GOBIERNO DE ESPAÑA
MINISTERIO DE AGRICULTURA, PESCA Y ALIMENTACIÓN

Dirección General de Sanidad de la Producción Agroalimentaria y Bienestar Animal

Subdirección General de Laboratorios de Sanidad Animal y Vegetal

African Swine Fever laboratory diagnosis in Spain

https://www.mapa.gob.es/es/ganaderia/temas/laboratorios-sanidad-genetica/

Laboratorio Central de Veterinaria

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Laboratorios de Referencia
Sanidad-Higiene-Genética Animal y Vegetal

Content

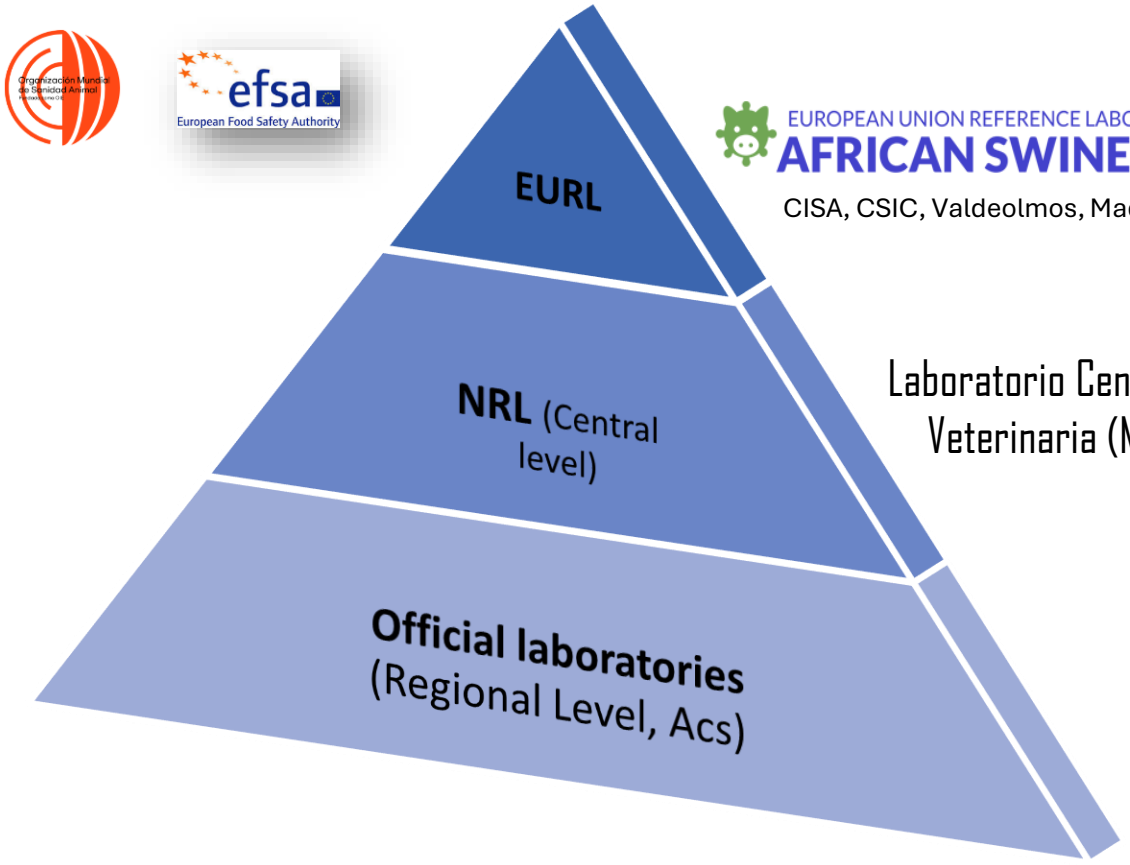
1. African Swine Fever laboratory network
2. Laboratory diagnostic workflow in the network
3. Laboratory diagnosis, characterization and monitoring of ASF outbreak
4. Origin of virus strain: hypothesis and lab. Investigation
5. Take-home messages

Content

1. African Swine Fever laboratory network (I)

Comisión Europea

efsa European Food Safety Authority



EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER

CISA, CSIC, Valdeolmos, Madrid (SPAIN)

Laboratorio Central de Veterinaria (MoA)

Framework: **Regulation (EU) 2017/625**

Laboratorios de Referencia

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1. African Swine Fever laboratory network (II)

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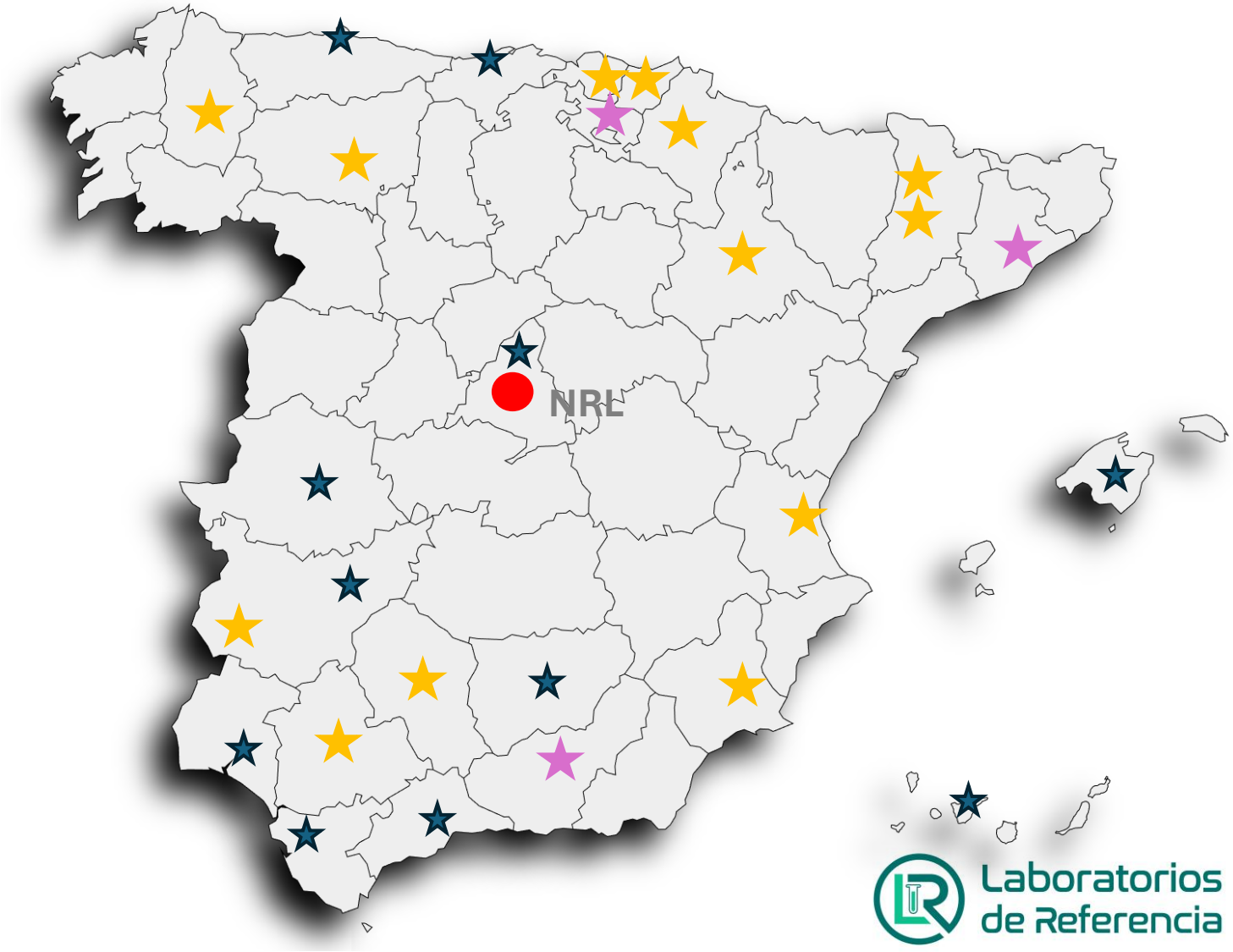
The entire network of laboratories accredited in accordance to ISO 17025

ASF laboratory network: 29 official labs + 1 NRL

Labs carrying out only ELISA 12
Labs carrying out only PCR 3
Labs carrying out ELISA + PCR 14



NRL ● Laboratorio Central de Veterinaria

Laboratory diagnosis at the NRL:

- Screening tests (ELISAs, IPT)
- Confirmatory methods (PCR, virus isolation, Sanger sequencing)

All methods accredited (ISO 17025)

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1. African Swine Fever laboratory network (III)

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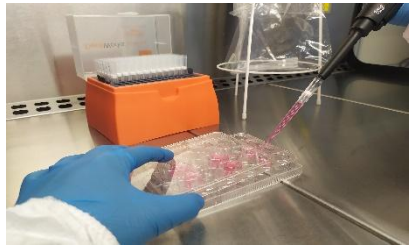
National Reference Laboratory: tasks and responsibilities (Regulation (EU) 2017/625, Law 8/2003)

Harmonization of diagnostic methods

Technical advice to OVS

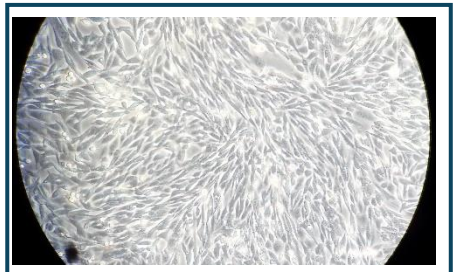
Control of diagnostic reagents



Confirmation of the diagnosis

Coordination with EURL (WS, participation in PTs, etc.)

Organization of Proficiency tests



Dissemination of information from EURL to OC labs.

Training courses, if neccesary

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Sanidad-Higiene-Genética Animal y Vegetal

Content

2. Laboratory diagnostic workflow

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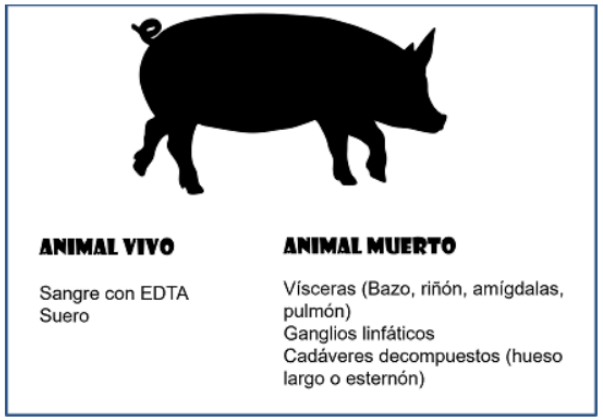
Subdirección General de Laboratorios de Sanidad Animal y Vegetal

FLOW DIAGNOSTIC CHART

AR of CATALONIA

Muestras de elección para el diagnóstico



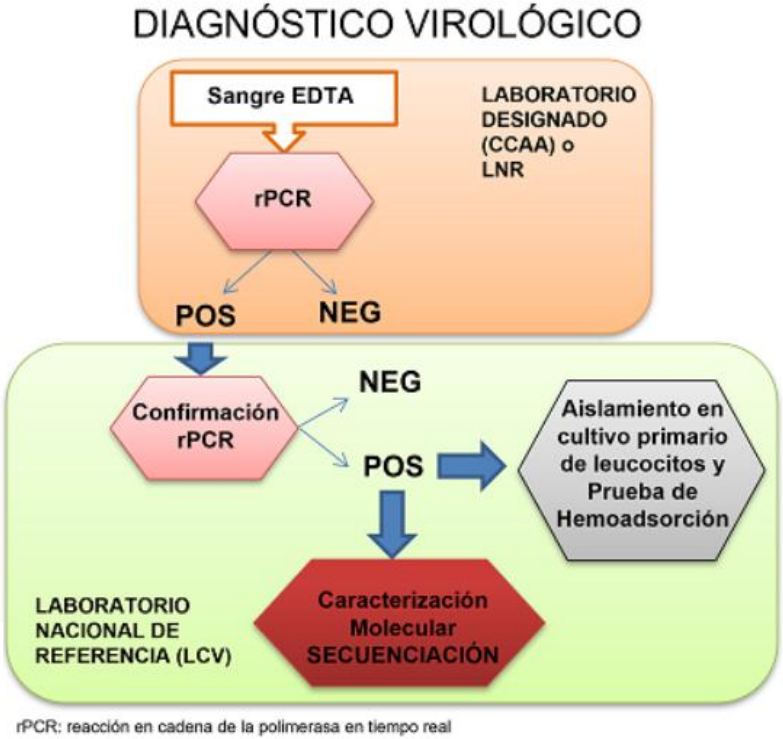
Dead animals:
EDTA blood
Tissues (organs, bone marrow)

Regional official lab (IRTA CReSA):
Necropsies and sampling
Screening by qPCR

If **POS/Non conclusive**

Samples sent to **NRL** for
Confirmation by qPCR&
Characterization (Sanger sequencing)

Ongoing collaborative serological study



Active and pasive surveillance in other Autonomous Regions

Serology and/or PCR by official labs, and confirmation at the **NRL**

Laboratorios de Referencia
Sanidad-Higiene-Genética Animal y Vegetal

Content

3. Laboratory diagnosis and characterisation and monitoring of ASF outbreak 2025-2026 (I)

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Subdirección General de Laboratorios de Sanidad Animal y Vegetal

Laboratory diagnosis of index case at the NRL (LCV- Algete): outbreaks 1 and 2.

November 26th (evening): Notice that we will be receiving samples that have tested PCR positive in the official laboratory (**IRTA-CreSa**, AR of Catalonia)

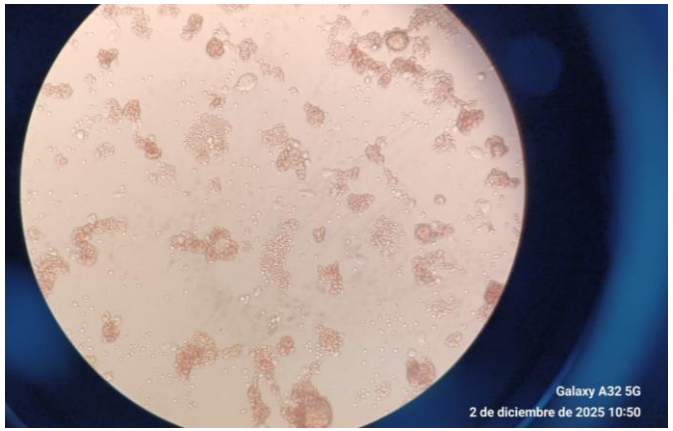
November 27th (18:30): **two EDTA blood samples** received at the NRL

(21:30): PCR results on EDTA blood samples (confirmed as **POSITIVE)**

November 28th (09:00): PCR results report is issued

(15:00): Partial sequencing Gen B646L (p72), Gen E183L (p54) and Gen B602L (CVR). Characterized as **GENOTYPE II**

(morning): primary monocyte culture preparation



December 01st (morning): inoculation on primary monocyte culture (**confirmed as POS next day**; presence of **Haemadsorbant Virus**

Rapid response: confirmation in 3 hours, genotyping in around 20 hours, isolation in 2 working days.

Laboratorios de Referencia
Sanidad-Higiene-Genética Animal y Vegetal

3. Laboratory diagnosis and characterisation and monitoring of ASF outbreak 2025-2026 (II)

Collaborative studies between NRL and EURL

- **Molecular characterisation** for the follow up of outbreaks (Sanger, WGS)
- **Serological** studies

EURL: full molecular characterization of ASF virus SPAIN 2025:

MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

CSIC Consejo Superior de Investigaciones Científicas

INIA Centro Nacional Instituto de Investigación y Tecnología Agraria y Alimentaria

African Swine Fever (ASF) confirmatory diagnosis and molecular characterization

REPORT ISSUED BY THE EUROPEAN UNION REFERENCE LABORATORY FOR AFRICAN SWINE FEVER (EURL-ASF), INIA-CISA

DATE: 05rd December 2025

Virus sequence is already published at **GenBank** (https://www.ncbi.nlm.nih.gov/). Accession number: **PZ023911**. (https://www.ncbi.nlm.nih.gov/nuccore/PZ023911.1)

EURL
December, 5. 2025

4. Monitoring of disease outbreaks: molecular markers

First detection:

- **qPCR** for the detection of ASFv (Fernández-Pinero *et al.*, 2013; WOAH, 2025)
- **partial sequencing** (Sanger) for genotype determination: gen B646L (p72), gen E183L (p54) and gen B602L (CVR) (Genotype II)

Disease outbreaks monitoring:

- **qPCR** for the **detection** of ASFv (Fernández-Pinero *et al.*, 2013; WOAH, 2025)

- **Unique molecular markers** of ASFv SPAIN 2025 used **to track** disease outbreaks at the **NRL:**
  •SNP in gen E183L (**p54**): Partial sequencing by Sanger (Gallardo et al., 2009) (635 pb)
  •SNP in **MGF505 9R/10R** intergenic region: Partial sequencing by Sanger (Giammaroli et al., 2014) (551pb)
  •**Large deletion** in the terminal left end of the viral genome: qPCR (EURL 2026, unpublished)

Content

5. Origin of virus strain: hypothesis and lab. investigation

Hypothesis of virus origin and probability of occurrence

Facts:

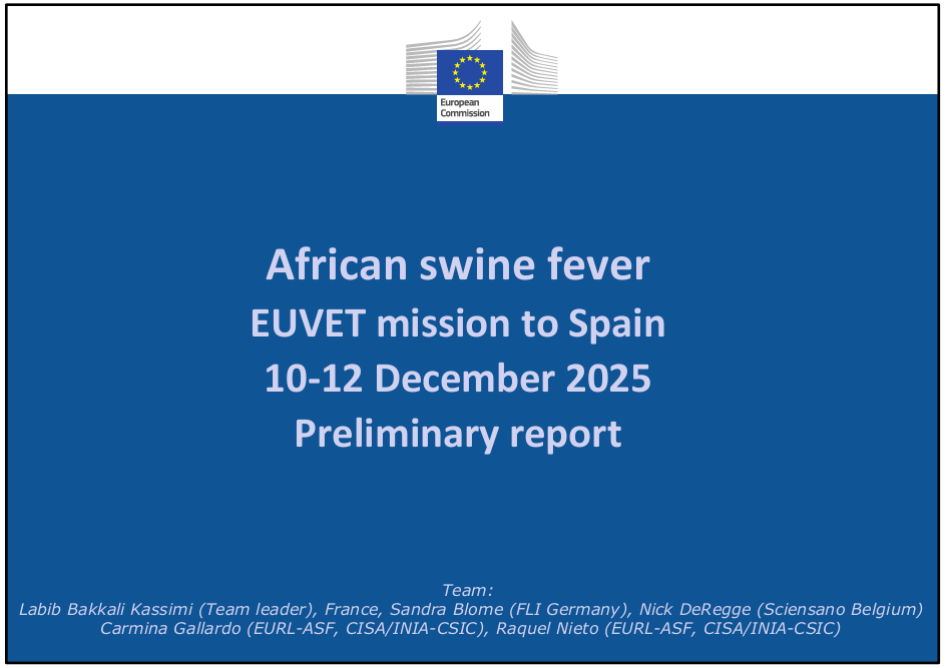
- The **geographically closest active European outbreak is located 500–600 km far** from Catalonia (Piedmont region, Italy)
- **Countries** located between the affected areas and Catalonia maintain **intensive wildlife surveillance** systems.
- The **genetic profile** of the virus detected in Spain did not show a close relationship with the dominant lineages in the European Union outbreaks. The virus has a large structural deletion in the left variable region of the genome, along with a genetic profile not previously described in European outbreaks. From a virological point of view, it is therefore an isolate with uncertain biological behavior, which reduces its predictability in terms of transmission and pathogenicity.
- Although **the 2 first outbreaks** detected were **closed to a ASF research laboratory**, **laboratory tests** results have **not found relationship** between the samples (**81** samples investigated for 3 specific markers, **17** virus strains studied by WGS) held at this facility and the virus responsible for the outbreak in Spain
- Location of the outbreak in a highly connected environment, with **high human mobility** and a dense **road and rail infrastructure network**.

- Hypothesis 1. **Accidental release** from a research laboratory. **Unlikely**
- Hypothesis 2. Introduction from active European sources through **natural or progressive transmission**. **Unlikely**
- Hypothesis 3. **Deliberate introduction**. **Unlikely**

- Hypothesis 4. **Introduction over long distances mediated by human activities** (contaminated meat products or food waste). **Most likely hypothesis:** This route of introduction is the most common mechanism for the long-distance spread of ASFv and is widely documented in the epidemiology of the disease, having been responsible for numerous episodes of transcontinental and long-distance introduction both in Europe and on other continents.

5. Origin of virus strain: hypothesis and lab. investigation

EU Veterinary Emergency Team (EUvet) - laboratories.



PAFF Animal Health and Welfare committee
December 15-16, 2025

Main conclusion: "Based on the data presented, the **EUVET** team **could not identify any obvious route** by which the **virus could have been released from the IRTA-CReSA facilities**".

Main recommendations:

- Perform **sequencing analysis** of samples collected from **outbreaks** to **follow up the virus variation** (ongoing by the NRL and EURL)
  - Analytical approach: Implementation of partial (Sanger) sequencing of virus to identified **relevant mutations (SNPs)** and a real time PCR for detection and screening for the **deletion** observed in the 5' end of the virus genome.
- Perform sequencing analysis (Sanger and or WGS) of **relevant viruses available in IRTA-CReSA** to exclude any possibility of laboratory scape based on a selection of viral isolates and samples from animals experimentally infected with ASF virus in the last year (ongoing by the NRL and EURL)
- Perform sequence comparison of **relevant viruses kept in other laboratories** in Spain to exclude any possibility of laboratory scape (two laboratories under investigation).
- Address the critical points pointed in the facility or protocols by performing an **audit of CReSA** (done with satisfactory results)

5. Take-home messages

1. The **network of laboratories** is appropriately sized and **operates** in a coordinated manner in accordance with **EU (and WOAH) standards**.

2. This network is led by the **national reference laboratory**, which carries out **intensive work in confirming and characterizing outbreaks**.

3. **Coordination** between the NRL and the EU reference laboratory (EURL) has been **key** for tackling disease outbreaks.

4. **Extensive laboratory work** is being conducted to determine the **origin of the virus.**


Gracias
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Ngiyabonga
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Barka
Maraba
Moltes
Dankon
Maketai
Paldies
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Grazzie
Ntyox
Grazias
Bedankt
Thanks
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TashakkurBulgaro
Gunasakulila
Tapaidh
Webale
Rakhmat
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Khrap
Imela
Doh
Gmadlob
Obrigado
maith
Fafetai
Eskerrik
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Alla
Rahmet
Danke
Dêkuji
Ha'evete
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mamexes
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