Approved: 23 September 2024

DOI: 10.2903/j.efsa.2024.e221117

EU-FORA SERIES 7

Exploring frameworks for quantitative risk assessment of antimicrobial resistance along the food chain

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts

Abstract

Campylobacter is one of the most reported causes of bacterial gastroenteritis worldwide. Birds are the predominant reservoirs for thermotolerant *Campylobacter*, therefore consumption of contaminated and undercooked poultry products represents one of the major transmission routes for campylobacteriosis. In addition to foodborne diseases, another relevant public challenge is the silent pandemic of antimicrobial resistance (AMR), impacting also the food chain. The occurrence of antimicrobial-resistant *Campylobacter* in broiler meat poses a significant threat to public health. In this context, quantitative microbiological risk assessment (QMRA) might support policy-makers in addressing these challenges. Hence, this project aimed to describe the current status of knowledge on occurrence of AMR *Campylobacter* at a global scale, with focus in estimating the prevalence of fluoroquinolone-resistant *Campylobacter* isolated from broiler meat at retail level. Following the drafting of a dedicated protocol, a systematic literature review and meta-analysis were conducted. Based on the extracted data, after the determination of the proportion of AMR isolates, the proportions were compared by *Campylobacter* species, geographical regions, processing step and sampling matrices. Meta-analysis allowed the estimation of the pooled prevalences of resistant isolates by regions and species, combining the findings from different independent studies in a comparable way. In terms of future perspectives, the outcome of this project will support future risk assessment and provide valuable inputs for estimating consumer exposure to AMR *Campylobacter* via broiler meat consumption. In conclusion, this report will provide a general overview of activities, preliminary results and research performed during the EU-FORA fellowship programme (2023/2024).

KEYWORDS

AMR, broiler, *Campylobacter*, fluoroquinolones, quantitative microbiological risk assessment

CONTENTS

1 | INTRODUCTION

Campylobacter spp. are gram-negative bacteria with some species able to cause gastroenteritis in humans. The shape of *Campylobacter* is usually slender, spirally curved and it has corkscrew alike movements of flagellums that allow the bacteria to move effectively in viscous environments (Bolton, 2015). This is in line with their natural habitat and main reservoirs in gastrointestinal tracts of birds and mammals. *Campylobacter* are microaerophilic, prefer high temperatures, are sensitive to desiccation and do not form spores (Park, 2002). Yet, regardless of their very specific requirements and rather sensible nature, campylobacteriosis is the most reported foodborne diseases in Europe (EFSA and ECDC, 2022). *Campylobacter* spp., particularly *Campylobacter jejuni* and *Campylobacter coli*, are among the most common bacterial pathogens responsible for foodborne gastroenteritis worldwide. Symptoms of campylobacteriosis include diarrhoea, abdominal pain, fever and, in severe cases, can lead to long-term sequelae such as Guillain-Barré syndrome. Campylobacteriosis cases have a seasonal trend with peak in the summer months followed by lower peaks during winter (e.g. January; EFSA and ECDC, 2022). Infection with *Campylobacter* often results from the consumption of ready to eat or raw/undercooked contaminated food, often after the handling of raw poultry meat, which is a primary reservoir for the bacterium. There are two critical points of cross-contamination events along the broiler meat food chain; one at slaughtering and the other in home-kitchen, where even inanimate objects and surfaces can act as 'transmission routes' of pathogens (Iulietto & Evers, 2020, 2024). Taking this into account, exploring cross-contamination events and describing the prevalence of the pathogen along the food chain, provides valuable knowledge that can be used for defining preventive measures. Previous risk assessments on *Campylobacter* in chicken were published during the last decades (Chapman et al., 2016), paving the way on this field: i.e. assessments from Denmark (Christensen et al., 2001), from The Netherlands, from World Health Organization (WHO, 2009) and from Finland (Evira, 2016).

Another relevant aspect takes into consideration the fact that in response to considerable selective pressure, *Campylobacter* has developed resistance to one or more classes of antibiotics, with the consequent spread of multidrug-resistant isolates (MDR, i.e. resistant to three or more classes of antibiotics). In general presence of AMR can be either intrinsic resistance (e.g. naturally present or inherited), adaptive resistance (triggered by the environment and might be reverted once stress signal is removed) or acquired resistance. Acquired resistance is the resistance exhibited when a previously sensitive bacterium acquires a resistance mechanism by either a mutation or the acquisition of new genetic material from an exogenous source (horizontal gene transfer). There are three main ways for horizontal gene transfer: transformation, transduction and conjugation (Christaki et al., 2020). *Campylobacter* acquires fluoroquinolone (FQ)-resistance mainly via point mutations and horizontal gene transfer (Luangtongkum et al., 2009).

Taking into account this context, the emergence of FQ-resistant *Campylobacter* poses a serious public health challenge. Quinolones and fluoroquinolones (FQQ) are listed as Critically Important Antimicrobials for human medicine (WHO, 2019). FQ are broad-spectrum antibiotics with a history of use since the late 1980s. FQ differ from other quinolones by the replacement of the eighth carbon atom of the backbone with a nitrogen atom and the addition of a fluorine atom at the sixth position. At lower concentrations, FQ cause impaired DNA replication and at higher concentrations cell death. Their antibacterial activity is attributed to the ability to inhibit DNA replication either by inhibiting DNA gyrase and/or topoisomerase II (Redgrave et al., 2014).

There are several studies describing the resistance profiles of *Campylobacter* from various steps of broiler production, yet variability in study design, sampling methods and geographic focus contribute to inconsistent data, complicating efforts to assess the true extent of the problem.

Quantitative microbiological risk assessment (QMRA) allows estimation of the probability and severity of an adverse event due to a pathogenic microorganism being transmitted from farm via slaughter and retail to the consumer's home (Arnaboldi et al., 2023). It provides quantitative support to decision-making for reducing food safety risks and for prioritising efforts, using mathematical modelling techniques. QMRA can be applied to explore the risk associated to antimicrobial resistance (AMR), with a specific focus on the AMR transmission route(s) via the food chain. In this context, risk assessment (CAC, 1999) and in particular, the assessment of exposure to antibiotic-resistant *Campylobacter* is a valid tool for exploring the most effective control measures to reduce such exposure and increase consumer protection (Caffrey et al., 2019).

2 | DESCRIPTION OF THE WORK PROGRAMME

2.1 | Aims

The work programme focused on exploring frameworks for quantitative risk assessment of antimicrobial resistance along the food chain. In order to contribute to the estimation of the exposure to AMR *Campylobacter*, the aims were:

(i) to provide an overview on the global level of AMR (in particular fluoroquinolones and quinolones-resistance) of *Campylobacter* isolates from broiler meat within a 20 years' timeframe by conducting a systematic review;
(ii) to estimate the pooled prevalence of FQ-resistant isolates by performing a meta-analysis.

2.2 | Activities

The fellow was actively involved in the first part of a two-year project, collaborating with the risk assessment group. The work programme was divided into four work packages (WP).

WP1 aimed to provide theory and practical exercises to gain insights into the Risk Assessment framework following the Codex Alimentarius approach. Knowledge acquired during the EU-FORA programme induction training was further expanded, discussed and enhanced via dedicated tools and exercises, with the support of the supervisors sharing their experiences in the field.

WP2 aimed to build and improve competences regarding systematic literature review, protocol development, database creation, data analysis and meta-analysis on AMR *Campylobacter*.

WP3 provided the elements on mathematical modelling. The fellow studied how to design a model, the needed basic mathematics and the characteristics of standard software in order to be able to implement a simulation. The tutors provided examples concerning previous RA activities/models completed by them during their career.

WP4 comprised numerous food safety-related activities that supported the fellow's exploration of the risk assessment field, including drafting of scientific publications and participation in conferences and trainings (i.e. courses on Writing in the Science and use of software like RAYYAN and R).

At the beginning of the fellowship, a dedicated one-week programme was launched to kick off the project and to design together a detailed working plan. To let the fellow familiarise with the hosting institute, meetings and presentations with representatives of key departments were held. During on-site periods the fellow had daily meetings and discussions with supervisors and other members of the working group. While, during the online period, biweekly 1–3 h meetings and discussions were held online. In the first part of the fellowship, the meetings were more focused on theoretical aspects of risk assessment (WP1), QMRA and conducting systematic review (WP2); the theoretical part was always accompanied by well thought–through practical hands-on exercises e.g. modelling growth of pathogens (*Listeria monocytogenes*) along the food chain. The second part of fellowship was more focused on preparing and analysing data, on learning the programming environment R and on coworking in writing scientific publications (WP3). Moreover, on-field visits to water-buffalo farms, cheese-production factory and insects farm were organised by supervisors to better understand the complexity of food production chains, even in relation to the developing of RA models (WP4). Laboratory visits (i.e. National reference laboratory of AMR and food microbiology laboratory) were also performed to encompass the sources of the primary data which are collected and used to assess the food safety risks.

The collaboration between sending and hosting institutes was strong throughout the year; in February a mutual webinar of African swine fever was organised where experts from both institutes gave presentations and shared knowledge. A graphical overview of the activities is presented in Figure 1, while the list of additional training activities is presented in Appendix A.

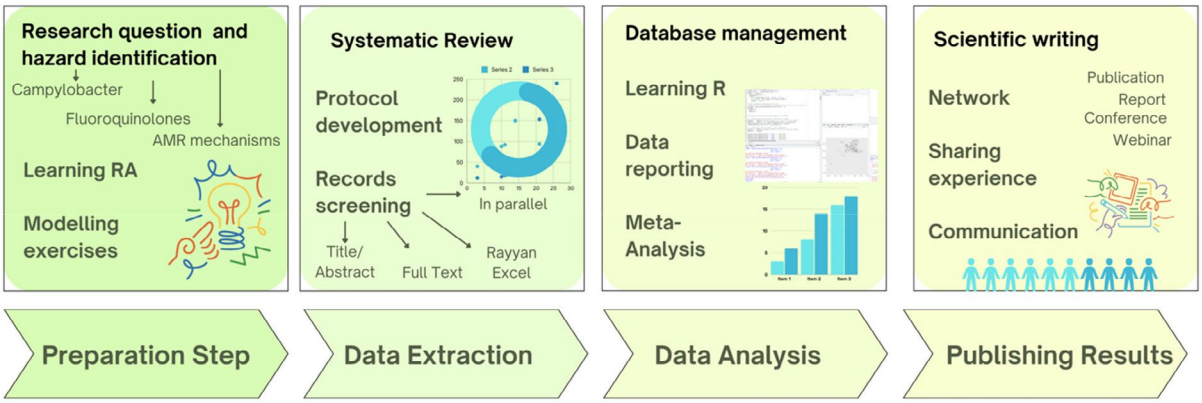

FIGURE 1 Graphical representation of the workflow.

3 | ANTIMICROBIAL RESISTANCE ALONG THE FOOD CHAIN: FOCUS ON *CAMPYLOBACTER*

3.1 | Data and methodologies

As the first activity foreseen by the work programme, an overview of the state of the art was performed, with an extensive literature review focusing on hazard identification: describing *Campylobacter*, antimicrobial resistance and mechanisms behind it, FQ-resistance, seasonal variability of *Campylobacter* and other relevant topics.

A systematic literature review was undertaken to collect evidence on the global level of AMR (in particular FQQ-resistance) of *Campylobacter* isolates from broiler meat. The literature search included the following electronic bibliographic databases: Scopus (Elsevier B.V. Portal) and Web of Science (WoS), including Science Citation Index Expanded, KCI-Korean Journal Database and SciELO Citation Index, Medline (Thomson Reuters). A dedicated Protocol was created (following S.

Visintini template) for performing the systematic literature review (including search string, recording the steps and intermediate results of the search, as well defining eligibility criteria and keeping track on timeframe) according to PECO framework and EFSA guidance on systematic reviews (EFSA, 2010). Title-Abstract screening was done in parallel by fellow and supervisor by using Rayyan (version 1.3.3, https://www.rayyan.ai/). Extraction was performed according to the pre-defined eligibility criteria. For evaluating the quality of studies, a modified version of JBI Critical Appraisal Tools was created (Moola et al., 2020; Munn et al., 2020). Systematic review was conducted and reported by guidelines of PRISMA (Page et al., 2021).

The chapter describing the body of evidence retrieved from systematic literature search was done using functions in Excel and the rest of analysis was conducted with Rstudio software (version 3.6.1, https://www.rstudio.org), for example R was used to create visuals like stacked bar charts and maps representing geographical info.

The resistance rate was calculated based on the sum of all resistant isolates divided by the sum of all tested isolates for each study separately. To find proportions for countries, the average of individual studies per country was calculated.

Meta-analysis is a mathematical approach to combine results of individual studies in a comparable way and it was carried out using the 'meta-prop' command in R statistical software (R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria)), using a random-effect model. Moreover, Double Arcsin transformation was applied to appropriately deal with studies delivering very low or very high prevalence values which allows them to give appropriate weight (Belluco et al., 2016). These techniques were used to estimate the pooled prevalences of FQQ-resistance by the geographic region, with 95% confidence intervals. Forest plots were created to represent the obtained results. For evaluating study bias, funnel plot and Eggers's test were used.

3.2 | Preliminary results

Out of nearly 3000 articles retrieved from the databases (WoS and Scopus), 5% were considered eligible and included in the final dataset (no. = 152 articles from which 302 studies were derived), with over 34,000 isolates tested for AMR. The number of relevant published articles between January 2003 and April 2024 showed an increasing trend (Figure 2).

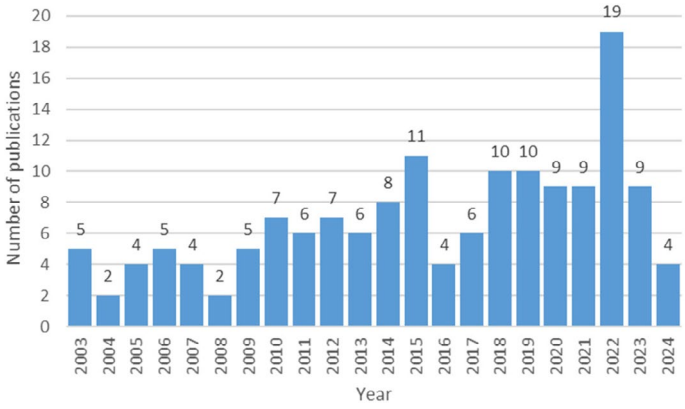

FIGURE 2 The number of eligible articles published on the topic by year from January 2003 to May 2024.

The geographical origin of the studies was uneven throughout the globe: 2/3 of were from Europe and Asia.

The data extraction phase consisted of collecting resistance information of *Campylobacter* isolates from broiler meat concerning about 39 different antimicrobial agents from 16 classes. The most studied antimicrobial classes were quinolones, tetracyclines, macrolides and aminoglycosides. For the database, no restrictions were set in acquiring data from the antimicrobials tested (based on class or effect of the antimicrobial); every agent mentioned at least in two studies was included into the final dataset, for further analysis. Usually from every class one or two flagship antimicrobials were presented in more studies (e.g. from aminoglycosides class gentamicin was presented in 103 studies, while 5 other agents more rarely). From the cephalosporins class, numerous agents were extracted, but all of them were described only by a few studies (max four). The proportion of resistant isolates out of all tested isolates by antimicrobial agent ranged widely, between 0% and 91%, with average of 37.7%.

Based on 8451 isolates from 100 studies, average proportion of multidrug-resistant isolates was 43.4%, 95% confidence interval (CI): 35.5–51.2.

3.2.1 | FQQ-resistant *Campylobacter*

A separate analysis was done regarding fluoroquinolones class (including ciprofloxacin, enrofloxacin, ofloxacin, levofloxacin, norfloxacin) and nalidixic acid. Results for ciprofloxacin and nalidixic acid were often presented together in studies and resistance about these antimicrobials derived from articles from all world regions. On the other hand, resistance to other fluoroquinolones were presented in fewer studies, mainly from European and Asian regions.

Preliminary results suggest that the more evident differences in the proportions of resistant isolates are in the geographical area; while the comparison of the proportions of resistant isolates by sampling matrix, processing step or species of *Campylobacter* show rather overlapping results. The most reported species was *C. jejuni*, followed by *C. coli* and only two eligible studies reported prevalence of *C. lari*. One quarter of studies did not present the results on the level of species, but on the level of genus. No significant differences in the levels or proportions of resistance were observed between the species except higher resistance of *C. lari*.

Ciprofloxacin and nalidixic acid were the more studied agents. The resistance rates in Europe were similar, for CIP 53.6% (95% CI: 39.7–67.5) and for NAL 54.3% (95% CI: 41.6–67.9). The third most studied agent was enrofloxacin with a resistance rates of 21.2% (CI: 15.5–26.8). Among the studied FQQ agents the highest reported resistance rates were for oflaxacin and levofloxacin, which also are the two less frequently investigated agents.

The resistant rates calculated by country are presented in Figure 3. Furthermore, the detailed results of meta-analysis will be presented in a dedicated publication (Table 1).

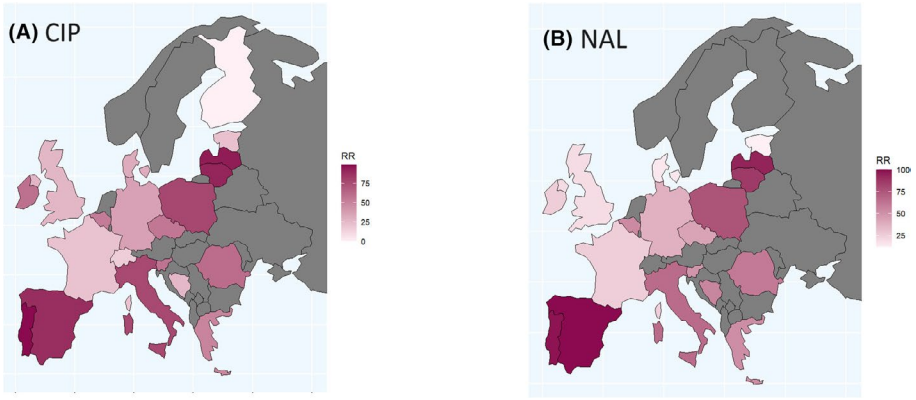

FIGURE 3 (A, B) Maps showing the resistance rates of *Campylobacter* spp. isolates from chicken meat to ciprofloxacin (A) and nalidixic acid (B) in Europe.

TABLE 1 Resistance rates of *Campylobacter* spp. isolates from chicken meat to nalidixic acid and ciprofloxacin in Europe.

Country	CIP RR	CIP iso	NAL RR	NAL iso
Belgium	53.1	1855	53.41	1855
Bosnia and Herz.	25.3	50	55.5	50
Czechia	55.4	189	42.7	101
Denmark	31.0	552	15.0	544
Estonia	21.3	71	11.3	35
France	20.5	137	25.1	137
Germany	36.0	391.8	37.0	116.8
Greece	49.2	359	51.9	315
Ireland	58.3	57	25.0	24
Italy	76.9	1024	66.1	582
Latvia	93.8	32	93.8	32
Lithuania	90.7	200	86.2	104
Poland	76.9	3130	76.2	1786
Portugal	98.1	130	98.1	130
Romania	60.2	32	60.2	32
Slovenia	57.2	55	48.5	55
Spain	87.7	99	100.0	74
Switzerland	15.1	282	na	na
United Kingdom	25.7	478	19.1	213

Abbreviation: Iso, number of tested isolates; na, not available; RR, resistance rate.

A very limited number of studies (55/302) presented also data on the prevalence of *Campylobacter* spp. from meat, allowing to determine the proportion of meat samples in which FQ-resistant *Campylobacter* was detected. This type of information would allow to assess the exposure of the consumer to FQ-resistant *Campylobacter*, reducing the uncertainty associated. To reduce uncertainty, it is advisable to consider designing a prevalence study which not only explores the

isolates but also maintains the link with the prevalence on the original sources: this approach would reduce under- or overestimation of the final consumer exposure due to variable isolate collection approaches applied through the world and presented by the different studies.

4 | CONCLUSIONS

This review aimed to address data gaps by conducting a systematic literature review and meta-analysis on the prevalence of quinolone and fluoroquinolone-resistant *Campylobacter* in broiler meat at retail and slaughterhouse level. AMR has a strong impact on global level and evidence from this research highlights high proportion of FQQ-resistant *Campylobacter* at retail and slaughterhouse level broiler meat in most of the regions of the world. These results will be integrated in a wider ongoing project as input parameters of a mathematical model for risk assessment that will provide science-based support to policy-makers, industry stakeholders and researchers, in identifying effective strategies to mitigate the spread of antibiotic-resistant *Campylobacter* and safeguard public health.

EU-FORA fellowship programme was an excellent opportunity to establish strong connections and collaboration between two European Laboratory and Research Institutes, one from North, the other from South, which did not have an official contact before. The programme is paving the way into future projects and further networking possibilities between two Institutes. Participation in programme also strengthened the relationships and partnership between Institutes and EFSA. The fellow gained deep and valuable knowledge about conducting big-scale systematic reviews and learned the science behind meta-analyses. Fellow developed practical and very needed skills in data analyses and programming with R software. EU-FORA fellowship stimulates critical thinking in relation to the issues that are going to be dealt with risk assessment approach and provides the capacity to analyse available data from different prospectives. Throughout the whole year, the fellow participated in activities proposed by hosting Institute, like meetings, trainings, monthly department training programmes and social activities and also stayed in touch with the sending Institute and transferred the gathered knowledge. The hosting site supervisors had chance to polish supervising and teaching skill, handle cultural differences and establish international relations. The collaboration and work on Risk Assessment will continue with dissemination of the results via conferences and publications in peer-reviewed journals.

ABBREVIATIONS

AMR	antimicrobial resistance
AST	antimicrobial susceptibility testing
CIP	ciprofloxacin
FQ	fluoroquinolones
FQQ	fluoroquinolones and quinolones
IZSLT	Istituto Zooprofilattico Sperimentale Del Lazio e Toscana
LABRIS	National Centre for Laboratory Research and Risk Assessment
MDR	multidrug resistance
QMRA	quantitative microbiological risk assessment
NAL	nalidix acid
RR	resistance rate
WHO	World Health Organization
WP	work package

ACKNOWLEDGEMENTS

This report is funded by EFSA as part of the EU-FORA programme. The Authors wish to thank the Risk Assessment Department in National Centre for Laboratory Research and Risk Assessment (LABRIS), Estonia; the Epidemiological Unit of Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (IZSLT) and National Reference Laboratory for Antimicrobial Resistance (NRL-AR) in Italy for the support provided to this scientific output.

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How to cite this article: Mandel, T., Iulietto, M. F., Reinik, M., & Condoleo, R. (2024). Exploring frameworks for quantitative risk assessment of antimicrobial resistance along the food chain. *EFSA Journal*, *22*(S1), e221117. https://doi.org/10.2903/j.efsa.2024.e221117

APPENDIX A

A.1 | LIST OF ADDITIONAL TRAINING ACTIVITIES

	Title /description	Date
Conference	International Conference on Using Epidemiological Studies in Health Risk Assessments: relevance, reliability and causality. Participating in preconference Workshops (OHAT approach and raRoB tool). In Berlin, organised by BfR and EFSA	9–10.11.2023
Trainings	Training to use STATA software (on-site group training at ISZLT, provided by STATA team)	26.10.2023
	EFSA e-learning training course on horizontal scientific assessment methodologies 'Protocol Development'	22.8–13.10.2023
	Writing in the Sciences, Standford University, Coursera e-learning	28.4–30.7.2024
Organised webinar	Webinar and Discussion between Experts on African Swine Fever, LABRIS and IZSLT	13.2.2024
Webinars	Communicating Antimicrobial Resistance, ECDC	26.3.2024
	ENVIRE Seminar. Interventions to control the dynamics of antimicrobial resistance from chickens through the environment	14.11.2024
Meetings with departments	Meetings, presentations and discussions with departments in hosting Institute: Epidemiological Unit, Training Unit, Food Microbiology Unit, Risk Assessment Group, Cooperation Unit, Bee Health Unit, National AMR Reference Laboratory	25–29.9.2023
Meetings with scientific presentations	EFSA networks meeting in Tallinn, Focal Point	6.12.2023
	Workshop on extensive literature review; Systematic review – protocol development; Study Quality Assessment – (Maria Francesca Iulietto)	26.9.2023 14.2.2023 11.4.2023
	Generic Risk Assessment tools – An overview; Meta-analyses summary – (Roberto Condoleo)	20.6.2024 18.7.2024
	Overview of the dataflow in laboratory and national information systems	8.5.2024
Field visits	Production of Mozzarella: from farm to fork. A two-day fieldtrip to see the full production line of mozzarella cheese, starting from visit the buffalo farm, continuing in a production house and finishing at retail. Also visit the Laboratories of IZSLT in Latina	5–6.3.2023
	Visits to the AMR National reference Laboratory. Visits to the laboratory to understand the work- and dataflow specific to the AMR. From arrival of samples, sample preparation, analyses, MIC technique, reading the answers and delivering the results	6–16.5.2024
	Edible Insect Day. Trip to see activities regarding insect farming and related research, visit to the IZSLT laboratories in Viterbo	23.7.2024
Department Seminars	Presentation of the project 'Aquae Strength– Strengthening capacity on aquatic animal health and epidemiological surveillance', Pasquale Rombolà	20.2.2024
	One health: skills necessary at European level for the development of a work force – 2. One Health Contributions next Italian Epidemiology Conference – Paola Scaramozzino	12.3.2024
	Risk Communication: Theory, practice and ongoing projects in EU – Barbara Tiozzo – IZSVe	14.5.2024
	Expert knowledge elicitation – experience with WHO – Maria Francesca Iulietto	16.7.2024
Invited speaker – seminars	Presentation from EU-FORA fellow Tiina Mandel: Background, objective and expectations. OES seminar.	26.9.2023
	IZSLT internal Seminar: 'My Experience in IZSLT through EFSA EUFORA programme', Tiina Mandel	30.7.2024