



Technical Specifications for a EU-wide baseline survey of AMR in bacteria from aquaculture animals

P.-A. BELCÉIL
on behalf of the EFSA WG

Process of production of the Baseline Survey

Initial Proposal of a BLS on AMR in aquaculture animals (EFSA, the EFSA Network and the MSs)

Mandate of the European Commission to EFSA (2023)

EFSA Expert Working Group → **EFSA Technical Specifications** (issued in **July 2024**)

*Discussions about detailed **implementing measures** between the EU Member States and the European Commission*

Legislative provisions:
Decision of the European Commission (in 2025)

Preparatory activities at both EU and MS levels:
Drafting protocols and technical documents

Baseline survey
(planned in 2027)



Mandate received from the European Commission

In accordance with Article 31 of Regulation (EC) No 178/2002, the Commission requests EFSA to provide **TECHNICAL AND SCIENTIFIC SUPPORT** for the development of a **BLS** on the prevalence of **AMR IN BACTERIA** isolated **FROM EU PRODUCED AQUACULTURE ANIMALS**, considering the most recent scientific literature and technological developments, epidemiological trends, and **RELEVANCE FOR PUBLIC HEALTH**. EFSA is notably asked to propose **HARMONISED APPROACHES** for the collection and the analysis of AMR data from aquaculture animals by:

- a. proposing **PRIORITY COMBINATIONS** of **AQUACULTURE ANIMALS/TARGET BACTERIA** to be considered in the BLS;
- b. proposing a **COMPLETE SAMPLING FRAMEWORK** for the implementation of the BLS including the origins of bacterial isolates subject to AMR testing, the sampling design and the sample size;
- c. proposing protocols for **ISOLATION AND CHARACTERIZATION OF BACTERIA**;
- d. proposing protocols for phenotypical **ANTIMICROBIAL SUSCEPTIBILITY TESTING** of bacterial isolates;
- e. proposing protocols for the testing of bacterial isolates via **MOLECULAR TYPING METHODS**;
- f. providing guidance for **TECHNICAL REPORTING** of the BLS data collected by Member States to EFSA.



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Clarifications about the mandate

- a.
 - The mandate is not drafted from an animal health perspective
- b.
 - **Fishing from the shore** is **excluded** from the mandate
- c.
 - **Imported seafood** is **excluded** from the mandate
- d. proposing protocols for phenotypical **ANTIMICROBIAL SUSCEPTIBILITY TESTING** of bacterial isolates;
- e. proposing protocols for the testing of bacterial isolates via **MOLECULAR TYPING METHODS**;
- f. providing guidance for **TECHNICAL REPORTING** of the BLS data collected by Member States to EFSA.

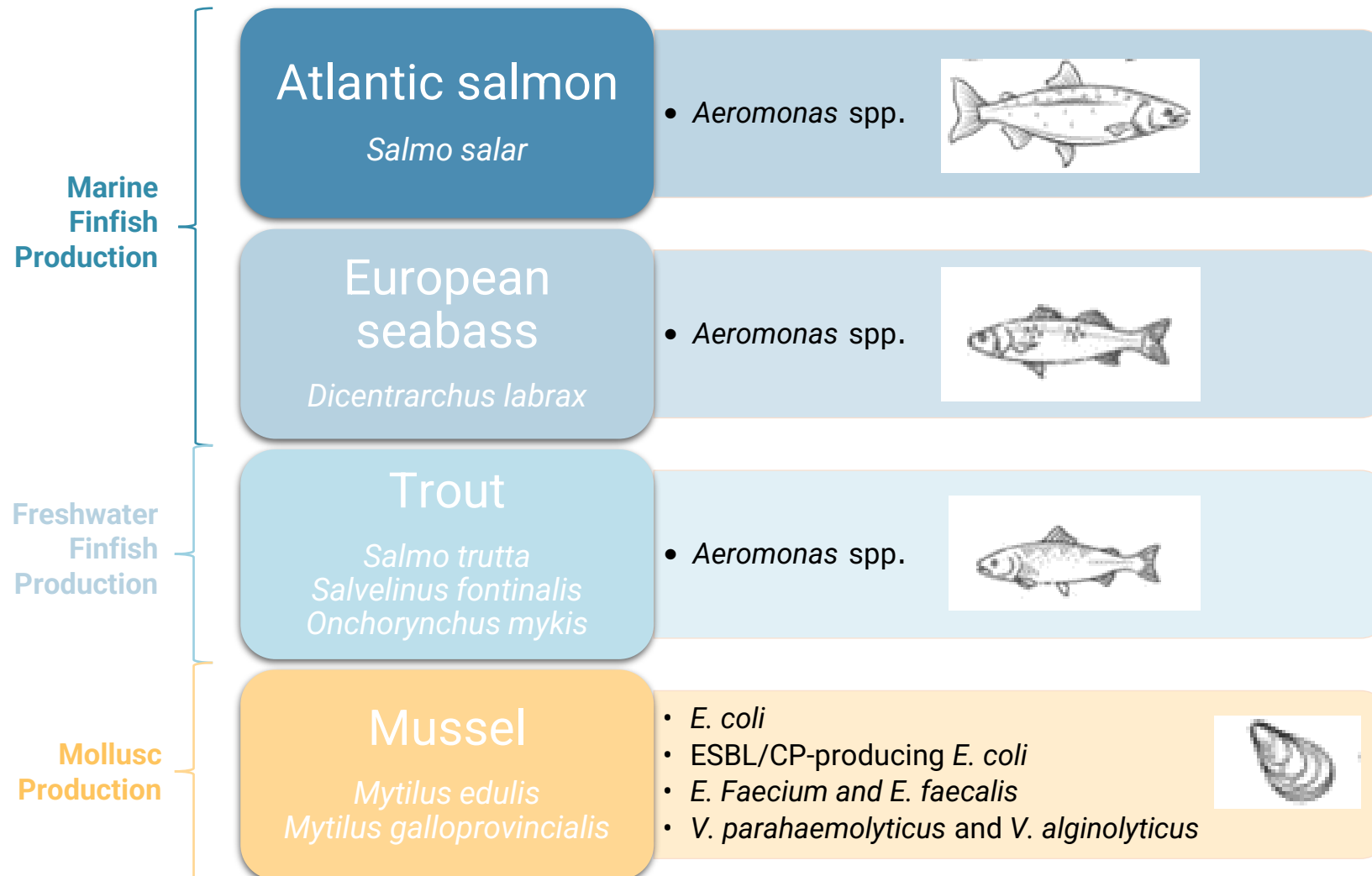


Objectives of the Baseline Survey

- The **primary objectives** of the survey are:
 - (Ia) To assess the **prevalence** of antimicrobial-resistant **microorganisms** and the **occurrence** and **diversity** of **AMR** in microorganisms from the main aquaculture productions **in the EU**
 - (Ib) To indirectly assess, through filter feeding molluscs produced within the EU, the degree of environmental anthropogenic **contamination** with **resistant bacteria** in **European production waters**
- The **secondary objective** of the survey is:
 - To **explore the link between AMC and AMR** in finfish aquaculture



Combinations of aquatic animal species/bacteria

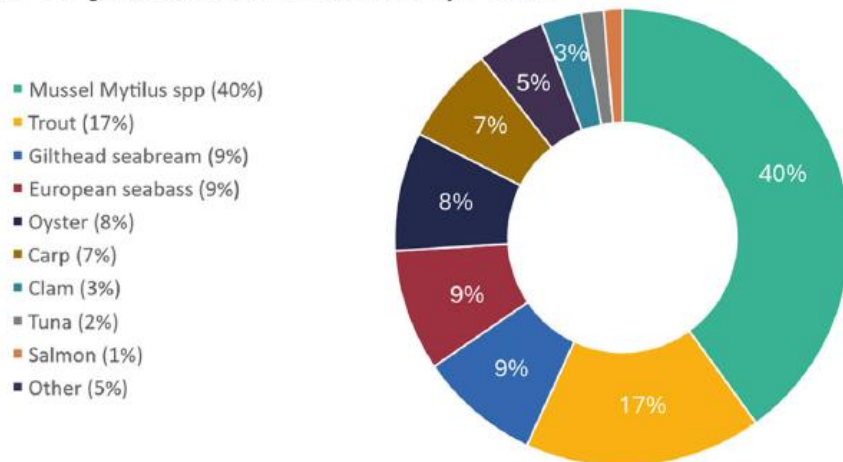


Rationale for the combinations targeted: Aquatic animal species

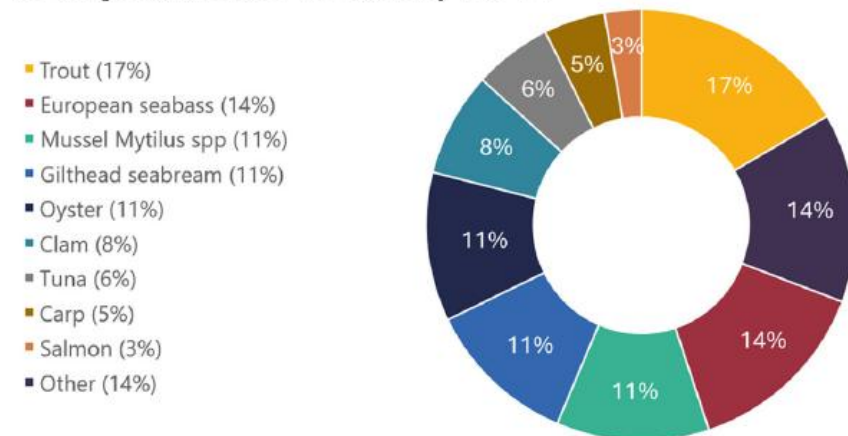
- The most important **aquaculture productions** in Europe
- Distribution of the productions across Europe (EU/EFTA MSs)
- **Highest number of countries** involved in the BLS

- **Aquaculture production data of fishery products:**

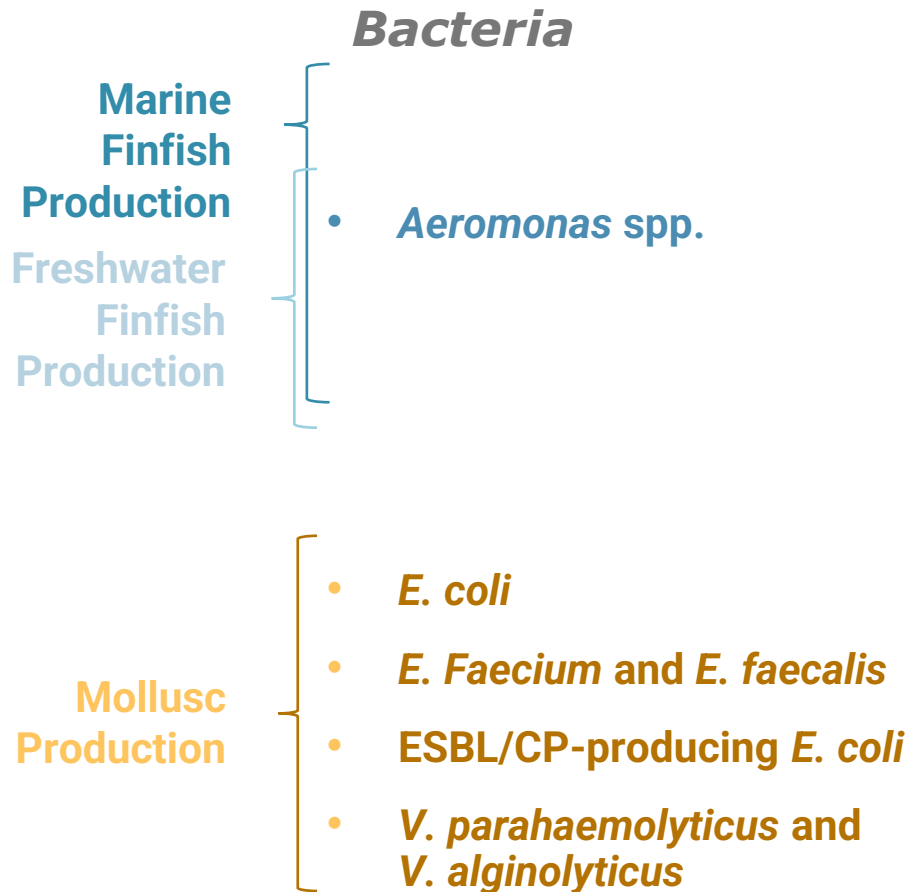
% of production in tonnes, 2021



% of production in euros, 2021



Rationale for the combinations targeted: Bacteria



Rationale

- Studied at the genus level, *Aeromonas* spp. is considered as an **indicator of the dissemination of AMR** in water or in fish.
- *Aeromonas* may also be an **opportunistic pathogen** for humans.
- Prevalence

- *E. coli* and *Enterococcus* sp. are typically used as **indicators of faecal contamination** in aquatic environments.
- *V. parahaemolyticus* as one of the species that should be included in monitoring and surveillance programmes of antibiotic susceptibilities of bacteria isolated from aquatic animals
- *V. alginolyticus* is considered as one of the most **common pathogenic species** for human



Survey design: stratified sampling approach

Hierarchical structure of the design

- **Stratified sampling** with main strata at the MS/Region level
- **Random sampling** of production units (**PU**)
- Sampling of production batches of animals (**PB**)
- **Harmonised samples**

	EU-strata	Production unit (PU)	Production batch (PB)	Minimal size of sample batch (SB) ^a
Shellfish	MS	Production area (PA)	At sampling point	≥ 15 mussel
Shellfish	MS	Dispatch centre (DC)	Packed & labelled	≥ 15 mussel
Marine finfish	(MS, Region)	Farm	Cage	≥ 5 fish
Freshwater finfish	(MS, Region)	Farm	Pond	≥ 5 fish

^aThe minimal size is based on the minimal biological material necessary for the testing.

Survey design: stratified sampling approach

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Sampling concept	Sampling of aquatic organisms			
	Marine finfish production: Seabass/salmon	Freshwater finfish production: Trout	Mollusc production: Mussels	
Target populations	EU/EFTA produced seabass/salmon ^a	EU/EFTA produced trout ^{a,b}	EU/EFTA produced mussels ^{a,c}	
Strata	(MS, Region) ^a	(MS, Region) ^a	MS	MS
Proportional allocation	Sample size (number of PBs) proportionate ^d to the stratum production, with a minimum and a maximum ^e number of PBs	Sample size (number of PBs) proportionate ^d to the stratum production, with a minimum and a maximum ^e number of PBs	Sample size (number of PBs) proportionate to the stratum production, with a minimum and a maximum ^e number of PBs	Sample size (number of PBs) proportionate to the stratum production, with a minimum and a maximum ^e number of PBs
Production Units	Random sampling of PUs (farms) per stratum ^{f,g}	Random sampling of PUs (farms) per stratum ^{f,g}	Random sampling of PUs (PA) per stratum ^{f,g}	Random sampling of PUs (DC) per stratum ^{f,g}
Epidemiological Units	PBs of seabass/salmon at slaughter	PBs of trout at slaughter	PBs at sampling point	PBs packed and labelled
Production Batches	Random sampling of 4 PBs per PU, approximately evenly distributed over the year	Random sampling of 4 PBs per PU, approximately evenly distributed over the year	Random sampling of 4 PBs per PU, approximately evenly distributed over the year	Random sampling of 4 PBs per PU, approximately evenly distributed over the year
Sampling	At farm/slaughter	At farm/slaughter	At sampling point ^h	At DC
Sample	Pooled sample of gills collected from 5 fish, randomly selected per PB	Pooled sample of gills collected from 5 fish, randomly selected per PB	Pooled sample of 15 mussels	Pooled sample of 15 mussels

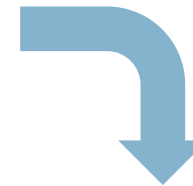


Sample size calculation

- The **sample size** (i.e. the number of isolates to be tested for susceptibility at each sampling time) should allow, **within a predetermined accuracy**, the calculation of **the occurrence of AMR** (proportion of antimicrobial resistance to a particular antimicrobial) for a given combination of bacteria/animal populations.

- **Standard calculation of the required number of isolates**

- Accounting for possible missing data and loss during storage
- Choices at the EU and the strata levels
- Multiple PB for the same PU
 - Intra-PU correlation



- **From isolate numbers to production batch numbers**
- **A sequential approach proposed**
(cf. MRSA BLS)



Illustrations: Freshwater Trout and Salmon / *Aeromonas* spp.

1. Objective

- To assess the **occurrence of resistance** at the **EU level**

2. Principles of the sampling design

- Stratified sampling** with EU/EFTA MSs-Region as strata
- Proportional allocation** using production volumes of strata
- Production batches** are the epidemiological units to be sampled

3. Parameters to calculate the required number of isolates

- Accuracy: 0.05**
- Confidence level: 0.95**
- Taking the **unknown occurrence of AMR** as **0.5**
- Extra 5%** for **missing observations**
- Extra 2%** for **losses**
- Quarterly measurements** on the same production unit
- Correlation 0.2**

-> The required
number of isolates

- When unadjusted: **388**
- When adjusted for missingness and losses: 416
- When additionally adjusted for multiple isolates from the same PU: 665
 - With rounding to integers (across the strata) **677 isolates**

Marine
Finfish
Production

Atlantic salmon
Salmo salar



Freshwater
Finfish
Production

Trout
Salmo trutta
Salvelinus fontinalis
Onchorynchus mykiss



Illustration: Freshwater Trout and *Aeromonas* spp. (1)

4. The conversion to the required number of batches (PB)

- Batch of size ≥ 5
 - Batch prevalence: 0.92
 - Within batch prevalence: 0.47
 - Intra-batch correlation: 0.18
 - Test sensitivity and test specificity taken as 1
 - Batch sensitivity: 0.88
- Derived from a dataset kindly provided by Anses (France)
- A **minimum** and a **maximum** number of **PUs** for any stratum is applied:
 - Minimum: 4 PUs and 16 PBs per MS
 - Maximum: 53 PUs or 2012 per MS

These values were applied to all strata.



Laboratory Methods

- Isolation methods
- Confirmatory testing and typing methods
- Antimicrobial Susceptibility Testing methods
 - Harmonised panel of substances
 - Harmonised criteria of resistance
- Whole Genome Sequencing

Laboratory
protocols
issued on the
EURL-AR
website



Data Reporting

- Data Models for Data Reporting

- Prevalence sample-based data model

- Detailed analytical results of samples taken (whether + or -)
 - EFSA standard for reporting laboratory results (SSD2)

- AMR isolate-based data model

- Isolate-level quantitative AMR data
 - WGS data

- Population data model

- Fish population size of the farms involved

Harmonised
Data
Models:



Expected Outputs of the survey

Expected Outputs



- Prevalence of targeted bacteria
- Occurrence of AMR
- Possible seasonal effect
- Diversity of AMR
 - ... at the batch level
 - ... at the EU level



Next steps

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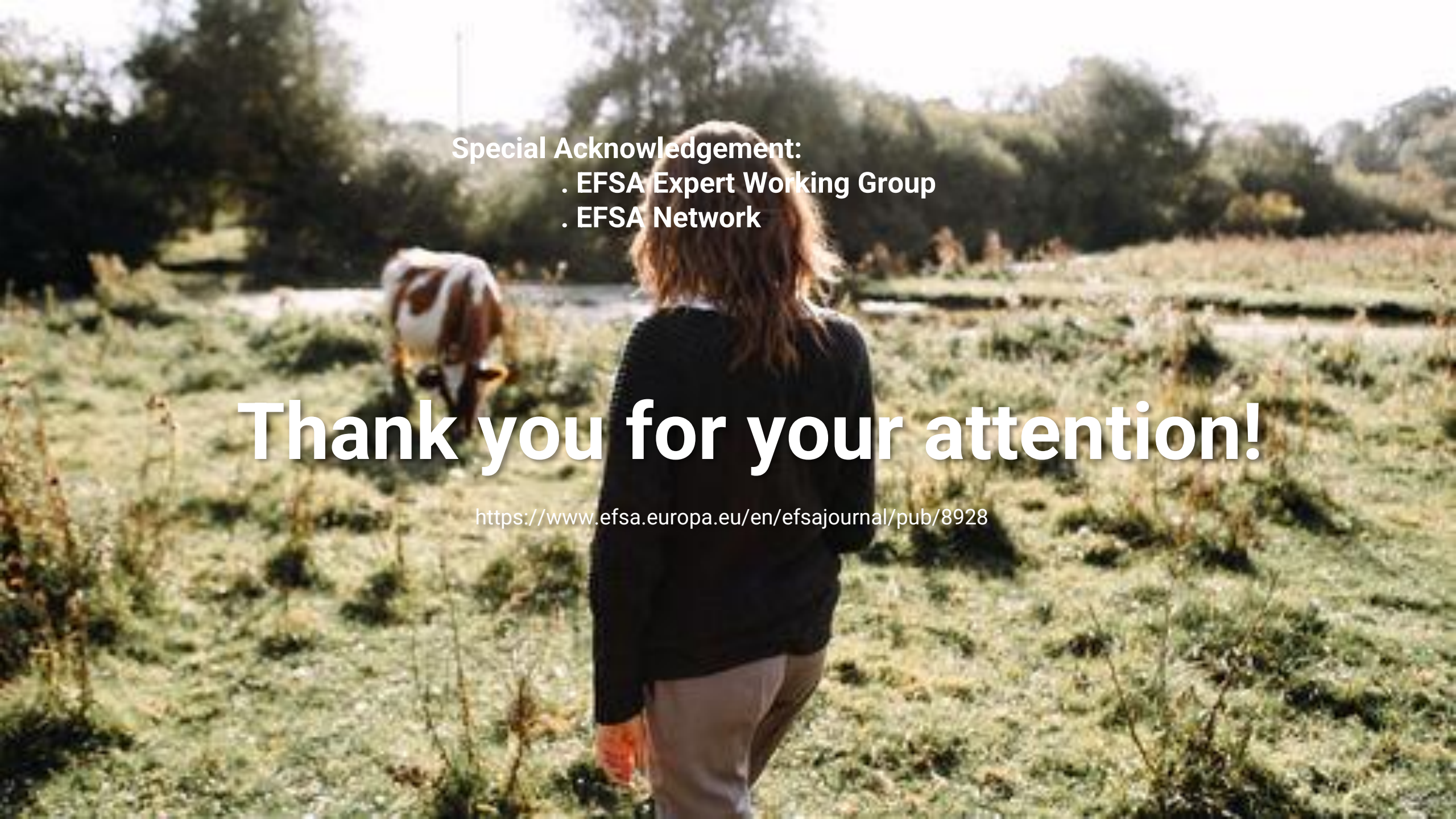
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Special Acknowledgement:
· EFSA Expert Working Group
· EFSA Network

Thank you for your attention!

<https://www.efsa.europa.eu/en/efsajournal/pub/8928>