Indagini di laboratorio in caso di sospetto /focolaio PSA

Alessandra De Felice
Osservatorio Regionale Sanità Animale- OREB
IZSM

Campioni idonei per la diagnosi di peste suina

- Sangue + EDTA (almeno 10 ml)
- Sangue per siero (almeno 5 ml)
- Tonsilla
- Linfonodi regione testa - collo
- Milza
- Rene
- Ileo
- Polmone
- Linfonodo gastro-epatico
- Linfonodi meseraici
- Midollo osseo (in caso di carcasse in avanzato stato di decomposizione)

Quali animali campionare?

Il tipo di animali da campionare dipende dall'obiettivo del campionamento

SORVEGLIANZA → ANIMALI MORTI /AMMALATI

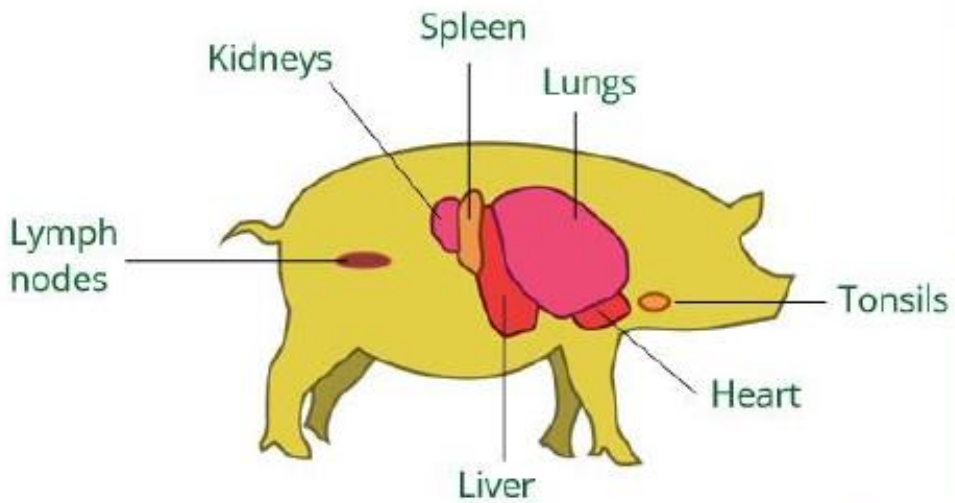
IN CASO DI FOCOLAIO → ANIMALI MORTI/AMMALATI

ANIMALI A CONTATTO

Sampling to detect ASF virus

Blood and **well perfused organs** are ideal to detect ASFV since they are likely to contain high loads of virus and/or viral DNA in infected animals. However, any organs or tissues can be used to check for the presence of ASFV (mainly in the acute and subacute forms of the disease).

The target organs for sampling are:



Of these, **spleen and lymph nodes are the most important** as they usually contain the highest amounts of virus. Bone marrow is an important sample to collect from decomposed carcasses.

Other bodily fluids, such as faeces, saliva or urine contain only low loads of virus. Therefore, chewing ropes to collect saliva have limited usefulness as a surveillance tool.

For further details, please explore table one of: Transmission routes of ASF to domestic pigs: current knowledge and future research directions, Guinat *et al.*, 2016.

Priority samples to collect: a summary

Whole blood EDTA

- Should be drawn from the jugular vein or inferior vena cava into an EDTA (purple top) vacutainer.
- For ASFV genome detection by PCR.

✗ **Do not** use heparinised blood! This will inhibit the PCR.

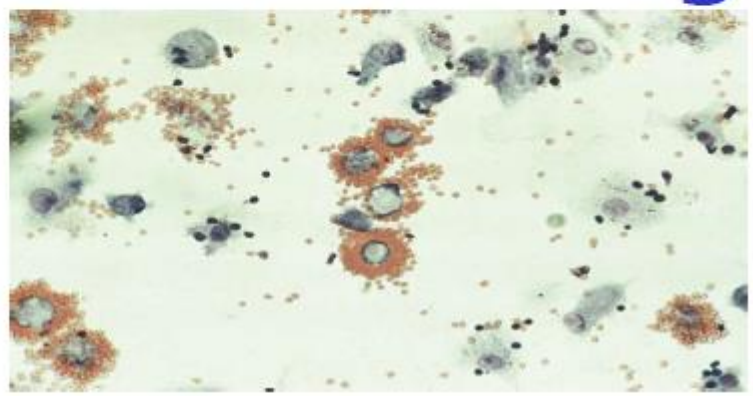
Serum

- Plain tube (red top vacutainer).
- Suitable for all diagnostic assays (and thus preferable when only one sample type is taken).
- Ideal for antibody detection.

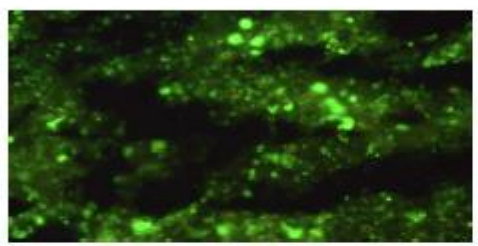
Organ/tissue

- Spleen, lung or lymph nodes most suitable for PCR and virus isolation.
- Bone marrow for decomposed carcasses.
- Small parts of organs can be mixed and put into a 2 ml cryovial.

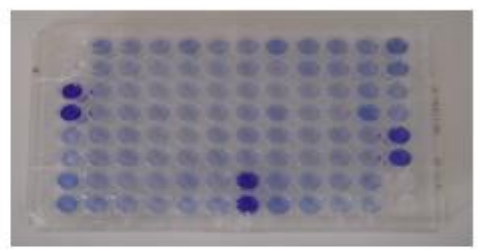
PSA diagnosi diretta



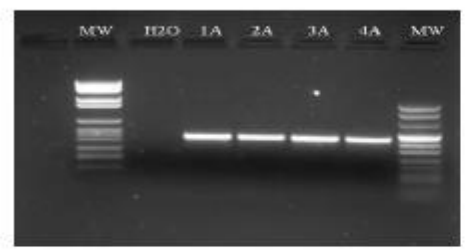
Malmquist, 1960

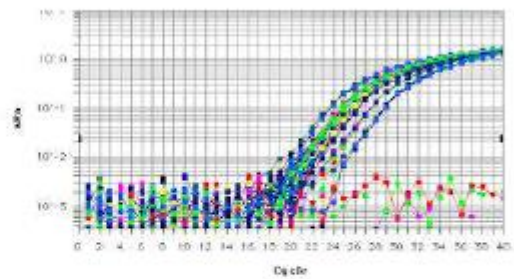


IFD



ELISA-Ag





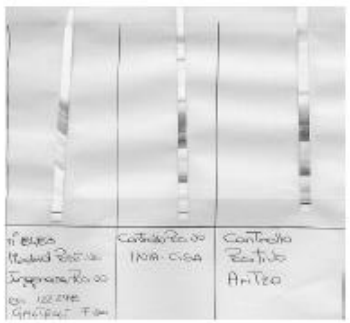
PCR & Real Time PCR

PSA diagnosi indiretta

Screening

ELISA-Ab

Pen side test (Ag&Ab)



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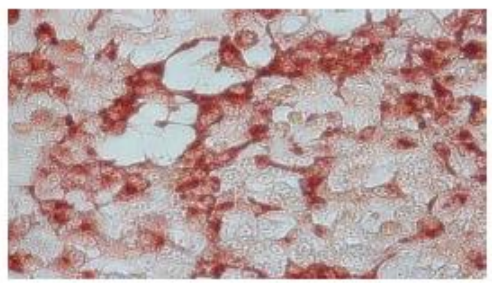
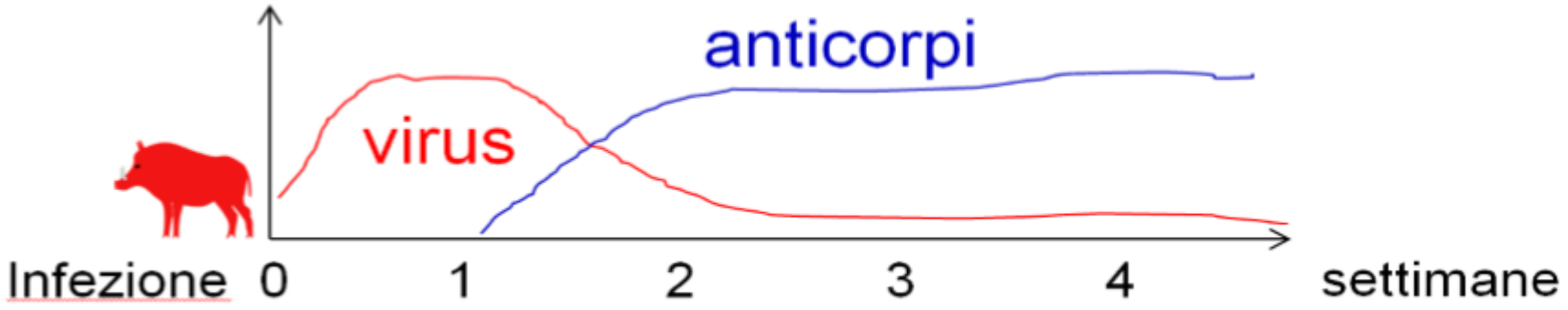


IPT

Conferma

Quali test sono disponibili per la diagnosi di conferma?

TEST	Ricerca di	MATRICE
TEST SIEROLOGICO	ANTICORPI	SANGUE
TEST VIROLOGICO	VIRUS	SANGUE/ORGANI (MILZA, RENI, LINFONODI)



Situazione di attesa:

test virologici

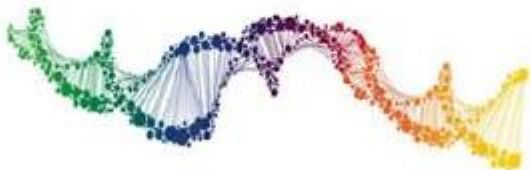
Obiettivo: cercare il primo caso

Situazione di infezione: test virologici e sierologici

Obiettivo: valutare la situazione epidemiologica

Polymerase chain reaction (PCR)

PCR is the tool of choice in the case of peracute, acute, or subacute ASF infections. It is used to detect the ASFV genome in samples (blood, organs, etc.) and ticks. Furthermore, since PCR detects the viral genome, it may be positive even when no infectious virus is detected by virus isolation.



- Small fragments of viral DNA are amplified by PCR to detect
- Tests may be conducted by conventional or real time PCR.
- Commercial systems are on the market, with validated meth

Click to read more:

+ Advantages of PCR

- Disadvantages of PCR

X

- Trained staff required.
- Prone to contamination (false-positive reaction).

PCR

VANTAGGI:

- PICCOLI FRAMMENTI DI DNA POSSONO ESSERE AMPLIFICATI
- PCR TRADIZIONALE O REAL TIME PCR
- METODI VALIDATI

SVANTAGGI:

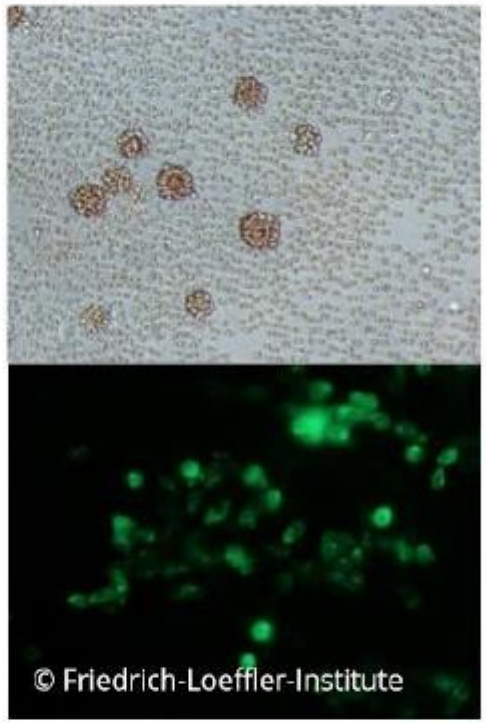
- PERSONALE QUALIFICATO
- POSSIBILI CONTAMINAZIONI (FALSI POSITIVI)

Virus isolation

Virus isolation is based on the inoculation of sample material onto susceptible primary cell cultures of porcine origin, monocytes, and macrophages.

If the ASFV is present in the sample, it will replicate in the susceptible cells, producing cytopathic effect (CPE) in the infected cells. Cell lysis and CPE usually occur after 48-72 hours of haemadsorption.

The importance of this finding relies on its specificity because none of the other pig viruses are capable of haemadsorbing in leukocyte cultures. When the virus replicates in these cultures, most of the ASFV strains produce the haemadsorption reaction (HAD) due to adsorption of pig red blood cells on ASFV-infected leukocytes forming crown-like "rosettes".



Click to read more:

+ Advantages of virus isolation

− Disadvantages of virus isolation

ISOLAMENTO VIRALE

VANTAGGI:

Test di conferma successivo a PCR, elisa e test anticorpi fluorescenti

Fornisce informazioni sullo STATUS DELL'ANIMALE: ANCORA INFETTO /INFETTIVO

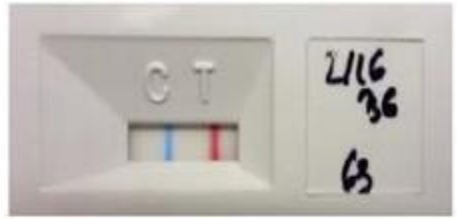
SVANTAGGI:

laboratorio Classe 3+
Personale specializzato

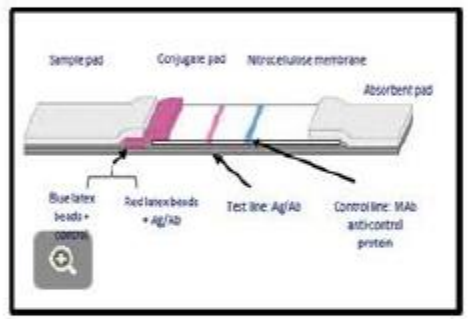
Tempi di risposta: 7-21gg

Lateral flow devices

The lateral flow device (also known as penside test) is used like a pregnancy test. The sample is prepared with a buffer and then added to the test window. Viral antigen (VP 72) is detected by use of monoclonal antibody (MAb) forming a latex-antibody-antigen immune complex. Results are interpreted 10 minutes after adding the sample.



The blue control line must always appear; otherwise, the test has to be considered invalid. If only one line appears, this is the control line and the sample is negative. If two lines appear, the sample is positive.



Click to read more:

Advantages of LFD

Disadvantages of LFD

TEST RAPIDO

VANTAGGI:
RISULTATO DISPONIBILE IN 10 MINUTI

NON RICHIEDE PERSONALE SPECIALIZZATO

NON RICHIEDE STRUMENTAZIONI

SVANTAGGI:

campioni liquidi: SIERO O SANGUE

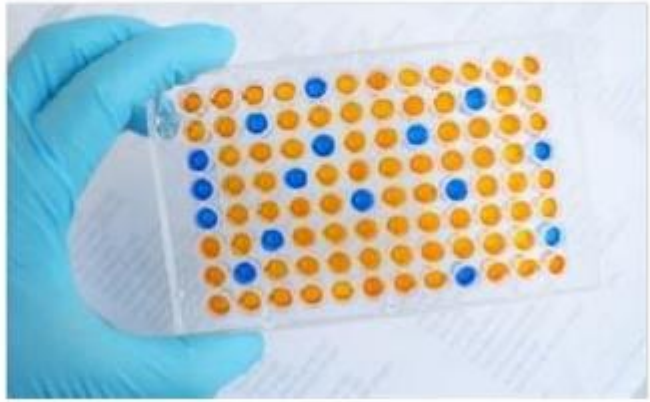
BASSA SENSIBILITA’
FALSI NEGATIVI

NON VALIDATO COME TEST DI CONFERMA, DEVE ESSERE CONFERMATO dalla PCR

Antibody ELISA

Enzyme Linked Immunosorbent Assay (ELISA) tests for antibodies are widely used for large-scale serological studies of many animal diseases.

A number of commercial ELISA tests for ASF antibodies are available. Blocking ELISA can be used to detect antibodies in serum samples while indirect ELISA can be used to detect antibodies in serum or meat juice samples.



Click to read more:

+ Advantages of antibody ELISA

− Disadvantages of antibody ELISA

ELISA

ALTA SENSIBILITA’ E SPECIFICITA’

VELOCE

COSTI BASSI

SEMPLICE INTERPRETAZIONE DEI

RISULTATI

CAMPIONI EMOLIZZATI POSSONO DARE FINO AL 20% DI FALSI POSITIVI

TUTTI I POSITIVI E I DUBBI DEVONO ESSERE CONFERMATI DA ALTRI TEST SIEROLOGICI (Es. IMMUNOBLOTTING, TEST PEROSSIDASI INDIRETTA)

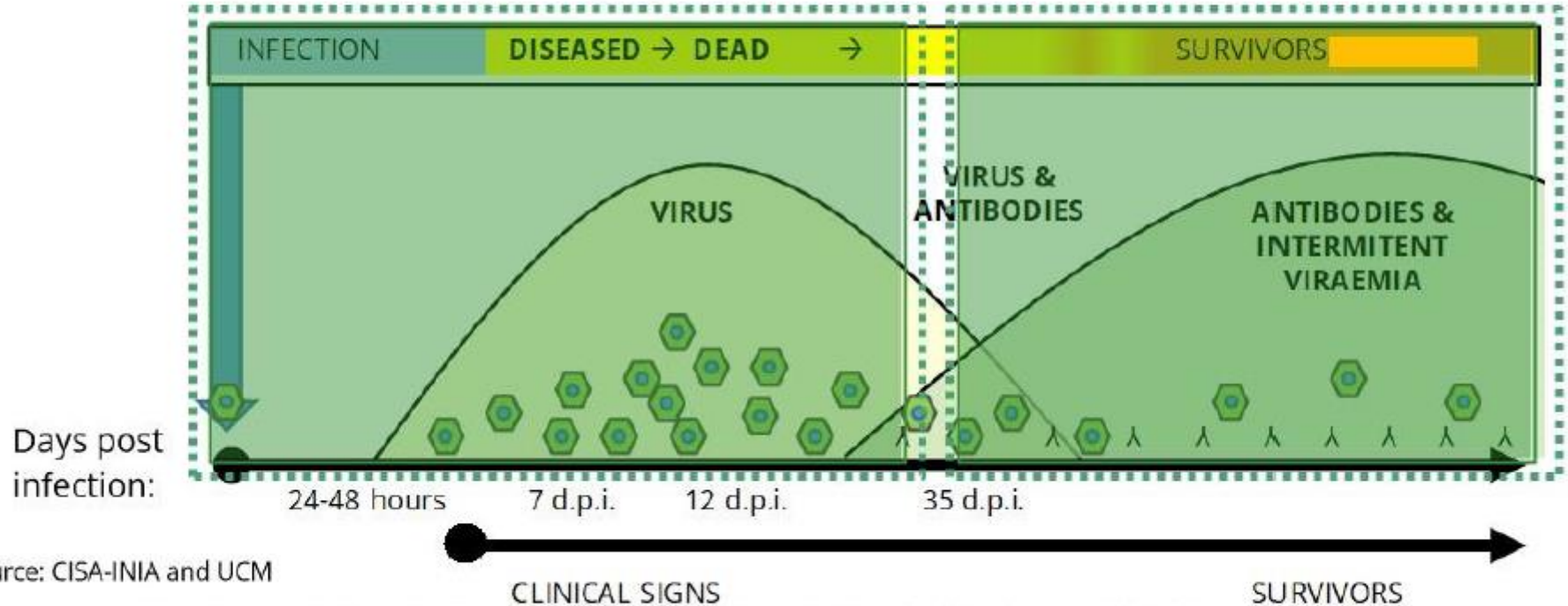
Self-test question

Here is the solution:

PCR		Virus isolation	Lateral flow device
The most sensitive of these tests	Prone to contamination resulting in false positives	Detects live virus	Not validated as a confirmatory test
Can detect viral genome in the absence of live virus		Results available in approximately seven days	Low sensitivity: false negatives are possible
Results available in five to six hours		Considered the "gold standard" confirmatory test	Results available in ten minutes

What do the results tell us?

The results of diagnostic tests should be interpreted on the basis of our understanding of the pathogenesis of ASF, as illustrated in the diagram below. *Click on each section to find out more;*



Source: CISA-INIA and UCM

Virus and antibody circulation in blood over time and in relation to the stage of ASF virus infection, as observed in European domestic pigs in the Iberian Peninsula and the Western Hemisphere (1960-1995).

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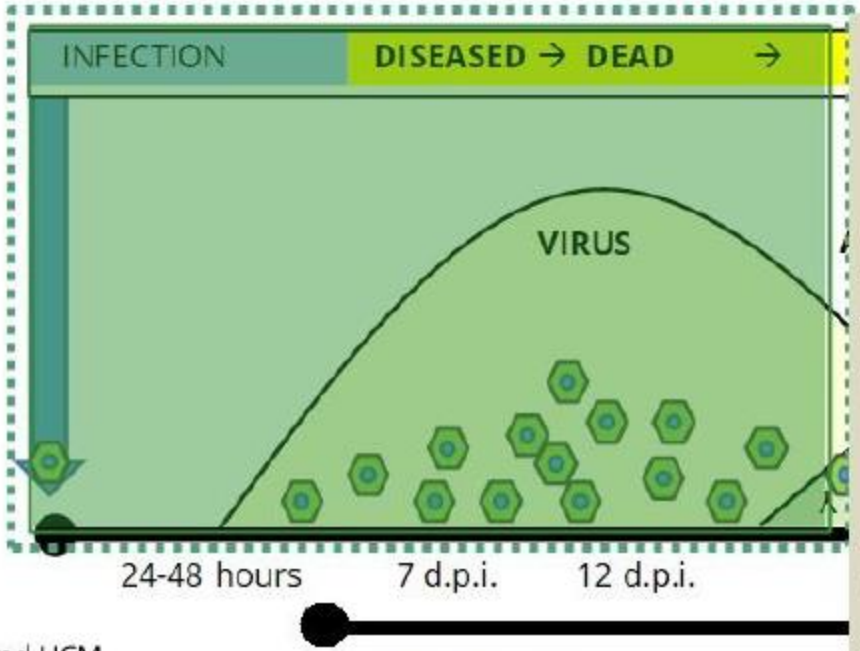

Source: CISA-INIA and UCM

Virus and antibody circulation in blood over time and in relation to [illegible] observed in European domestic pigs in the Iberian Peninsula and the [illegible]

X

Approximately two days before clinical signs develop, ASF-infected animals begin to shed large amounts of the virus; therefore animals which are clinically healthy may test positive for ASF virus.

A positive test for the presence of the virus (i.e. antigen) indicates that the tested animal was undergoing infection at the time of sampling.

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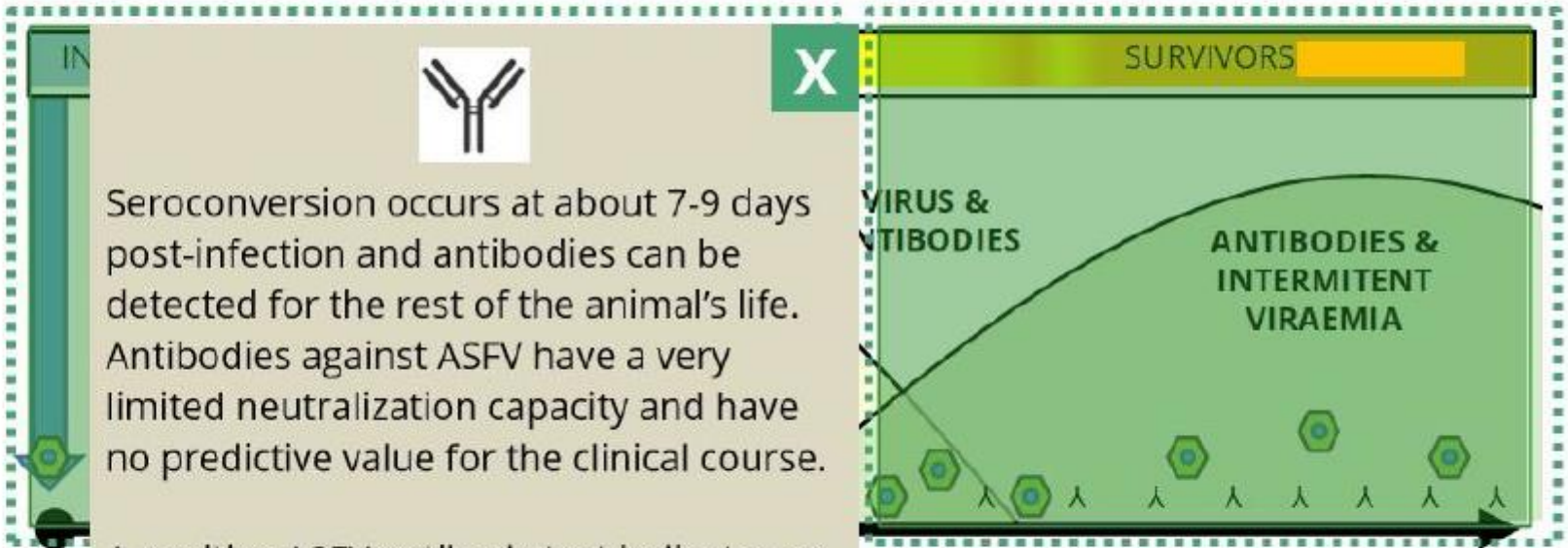

Seroconversion occurs at about 7-9 days post-infection and antibodies can be detected for the rest of the animal's life. Antibodies against ASFV have a very limited neutralization capacity and have no predictive value for the clinical course.

A positive ASFV antibody test indicates an ongoing or past infection, where the animals have recovered (and may remain seropositive for life).

Source: CISA-INIA and UCM

Virus and antibody c… the stage of ASF virus infection, as observed in Europea… e Western Hemisphere (1960-1995).

UNA CORRETTA INTERPRETAZIONE DEI RISULTATI AIUTA A STIMARE IL PERIODO DELL'INFEZIONE

Interpretation of results

Results of laboratory tests can give an indication how long the virus has been circulating in a given population.

Can you drag the stages of infection to the appropriate place in the table below?

PCR	Virus isolation	Antibody	Disease progression
+	+	-	FASE IPERACUTA < 10dpi
+	+	+	FASE ACUTA >10 dpi
+	-	+	STATO TARDIVO/ANIMALI CHE SOPRAVVIVONO >dpi
-	-	+	FASE SUBACUTA, ANIMALI SOPRAVVISSUTI

Peracute and early acute phase < 10 dpi

Subacute phase, survived animal

Survived animal > 30 dpi

Acute phase >10 dpi

Submit

Interpretation of results

In early stage of ASF infection, PCR and virus isolation (VI) can be positive, but antibody tests stay negative. This means, the infection is less than 10 days old.
Antibodies begin to be produced from 7 days post infection, but at this stage may only be detected by indirect immunoperoxidase test (IPT). Antibodies may be detected by ELISA from 12 days post infection.

If PCR is positive, but virus isolation negative and antibodies detected, that means that the animal is in a late stage or a surviving animal. In the blood, residues (genetic material) of the virus can be found, hence the PCR is positive but the virus is not infectious anymore, therefore the virus isolation is negative. A PCR-negative, VI-negative and antibody-positive result indicates that the animal has survived ASF.

Click the table to enlarge:

PCR	Virus Isolation	Antibody	Disease progression
+	+	-	Peracute and early acute phase < 10 dpi
+	+	+	Acute phase >10 dpi
+	-	+	Survived animal > 30 dpi
-	-	+	Subacute phase, survived animal

References

Beltrán-Alcrudo, D., Arias, M., Gallardo, C., Kramer, S. & Penrith, M.L. 2017. ***African swine fever: detection and diagnosis – A manual for veterinarians***. FAO Animal Production and Health Manual No. 19. Rome. Food and Agriculture Organization of the United Nations (FAO).

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Guinat, C., Gogin, A., Blome, S., Keil, G., Pollin, R., Pfeiffer, D. U., & Dixon, L. (2016). ***Transmission routes of African swine fever virus to domestic pigs: current knowledge and future research directions.*** The Veterinary record, 178(11), 262–267. https://doi.org/10.1136/vr.103593

Grazie per l'attenzione!

alessandra.defelice@izsmportici.it