# PREVALENCE OF VEROTOXIGENIC *Escherichia coli* SEROGROUPS AND VIRULENCE GENES OF RELEVANCE IN PUBLIC HEALTH IN FATTENING CALVES IN SPAIN

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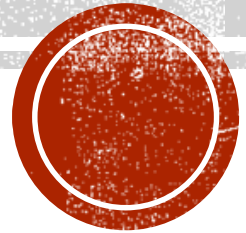

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# CENTRAL VETERINARY LABORATORY-ANIMAL HEALTH (LCV)

Entry

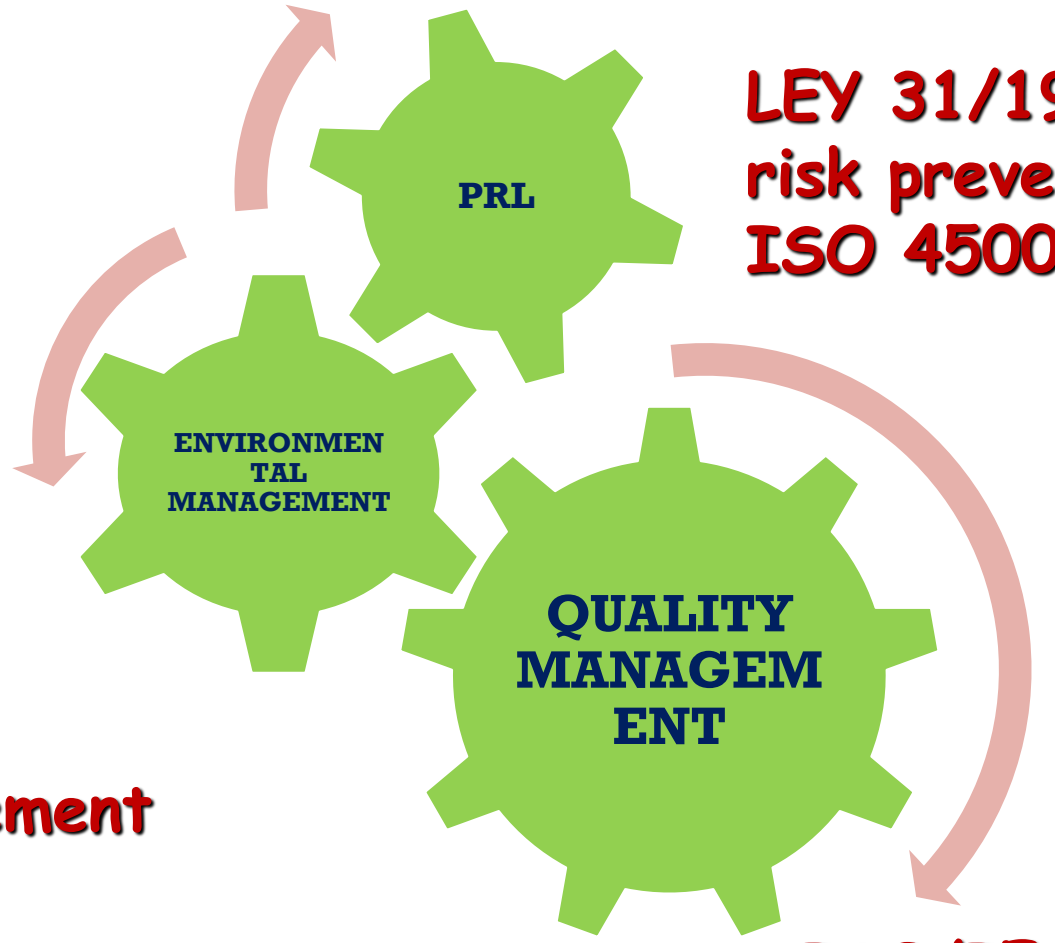
Main Building or MOLECULAR GENETICS Building



ANIMAL HEALTH buildings

LCV

# INTEGRATED MANAGEMENT SYSTEM

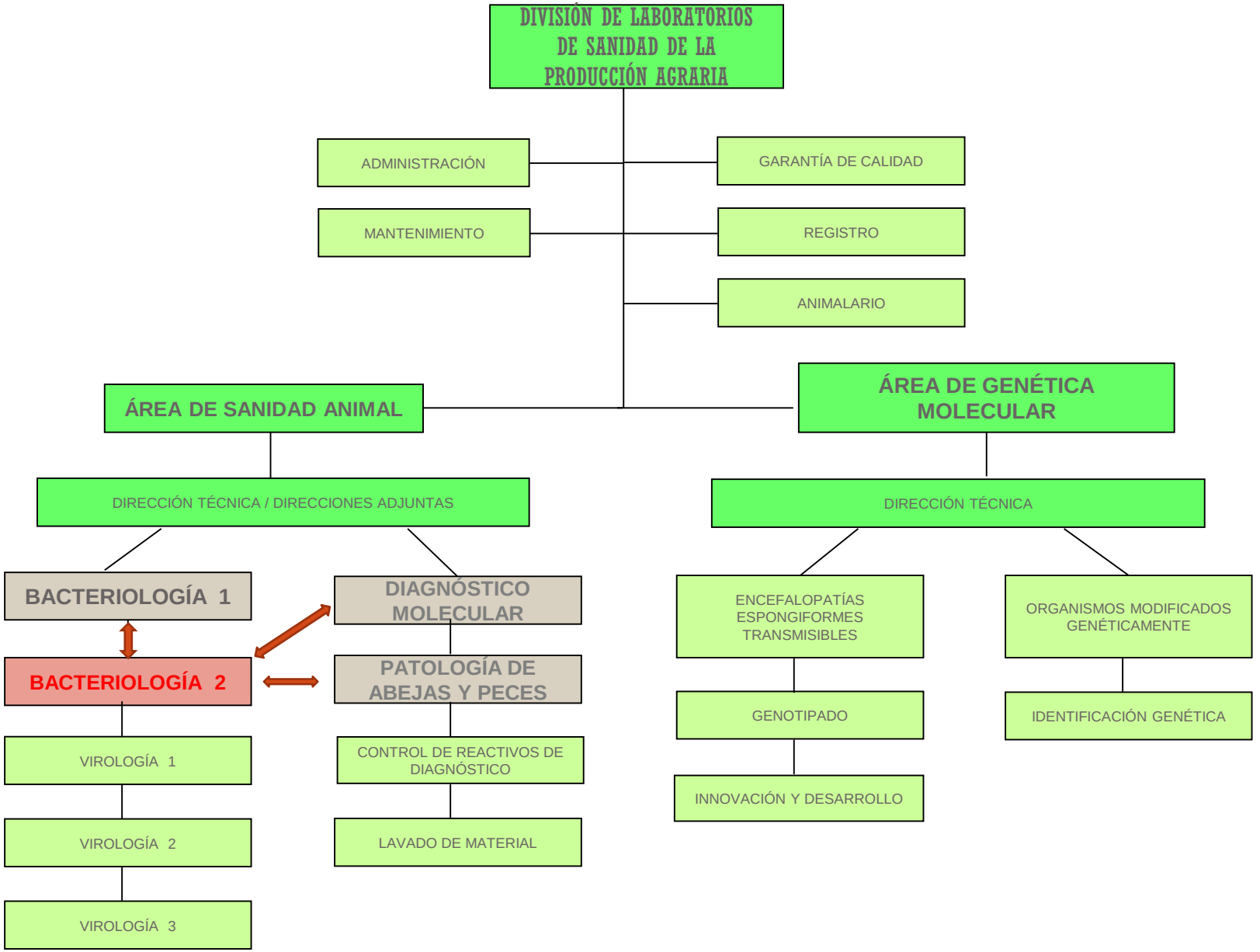



LEY 31/1995: occupational risk prevention
ISO 45001

ISO 14.001
-Certification-
Enviromental management system

ISO/IEC 17.025
-acreditation-

# ORGANIGRAMA LCV



# ACTIVITIES OF BACTERIOLOGY DEPARMENT 2

*Act as NLR of:*

- ✓ *Campylobacteriosis*
- ✓ **VTEC**
- ✓ Monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria and repealing Implementing Decision 2020/ 1729 /EU (isolation)
  - ✓ *Campylobacter coli*, *Campylobacter jejuni*
  - ✓ Indicator commensal *Escherichia coli* (E. coli)
  - ✓ *E. coli* producing the following enzymes:
    - ✓ Extended Spectrum β-Lactamases (ESBL)
    - ✓ AmpC β-Lactamases (AmpC);
    - ✓ Carbapenemases (CP)
- ✓ Leptospirosis
- ✓ Botulism
- ✓ Tularemia
- ✓ Contagious Equine Metritis
- ✓ Bacteriological honeybees diseases (American Foulrood, European Foulbrood)

# INTRODUCTION

Verotoxigenic *Escherichia coli* (VTEC) is a foodborne pathogen of relevance in Public Health in European Union (EU), being in **2020** the fourth most frequent bacterial agent detected in foodborne outbreaks with an increase between 2015 to 2019.

- **hospitalisation** for 35.8% of all confirmed VTEC cases in the EU in 2020 (EFSA Journal 2021;19(12):6971)

  **T**he most common serogroups among **HUS cases** were:

| % | O26 | O157 | O80 | O145 | ONT |
|---|---|---|---|---|---|
| 2019 | **38.7** | **23.0** | 9.0 | 9.8 | 4.7 |
| 2020 | **41.8** | 11.9 | **13.2** | 8 | 24.1 |

- Directive 2003/99/EC established that **verotoxigenic *Escherichia coli* (VTEC)** is a zoonotic agent to be included in monitoring (list A). In Spain it was transposed by RD 1940/2004 on the surveillance of zoonoses and zoonotic agents
- **In the EU, animal monitoring is not widely implemented or carried out with a harmonized sampling strategy**.

# OBJETIVE

- The objetive of this surveillance was determine **VTEC  prevalence in Spain in calves less than one year of age and strains characterization.**

- **Assist the investigation of foodborne outbreaks in humans**, providing support to the competent authorities under a One Health approach.

# SAMPLING

- ✓ Based on the technical specifications for the monitoring and reporting of VTEC on animals and food drafted by EFSA (EFSA Journal 2009; 7(11):1366).
- ✓ A nationwide monitoring in the **most representative national production slaughterhouses throughout the Spanish geography** (Sampling of AMR surveillance was taken advantage of)
- ✓ For **years 2019** and **2021.**
- ✓ Targeted population: **calves under one year of age**
- ✓ **Epidemiological unit:** herd. **One sample from each herd avoiding repetitions**
- ✓ A total of **498 hide swabs samples (breast)** were taken **throughout the year** avoiding seasonal bias.

efsa
European Food Safety Authority

EFSA Journal 2009; 7(11):1366

**SCIENTIFIC REPORT OF EFSA**

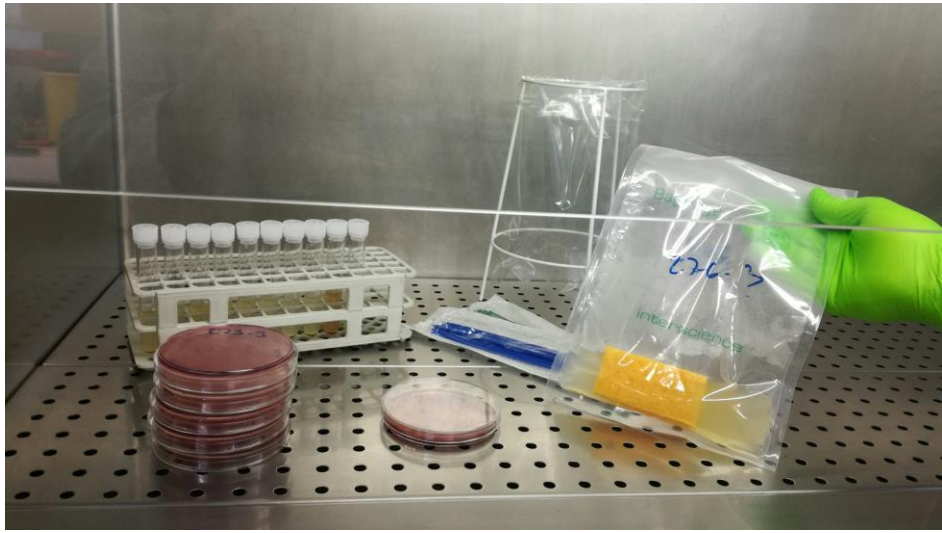
**Technical specifications for the monitoring and reporting of verotoxigenic *Escherichia coli* (VTEC) on animals and food (VTEC surveys on animals and food)**[1]

**European Food Safety Authority**[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

# LABORATORY ANALYTICAL METHODS

# 2019: INTERNAL METHOD ACCREDITED BASED ON ISO 13136:2012 FOR THE PRESUMPTIVE DETECTION, ISOLATION AND IDENTIFICATION.



## STEP 1: Microbiological enrichment of the sample

**1 sponge of hide calve**: 90 ml BPW
18-24 h (37°C ±1°C)

## STEP 2: Presumptive detection in BPW: real-time PCR

- **vtx1, vtx2 genes**
- **eae gene**
- **if positive to eae:** determine 6 of the more prevalent serogroups of VTEC in human health **(Top 5 (**O157, O26,O111, O103,O145) **+ O104)**

## STEP 3: Isolation, confirmation and characterization of strains

- Inoculation in **specific solid media** for *E. coli: TBX, MCA, CT-SMAC, CT-RMAC*
- Pick up to **50 colonies** with *E. coli* morphology
- Inoculate, dot or streak each colony in TSA
- **Test of pools of 10 colonies by real-time PCR** for the detection of virulence genes or markers associated with STEC (stx1/stx2).
- **Subculture of isolated colonies** for subsequent **confirmation and characterization by real-time PCR**

# 2019 SURVEILLANCE RESULTS

- **STEP1: Microbiological enrichment of the sample**

  **236 samples**

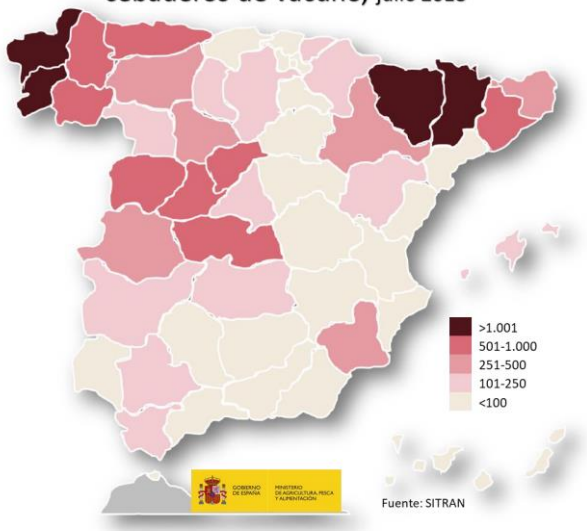
- **STEP2: Presumptive detection in BPW: real-time PCR**
  - **Results in 95,3%** positive samples.
    - **98,7%** from these were **eae positive.**
      - 97,8% of them were positive at least to one or more of the 6 investigated serogroups “Top 5” + O104)
- **STEP3: Isolation, confirmation and characterization of strains**
  - **prevalence of 21,2% (79 isolates)**
  - 32,9 % VTEC (vtx1 and/or vtx2, eae +, O-T (O157, O111, O26, O103)
  - 19,0 %: VTEC (vtx1 and/or vtx2, eae +, O-NT )
  - 48,1 % VTEC (vtx1 and/or vtx2, eae -)

Low efficency and high finantial and laboratory work cost

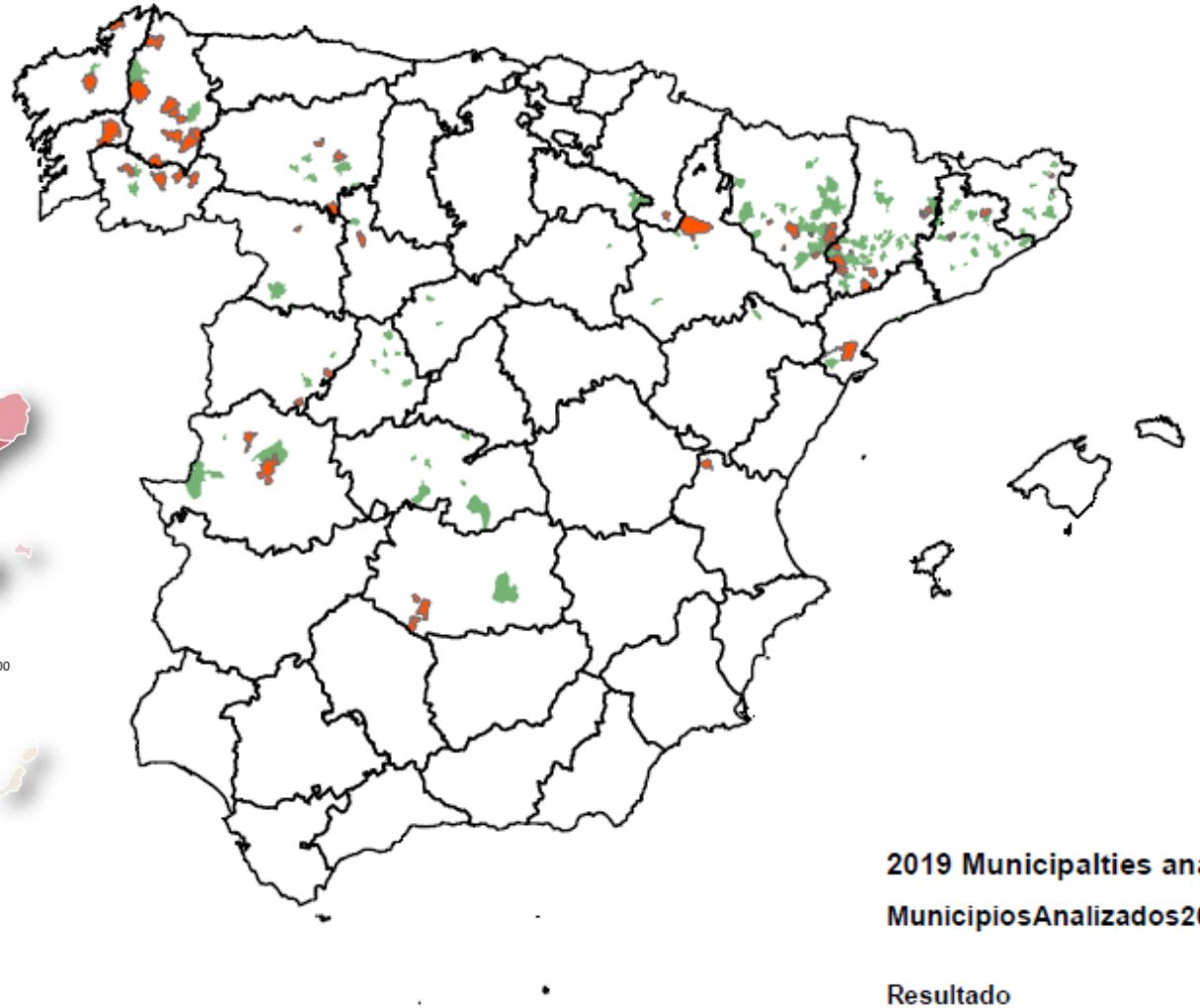
| Serogroups | 2019 |
|---|---|
| O157 VTEC | 19.0% |
| O26 VTEC | 7.6% |
| O103 VTEC | 5.1% |
| O145 VTEC | 0% |
| O111 VTEC | 1.3% |
| O104 VTEC | 0% |

| 2019 | vtx1,eae | vtx2,eae | vtx1,vtx2,eae | vtx1 | vtx2 | vtx1,vtx2 |
|---|---|---|---|---|---|---|
| O157 | 1,3% | 6,3% | 11,4% | | | |
| O26 | 6,3% | | 1,3% | | | |
| O103 | 5,1% | | | | | |
| O145 | | | | | | |
| O104 | | | | | | |
| O111 | 1,3% | | | | | |
| O-NT | 5,1% | 7,6% | 6,3% | 11,4% | 27,8% | 8,9% |

Distribución provincial del nº de cebaderos de vacuno, julio 2018




Nº de animales en cebaderos, julio 2018



**2019 Municipalties analyzed**

**MunicipiosAnalizados2019**

**Resultado**

Detectado

No detectado

# 2021: INTERNAL METHOD WITH REVIEW ACCREDITED FOR THE ISOLATION AND IDENTIFICATION OF SHIGATOXIN-PRODUCING *Escherichia coli* (VTEC) AND DETERMINATION OF SEROGROUPS 0157, 0111, 026, 0103, 0104 AND 0145.

**STEP 1: Microbiological enrichment of the sample**

1 sponge of hide calve: 90 ml BPW

18-24 h (**41,5°C** ±1°C)

**STEP 2: Isolation of 100% of the samples, confirmation and characterization.**

# 2021 SURVEILLANCE RESULTS

- **STEP 1: Microbiological enrichment of the sample in BWP**

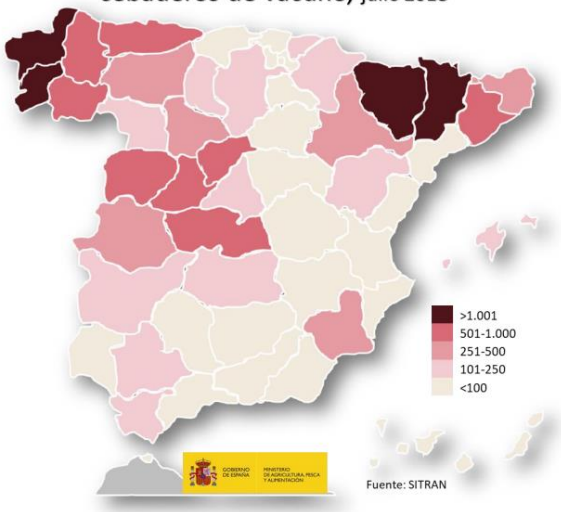
  **262 samples**

- **STEP 2: Isolation, confirmation and characterization**
  - **prevalence of 36,6 % (112 isolates)**
  - 27,7% VTEC (vtx1 and/or vtx2, eae +, O-T (O157, O26, O103,O145)
  - 14,3 %: VTEC (vtx1 and/or vtx2, eae +, O-NT )
  - 58,0 % VTEC (vtx1 and/or vtx2, eae -)

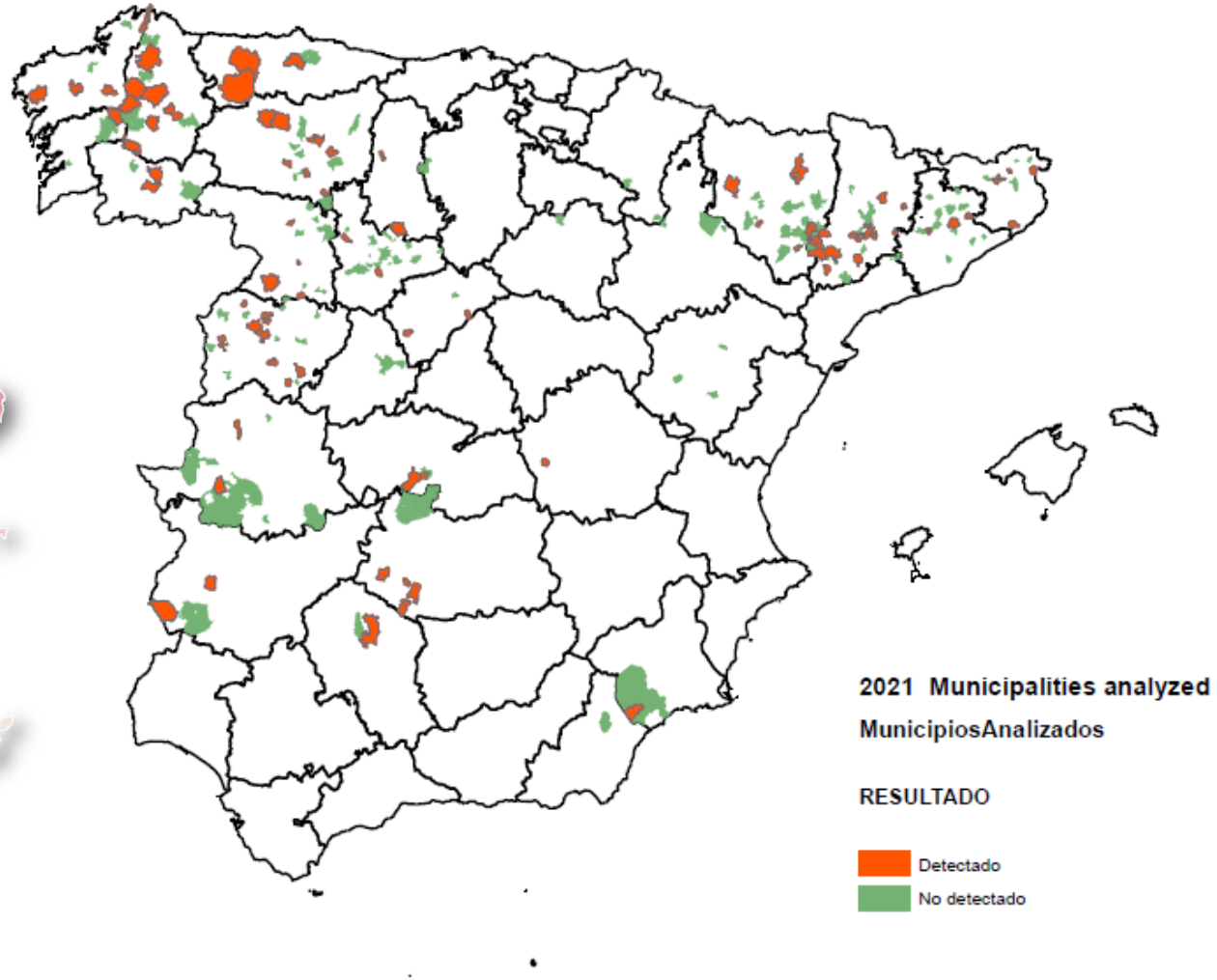
| Serogroups | 2021 |
|---|---|
| O157 VTEC | 15.2 % |
| O26 VTEC | 8.9 % |
| O103 VTEC | 1.8 % |
| O145 VTEC | 1.8 % |
| O111 VTEC | 0 % |
| O104 VTEC | 0% |

| 2021 | vtx1,eae | vtx2,eae | vtx1,vtx2,ea | vtx1 | vtx2 | vtx1,vtx2 |
|---|---|---|---|---|---|---|
| O157 | 0,9% | 10,7% | 3,6% | | | |
| O26 | 5,4% | 0,9% | 2,7% | | | |
| O103 | | 1,8% | | | | |
| O145 | | 1,8% | | 0,9% | 0,9% | |
| O104 | | | | | | |
| O111 | | | | | | |
| O-NT | 7,1% | 3,6% | 3,6% | 17,9% | 35,7% | 2,7% |

Distribución provincial del nº de cebaderos de vacuno, julio 2018




Nº de animales en cebaderos, julio 2018



# CONCLUSIONS 1

- ✓ **Wide effort in sampling and laboratory analysis:** a total of 498 samples (cattle sampling units were analysed in 2019 and 2021)
- ✓ The **detection** increase from 2019 to 2021 from **21.2%** to **36.6%.**
  - **May be due to the optimisation of the temperature of enrichment in BPW (changed from 37ºC to 41,5ºC).**

SCIENTIFIC REPORT

ecdc ej EFSA Journal

  - **EU 2019**: 17,1% positive herds (9 MS reported data, 1493 cattle sampling units)
  - **EU 2020**: 5,2% positive herds (3 MS reported data, 678 cattle sampling units)
- ✓ **Sampling in primary production**: it could be used at EU level taking advance of the sampling of AMR surveillance every two years (calves).

# CONCLUSIONS 2

- ✓ The more prevalent serogroups in calves (<1 year old) in Spain were **O157**, **O26**, **O103** and **O145**, representing the **17.1%**, **8.3%**, **3.4%** and **0.9%** of the isolates respectively. **Not detection for O104.**
  - ✓ In line with human HUS cases
- ✓ **Future**: subtyping
- ✓ The periodic surveillance in targeted animal populations could assist the investigation of foodborne outbreaks in humans, providing support to the competent authorities under a One Health approach.

GOBIERNO DE ESPAÑA
MINISTERIO DE AGRICULTURA, PESCA Y ALIMENTACIÓN



# Without all of them...

# it would not be possible!