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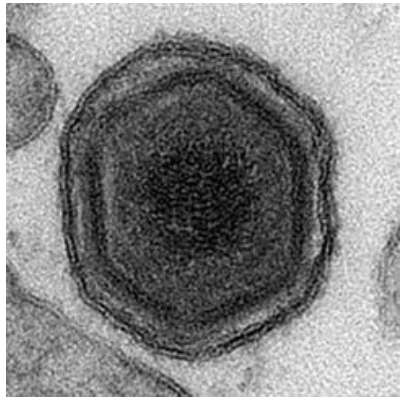
VACCINATION FOR ASF, HOW FAR WE ARE?



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Beijing, 9th April 2019

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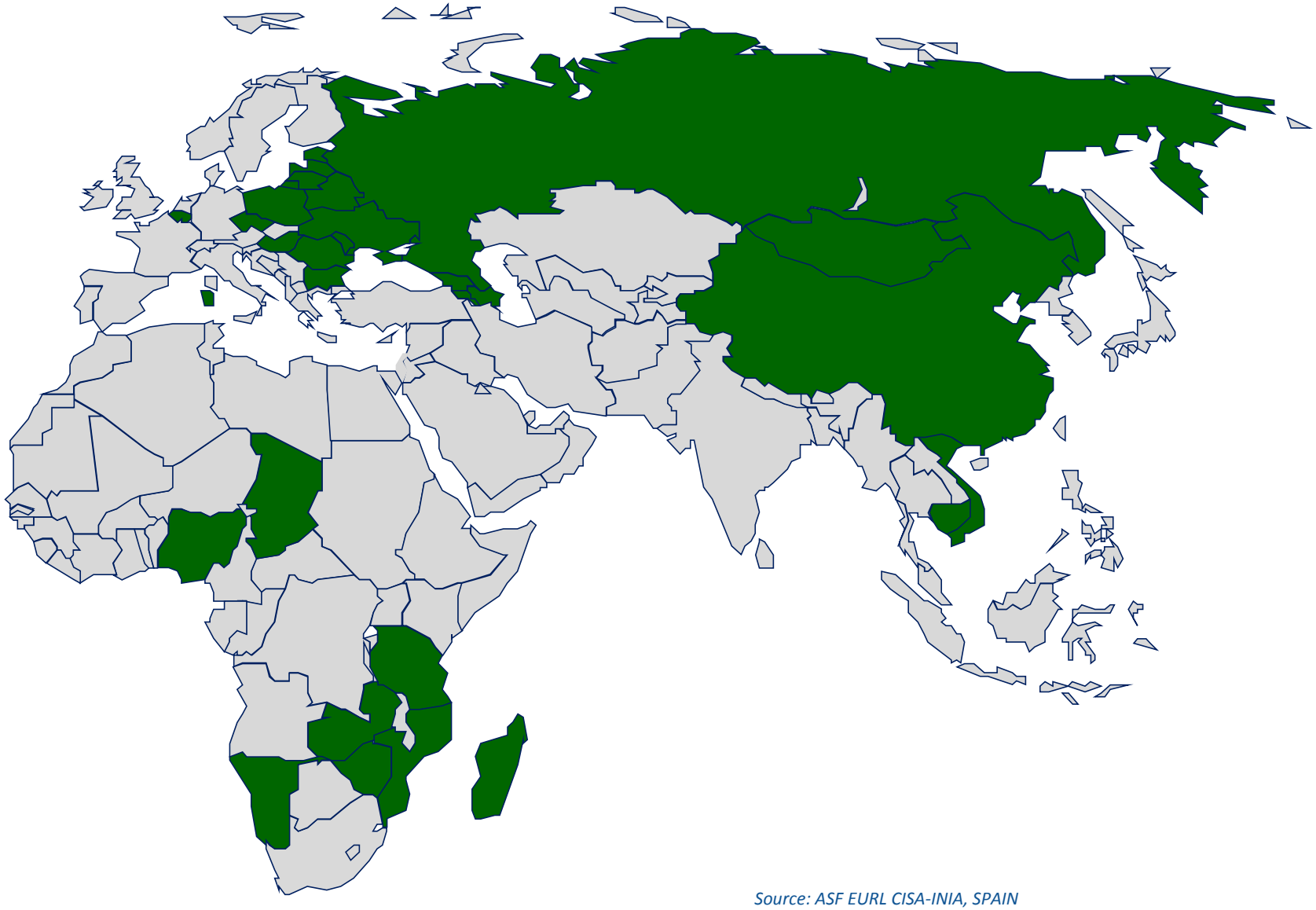
Epidemiological situation of ASF in the world



Source: ASF EURL CISA-INIA, SPAIN

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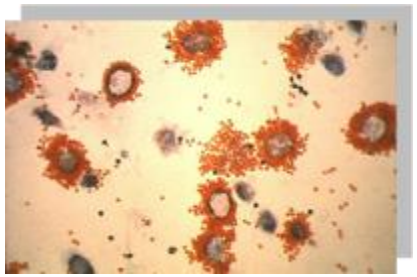
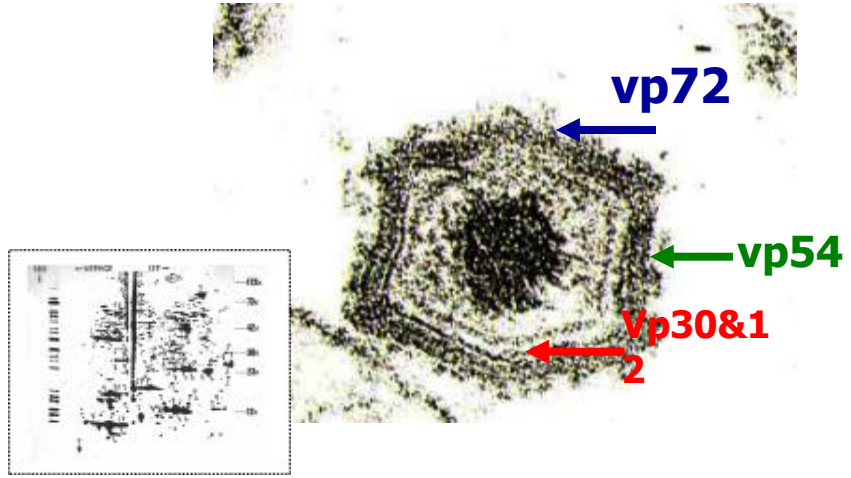
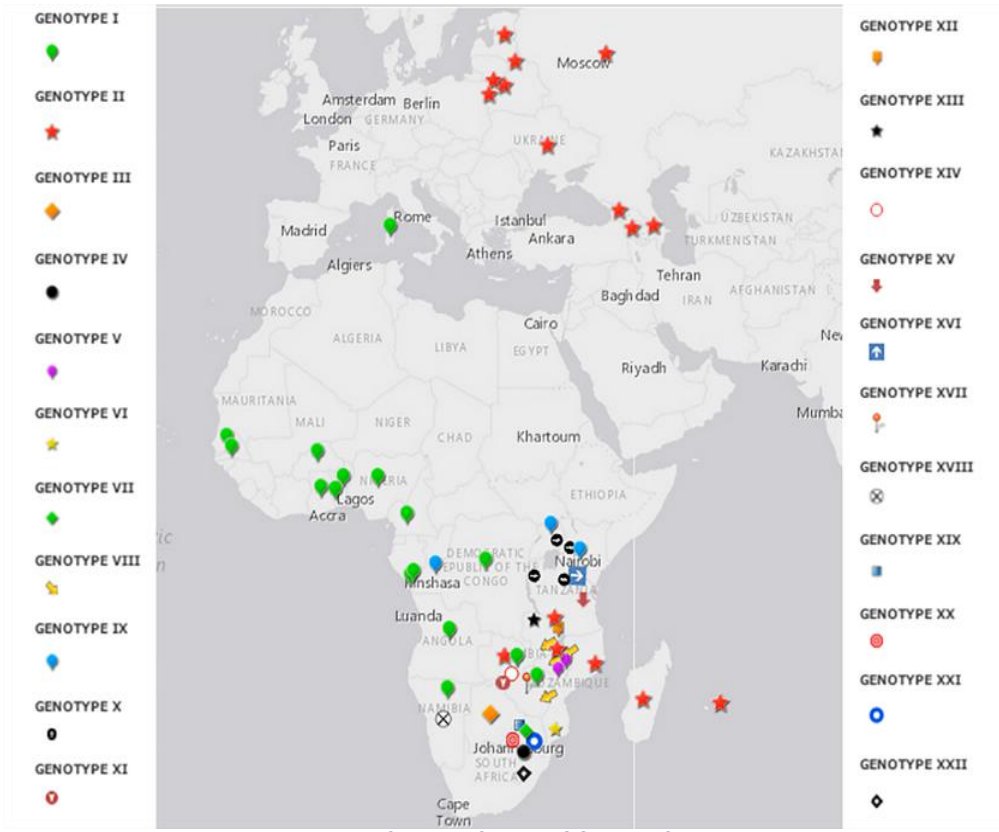
THE VIRUS

- Enveloped virus (the only one of Asfarviridae family);
- Very complex and large virus (big size, 200 nm);
- 54 structural proteins described; with more than 100 infection proteins;
- Main target cells: macrophages and monocytes;
- Doesn't produce fully neutralizing antibodies;
- There are 24 p72 genotypes, and many different strains;
- Protective immunity still poorly characterised



LACK OF VACCINE





Source: ASF EURL CISA-INIA, SPAIN

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THE HOST

DOMESTIC PIGS
SUSCEPTIBLE to ASFV infection

WILD SUIDS:

- **EUROPE: Wildboar: SUSCEPTIBLE.**
- **AFRICA: warthogs, bushpigs, giant forest hogs are infected, RESISTANT to the disease , no clinical signs.**

SOFT TICKS BIOLOGICAL RESERVOIRS

Ornithodorus genus

AFRICA:
O. moubata
Transtadial and Transovarial transmission

EUROPE
O. erraticus
Transtadial Transmission

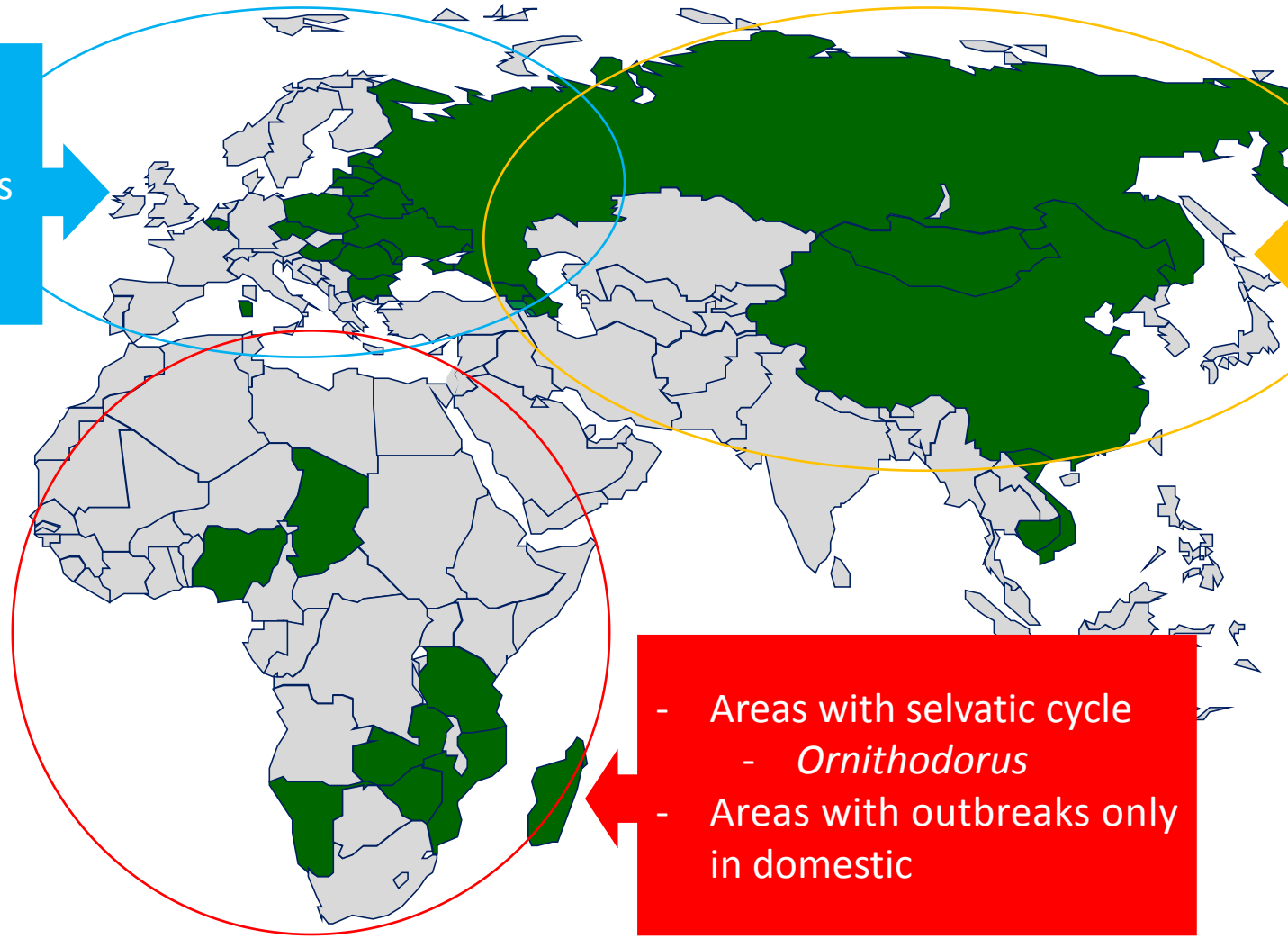
Source: ASF EURL CISA-INIA, SPAIN

COMPLEX EPIDEMIOLOGY

Not always all the 3 Actors are present.
Even present, not always play an active role.

Use of the vaccine: different scenarios

ASF viral circulation maintained in wild boars



ASF infection maintained in domestic pig holdings. Wild boars?

- Areas with selvatic cycle
  - *Ornithodorus*
- Areas with outbreaks only in domestic

Source: ASF EURL CISA-INIA, SPAIN

Vaccine development strategies

Different strategies have been followed in the past and some others are currently under research:

- Inactivated vaccines
- Subunit vaccines
- DNA-based vaccines

→ All attempts have failed to confer full protection against lethal viral challenge

- **Live attenuated vaccines:** most promising approaches are those based on stimulating cytolytic CD8+ T-cell and Antibody response.

Attenuation of virulent virus isolates, with deleted or interrupted genes involved in inhibition of Type I interferon response.

Deletion Mutants from virulent or low virulent isolates: by deletion of genes involved in virulence, cellular transport, etc.

Vaccine development strategies

VACCINE TYPE	PROTECTION	SIDE EFFECTS	REFERENCE
Live attenuated (based on passages in Bone Marrow cells)	Partial / full protection	Yes (pneumonia, arthritis, joints inflamation, fever…)	Petisca, 1965 (Tested in field in Spain and Portugal in the 60's)
Inactivated vaccines (with coadyuvants)	No protection	Not applicable	Stone and Hess, 1967; Bommeli et al., 1981; Mebus, 1988; Blome et al., 2014

- Lack of T cell response.
- More than 50 proteins in several layers.
- Two infectious forms (intracellular mature and extracellular viruses).
- Not effective virus neutralization. Very difficult to achieve.
- Possibility of antibody mediated enhacement of infection.

Vaccine development strategies

Genes / proteins delivered	Type of vaccine	Protection	Reference
p54/E183L, p30/CP204L	Baculovirus expressed proteins	Partial protection (delay of infection)	Gómez-Puertas, et al. Virology 1998
p54/E183L, p30/CP204L, p72/B646L	Baculovirus expressed proteins	No protection	Neilan, et al. Virology 2004
CD2v/pEP402R	Baculovirus expressed proteins	Partial protection (delay of infection)	Ruiz-Gonzalvo et al. Virology, 1996

- ASF encoding up to 167 proteins
- Difficult to select candidate antigens. Most commonly used are those reported as target for for virus neutralization

Vaccine development strategies

Genes / proteins delivered	Type of vaccine	Protection	Reference
p54/E183L, p30/CP204L	Baculovirus expressed proteins	Partial protection	Gómez-Puertas, et al. Virology, 1998
p54/E183L, p30/CP204L, p72/B646L	Baculovirus expressed proteins	No protection	Neilan et al. Virology, 2004
CD2v/pEP402R	Baculovirus expressed proteins	Partial protection	Ruiz-Gonzalvo et al. Virology, 1996
p54/E183L, p30/CP204L	DNA vaccination	No protection	Argilaguet et al. Vaccine, 2011, and PLoSOne 2012
Ubiquitin-CD2v p54/E183L-p30/CP204L	DNA vaccination	Partial protection (delay of infection)	Argilaguet et al, Vaccine 2011
DNA expression library	DNA vaccination	Partial protection (delay of infection)	Lacasta et al., J Virol 2014

Vaccine development strategies (LAVs)

Vaccine type	Protection	Side effects	Reference
Naturally attenuated virus isolates from field	Partial / full protection (against homologous & heterologous)	Yes	Leitao et al., 2001 Boinas et al., 2004 King et al., 2011 Gallardo. et al 2019; Sánchez-Cordón et al, 2016.
Live attenuated deletion mutants (virulent isolates)	Partial / full protection (against homologous & heterologous)	Yes	Rodríguez, et al,2015; O´Donnell et al, 2016; Reis et al. 2016.
Live attenuated deletion mutants (attenuated isolates)	Full (against homologous) / partial (against heterologous)	Yes	Gallardo et al., 2015

Table 1: Promising progress towards the development of a ASFV LAV.

Parental ASFV	Vaccine type	ASFV vaccine	Cell production system	PROTECTION	References
NH/P68 (att)	Naturally attenuated	NHV/P68	PBM	HETEROLOGOUS STRAIN (L60, Arm07)	Leitao *et* al., 2001; Gallardo *et* al., 2012
OURT88/3 (att)	Naturally attenuated	OURT88/3	BM	HOMOLOGOUS/ HETEROLOGOUS STRAIN (OURT88/1, Ug65)	Boinas *et* al., 2004, King *et* al., 2011, Sanchez-Cordon et al 2016)
Georgia07 (vir)	Genetically modified	Georgia07Δ9GL &UK	PAM	HOMOLOGOUS STRAIN	O´Donnel et al 2016
Ba71 (vir)	Genetically modified	Ba71ΔCD2	COS	HOMOLOGOUS AND HETEROLOGOUS STRAIN (E75, Georgia07)	Patent. Fernando Rodriguez and Maria Luisa Salas WO 2015091322 A1
Benin (vir)	Genetically modified	BeninΔMGF	BM	HOMOLOGOUS STRAIN	Reis et al., 2016
Benin (vir)	Genetically modified	BeninΔDP148R	BM	HOMOLOGOUS STRAIN	Reis et al 2016
NH/P68 (att)	Genetically modified	NHV/P68 TETβGUS,	COS + 4 passages in PAM	HOMOLOGOUS AND HETEROLOGOUS STRAIN (Arm07)	Revilla Y. unpublished data
NH/P68 (att)	Genetically modified	NH/P68ΔA238L	COS + 4 passages in PAM	HOMOLOGOUS AND HETEROLOGOUS STRAIN (Arm07)	Gallardo et al 2015

Att = attenuated, Vir = virulent

Cell systems: Porcine blood monocyte/ macrophages (PBM), pig bone marrow cells (BM), monkey kidney tissue derived cells (COS) or porcine alveolar macrophages (PAM)

Basic conditions of the vaccine

- **Safety** → without side effects; not infecting other animals.
  - Some LAVs based on naturally attenuated strains of ASFV have some side effects (vaccine tested in Portugal and Spain, 1962-1964), and animals develop ASF chronic form.
  - In LAVs based on virulent ASF viruses containing engineered deletions, animals may develop undetected subclinical infection with later possible recombination with the natural strain.
- **Efficacy** → immunity to different strains within the same or different genotypes.
- **Necessary equilibrium between SAFETY and EFFICACY**: Too much attenuation in LAVs could lead to nonpathogenic viruses that are non-efficient for vaccine purposes but too low attenuation would result in avoiding its use in the field for safety reasons.
- Convenience of being a **DIVA** vaccine: based on negative markers (with deletion of targeted virulent factors).
- **Commercial production:** cell lines instead of primary cell cultures
- Wild boars use: **Stability** in the external environment to avoid losing potency when it is exposed to low and hot temperatures, sunshine, etc., and in oral administration route (baits).

Use in wild life

- Previous experience in CSF, rabies…
- Some important questions regarding its use in wild boars:
  - **Necessary previous testing on wild boar populations** → risk of spreading ASF virus in the environment.
  - **What dose**?
    - Number: One, two or more doses (onset of immunity)
    - Titer
    - Revaccination (duration of immunity) and overdose (safety tests)
  - **Effect on specific age-group**?
  - **Routes of administration**? → Possible use of baits to administrate orally the vaccine (safer use; but possible ingestion by other species)

SOME IMPORTANT GAPS FOR ASF VACCINE DEVELOPMENT

VIRUS PATHOGEN

- Role of multigene families in antigenic variability and evasion of immune response
- Identify genes related to host protection
- Understanding the evolution of circulating viruses (endemic regions).

IMMUNE RESPONSE

- Role of viral proteins in inducing effective immune mechanisms in surviving animals
- Mechanisms of viral persistence in the host
- Interactions between ASFV, macrophages and other cells in host.
- Knowledge on mechanisms to evade immune response, induce protection and pathogenicity

IMPORTANT GAPS FOR ASF VACCINE DEVELOPMENT

VACCINE DEVELOPMENT

- Studies on existing promising live attenuated vaccine candidates: further investigation on side effects, doses and other parameters of safety.
- Selection of targeted virulence genes to be deleted.
- Cell lines for replacing primary cell cultures (five potential: ZMAC, IPAM WT, IPAM –CD163, WSL, CA2+, COS).
- Research on vaccine candidates: new types and strategies.
- DIVA test accompanying vaccines.

Main conclusions

- Vaccination would help to re-inforce control and eradication strategies of ASF.
- Use in different scenarios according to their particular situation.
- Complexity of the virus and its epidemiology → difficulties to develop the vaccine.
- Still more time to get the vaccine, although a big effort in its research is being made.
- LAVs seem to be the most promising, but there are some gaps that might constrain its development in the mid-term.

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THANK YOU FOR YOUR ATTENTION

Blueprint and Roadmap on the possible development of a vaccine for ASF prepared by the ASF-EURL on Commission request

https://ec.europa.eu/food/sites/food/files/safety/docs/cff_animal_vet-progs_asf_blue-print-road-map.pdf