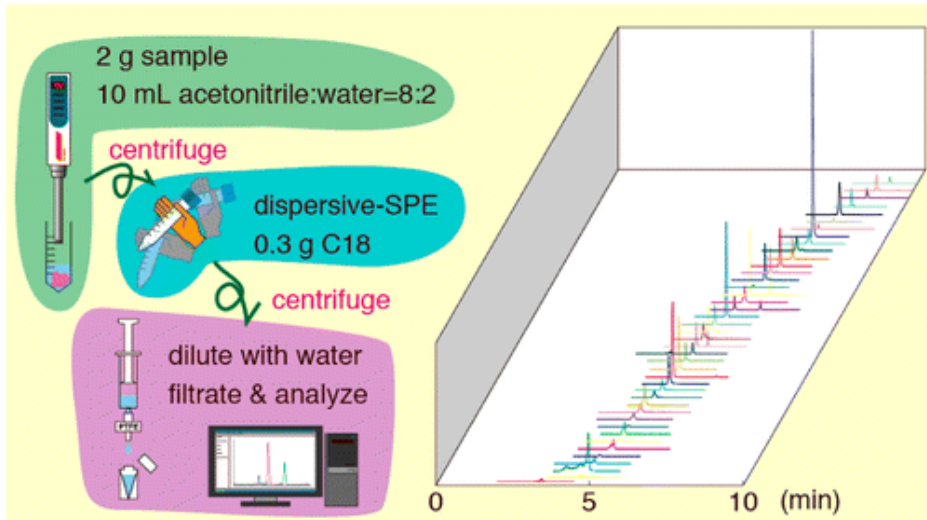
Absorb onto SPE cartridge at each site



Transport to Osaka University



LC/MS/MS and HPLC analysis



Analytes

Totally 66 chemicals
41 for food
45 for environment

sulfonamide (17)

	Food	Water
sulfabenzamide	✓	
sulfacetamide	✓	
sulfachlorpyridazine	✓	✓
sulfaclozine	✓	✓
sulfadiazine		✓
sulfadimethoxine	✓	✓
sulfadoxine	✓	✓
sulfamethazine		✓
(sulfadimidine)	✓	✓
sulfamethoxazole	✓	✓
sulfamethoxypyridazine	✓	✓
sulfamerazine	✓	✓
sulfamonomethoxine	✓	✓
sulfanitran		✓
sulfapyridine	✓	✓
sulfaquinoxaline		✓
sulfathiazole	✓	
sulfisozole	✓	

folic acid antagonist (2)

	Food	Water
ormethoprim		✓
trimethoprim	✓	✓

quinolone (12)

	Food	Water
ciprofloxacin	✓	✓
danofloxacin	✓	
difloxacin	✓	✓
enrofloxacin	✓	✓
flumequine	✓	✓
marbofloxacin	✓	
nalidixic acid	✓	✓
norfloxacin	✓	✓
ofloxacin	✓	✓
orbifloxacin	✓	
oxolinic acid	✓	✓
sarafloxacin	✓	

β-lactam (17)

	Food	Water
amoxicillin	✓	✓
ampicillin	✓	✓
aspxocillin	✓	✓
cefapirin	✓	✓
cefazolin		✓
cefotaxime		✓
cefoperazone	✓	
cefquinome	✓	
ceftiofur		✓
cefuroxime		✓
cephalexin		✓
cephalonium		✓
cloxacillin		✓
dicloxacillin		✓
oxacillin	✓	✓
penicillin G	✓	✓
penicillin V	✓	✓

macrolide (3)

	Food	Water
spiramycin	✓	
tilmicosin	✓	
tylosin	✓	

tetracycline (3)

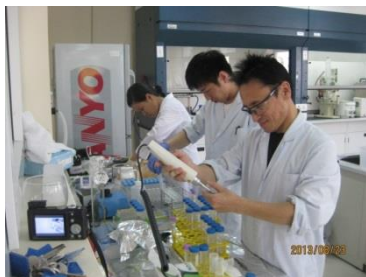
	Food	Water
chlortetracycline		✓
oxytetracycline		✓
tetracycline		✓

chloramphenicol (2)

	Food	Water
chloramphenicol		✓
tiamphenicol		✓

other (β-agonist etc. 10)

Sample Preparation Protocol for LC/MS/MS

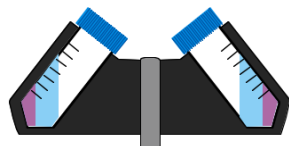


sample 2 g

50 mL tube

- meat : 80% acetonitrile 10 mL
- egg : acetonitrile 8 mL
- fishery : acetonitrile + 2% formic acid 8 mL

homogenize



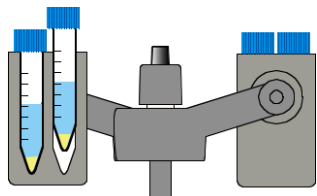
centrifuge

15 mL tube
(fill up to 10 mL exc. meat)

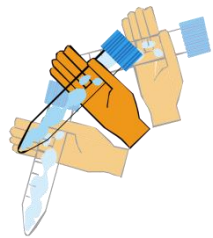
sediment

supernatant

dispersive-SPE C18 0.3 g



centrifuge



sediment

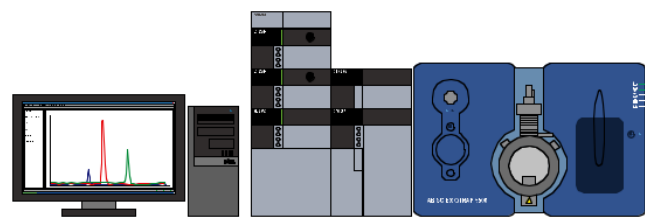
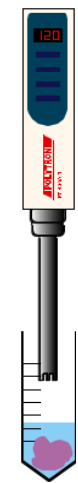
supernatant

aliquot 1 mL

+ distilled water 1 mL

LC/MS/MS

filtration
(hydrophilic PTFE, 0.20 μm)



Development of Protocol for LC/MS/MS Screening

LC/MS/MS conditions

Instrument

LC: UHPLC Nexera XR (Shimadzu), MS: 5500QTRAP (AB SCIEX)

LC

Column : LUNA C18 columns (100 X 3 mm i.d., 2.5 μ m, Phenomenex)

Column Temp. : 40°C

Flow rate : 0.4 mL/min

Eluent (A) 0.1%(w/w) formic acid / (B) AcCN

Injection volume : 5.0 μ L

Gradient : B (%) :5 (0 min) – 95 (10 min) – 95 (18 min)

MS

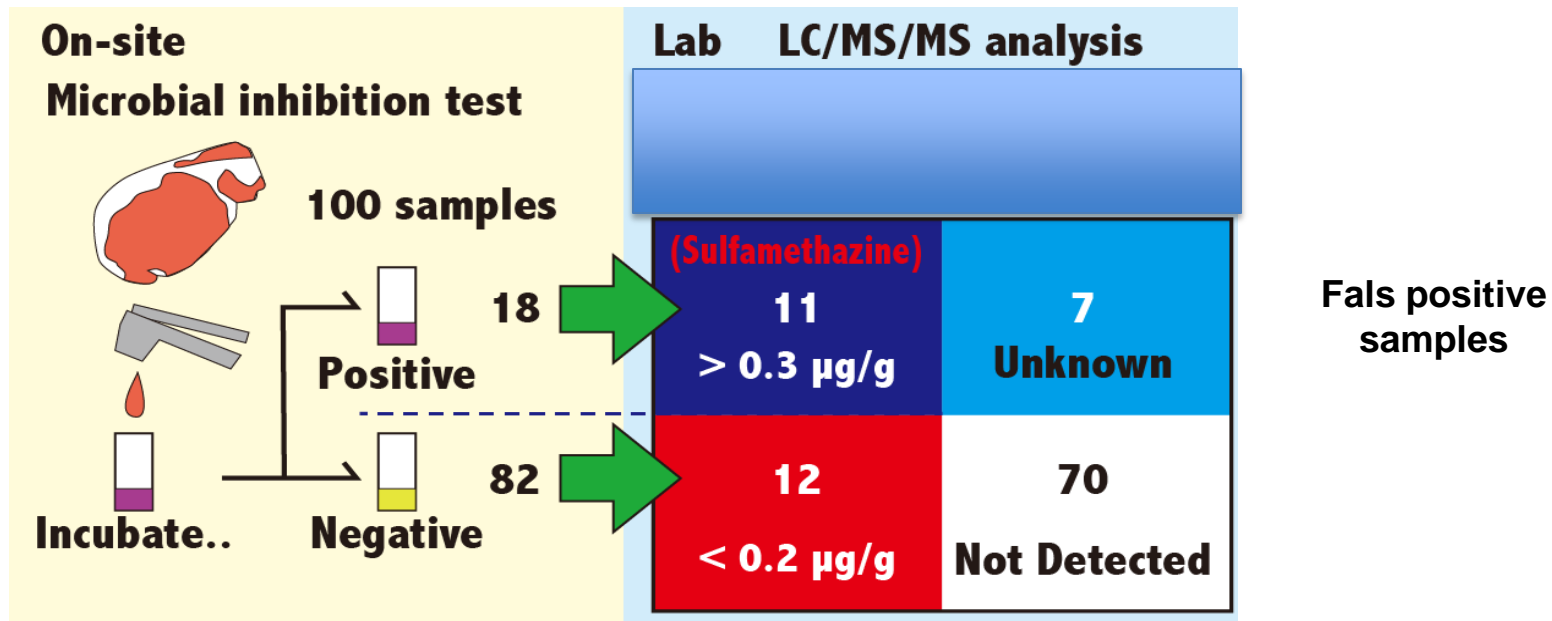
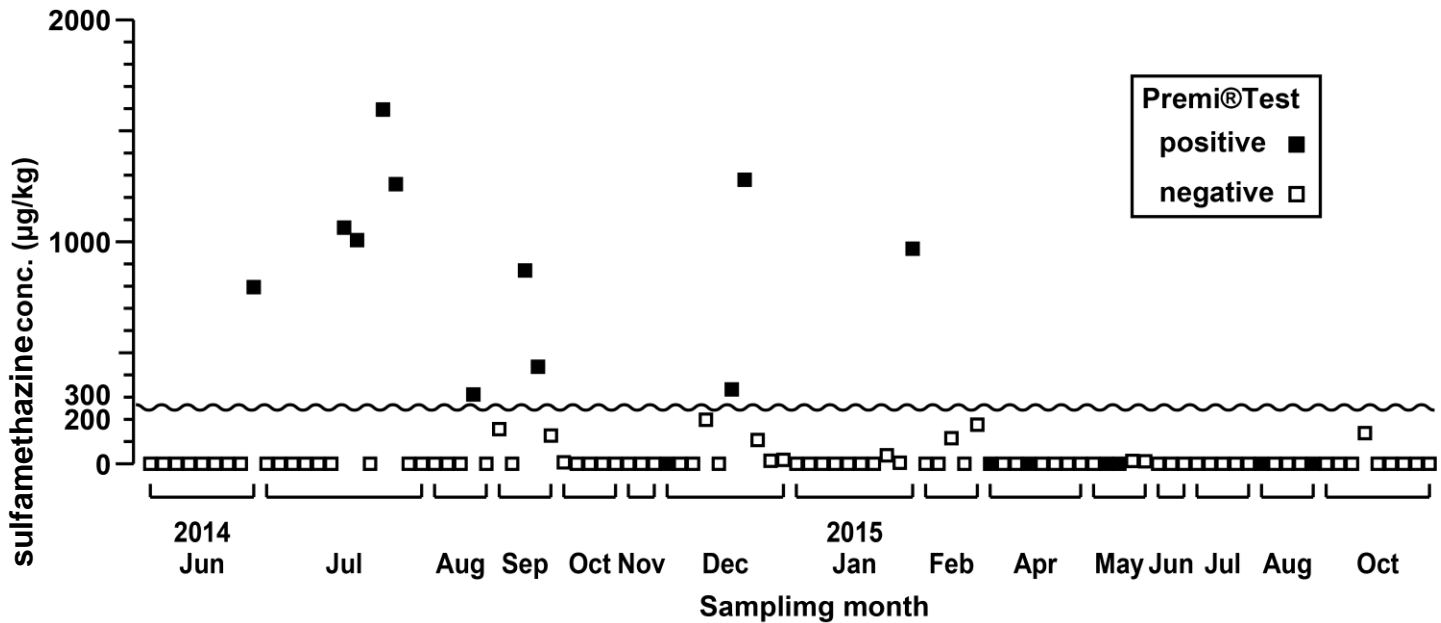
Ionization : Electrospray ionization (positive)

Capillary voltage : 4.5 kV

Selected Reaction Monitoring (SRM)



Comparison of results from PremiTest with LC/MS/MS



Antibiotic residues in food materials (2012 – 2015)

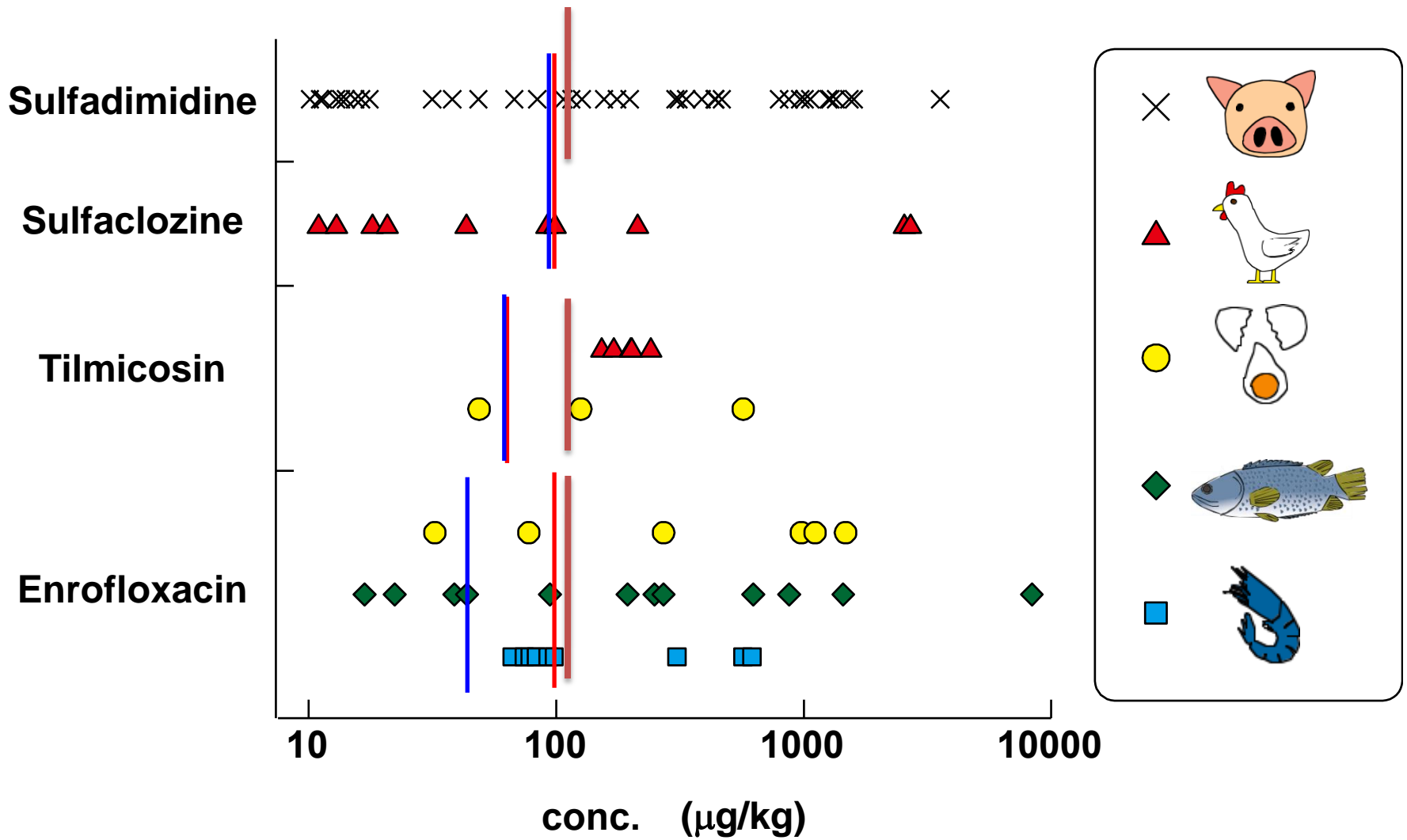
Summaries of tested and positive sample numbers and detection ratios of residual antibiotics in food materials by LC/MS/MS

	Thai Binh		Nha Trang		HCMC		Can Tho		Total	
	Positive / Sample	Ratio (%)	Positive / Sample	Ratio (%)	Positive / Sample	Ratio (%)	Positive / Sample	Ratio (%)	Positive / Sample	Ratio (%)
chicken	11 / 66	17	10 / 84	12	17 / 127	13	2 / 21	10	40 / 298	13
pork	10 / 74	14	3 / 85	4	43 / 232	19	4 / 16	25	60 / 407	15
beef	-	-	-	-	5 / 68	7	-	-	5 / 68	7
fish	0 / 62	0	-	-	38 / 203	19	-	-	38 / 265	14
shrimp	1 / 56	2	1 / 31	3	13 / 161	8	-	-	15 / 248	6
egg	-	-	-	-	13 / 125	10	-	-	13 / 125	10
Total									171 / 1411	12

Antibiotics detected in food materials

	total (1411)	chicken (298)	pork (407)	beef (68)	fish (265)	shrimp (248)	egg (125)
sulfamethazine	70		58	4	3	2	3
enrofloxacin	56	9	1		28	12	6
ciprofloxacin	20				14	2	4
tilmicosin	19	15					4
sulfaclozine	13	13					
norfloxacin	6	3			1		2
difloxacin	3	1		2			
others	17	1	1		10	2	3

Residue Levels of Major Antibiotics (HCMC)

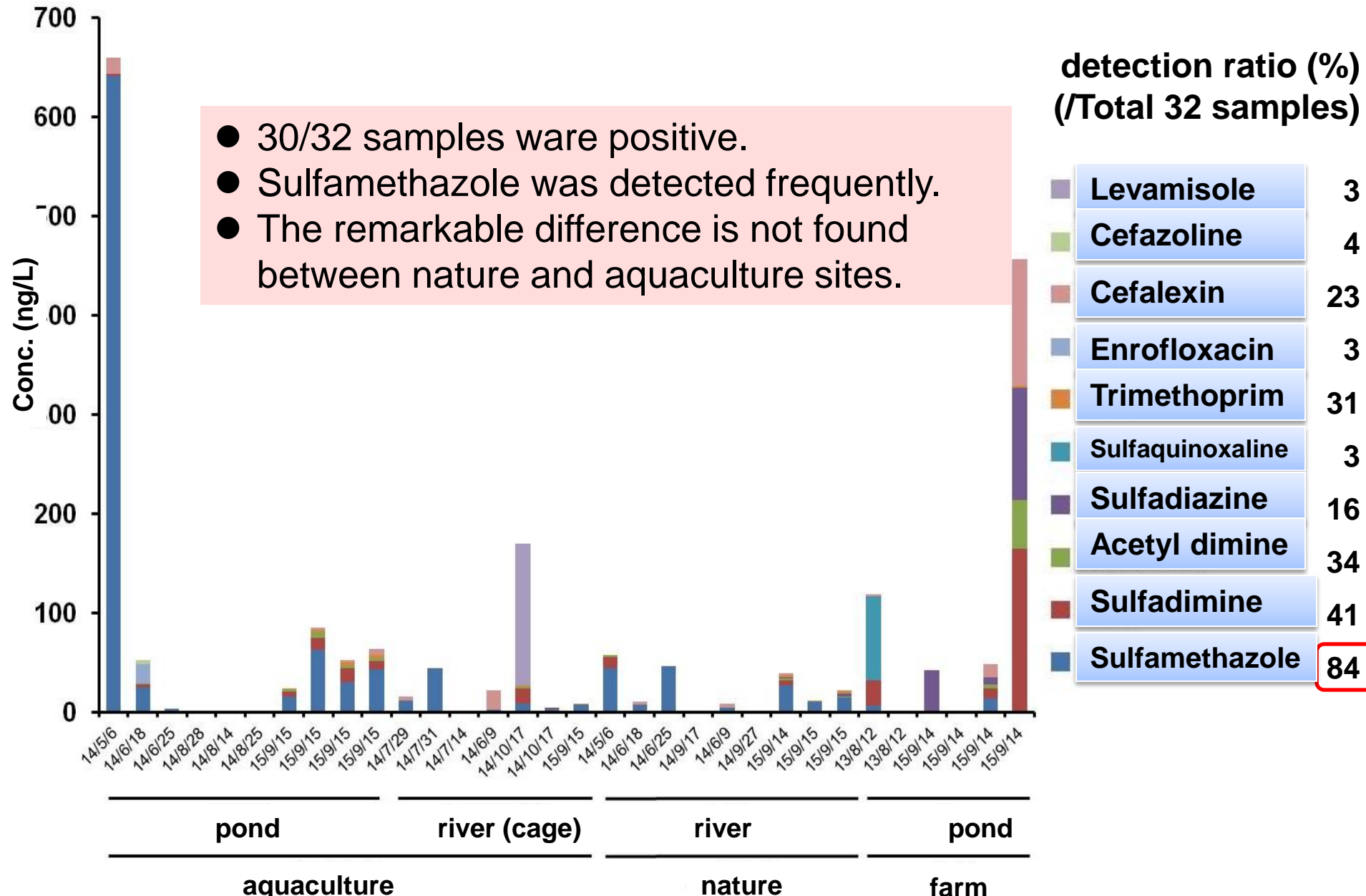


MRL — EU, — Japan — VN

Number of positive sample over the MRL

	MRL ($\mu\text{g}/\text{kg}$)	chicken (298)	pork (407)	beef (68)	fish (265)	shrimp (248)	egg (125)
sulfamethazine	100	0	37 (9%)	0	0	0	0
tilmicosin	(chicken, egg) 150	10 (3%)	0	-	-	-	1 (1%)
	(pork) 100						
sulfaclozine	-	-	-	-	-	-	-
enrofloxacin	(fish, shrimp) 100	-	-	-	13 (5%)	4 (2%)	-
ciprofloxacin	-	-	-	-	-	-	-
norfloxacin	-	-	-	-	-	-	2
difloxacin	(fish, shrimp) 300	-	-	-	0	0	-

Result the antibiotic residual levels in environmental water



THANK YOU FOR YOUR ATTENTION