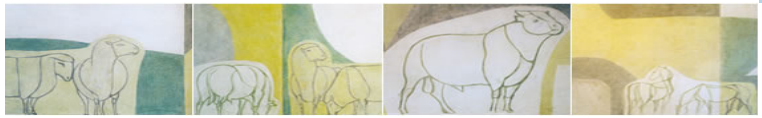
EQUINE INFECTIOUS ANAEMIA:
LESSONS LEARNED FROM THE SIX-YEAR APPLICATION OF THE NATIONAL ITALIAN SURVEILLANCE
Rome 1st October 2012

DYNAMICS OF EQUINE INFECTIOUS ANEMIA VIRUS INFECTION IN NATURALLY INFECTED MULES

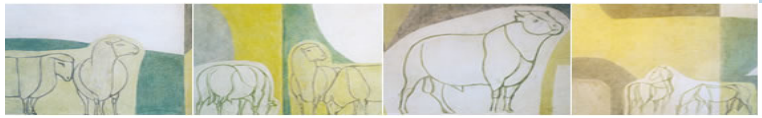
Study conducted within Research Project IZSLT 07/08 RC, approved and funded by the Italian Ministry of Health

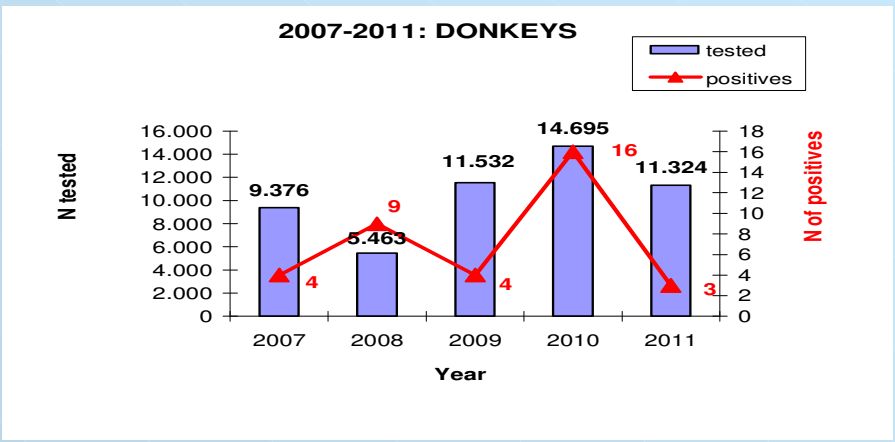


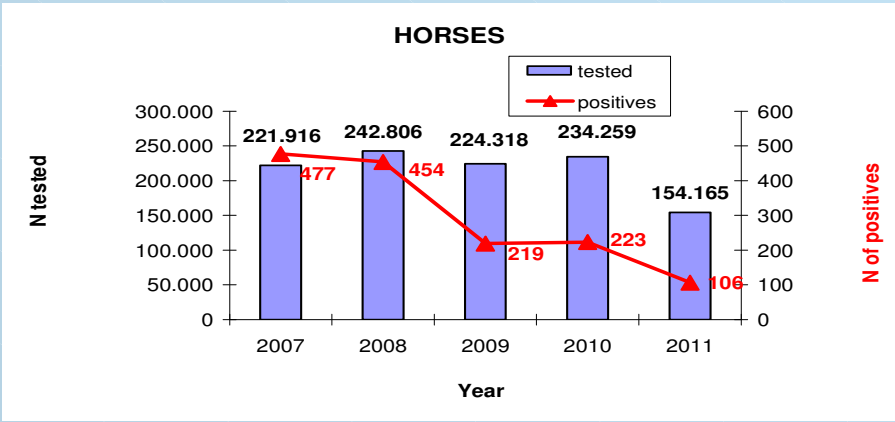
Distribution of EIA cases among the species examined during the surveillance period 2007-2011

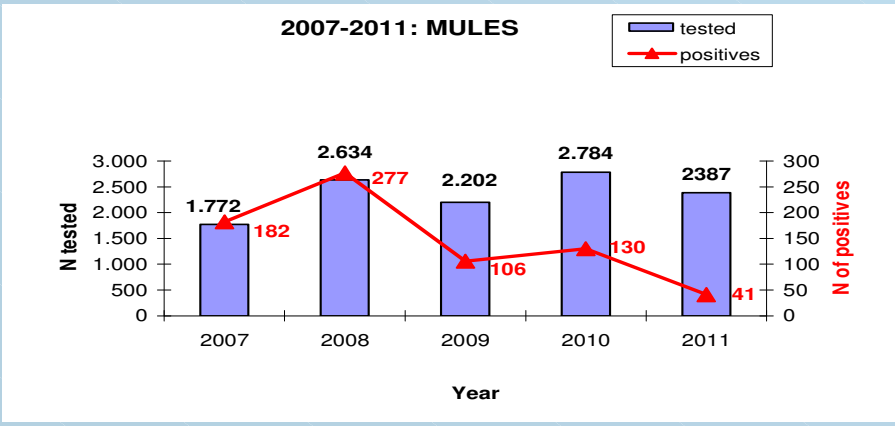
http://www.izslt.it/izslt/

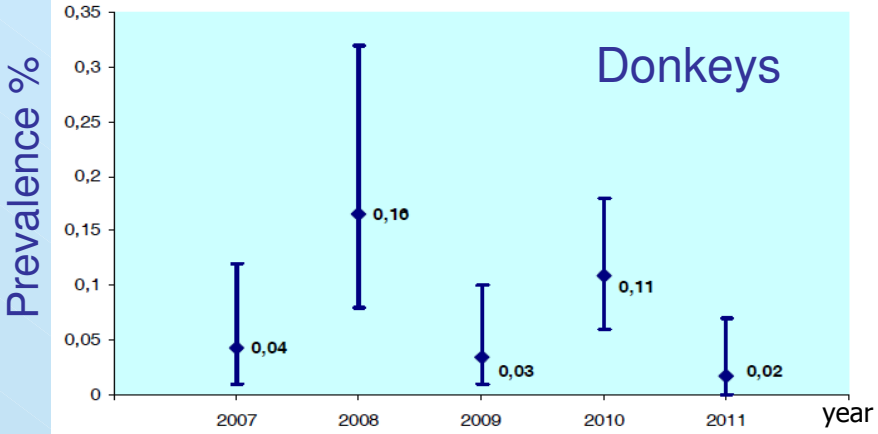
2007-2011 EIA surveillance activity report of the Italian National Reference Centre for Equine Diseases & Equine Infectious Anaemia

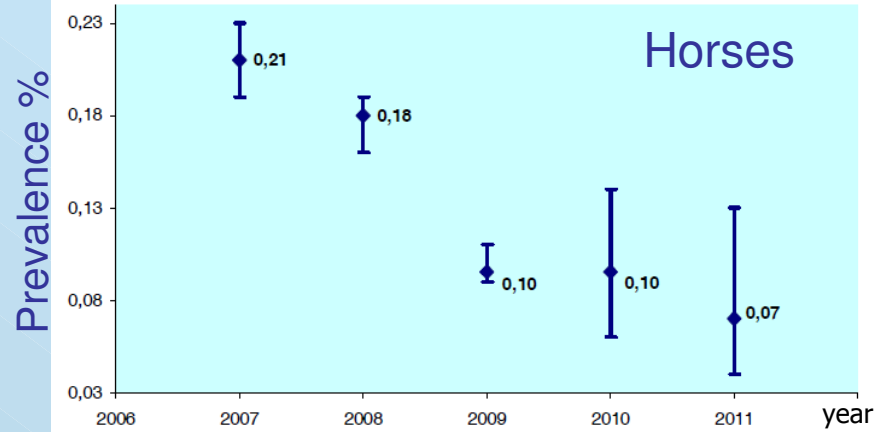


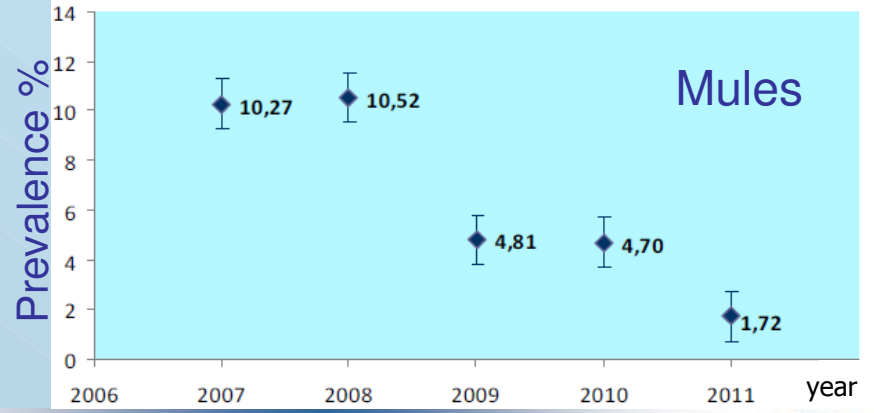

2007-2011: DONKEYS
tested
positives
N tested
N of positives
16.000
14.000
12.000
10.000
8.000
6.000
4.000
2.000
0
18
16
14
12
10
8
6
4
2
0
9.376
5.463
11.532
14.695
11.324
4
9
4
16
3
2007
2008
2009
2010
2011
Year

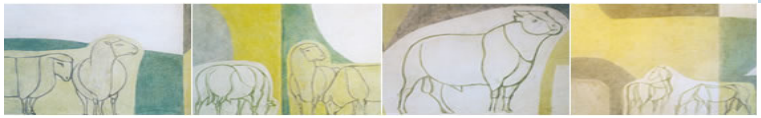

HORSES
tested
positives
N tested
N of positives
300.000
250.000
200.000
150.000
100.000
50.000
0
600
500
400
300
200
100
0
221.916
242.806
224.318
234.259
154.165
477
454
219
223
106
2007
2008
2009
2010
2011
Year


2007-2011: MULES
tested
positives
N tested
N of positives
3.000
2.500
2.000
1.500
1.000
500
0
300
250
200
150
100
50
0
1.772
2.634
2.202
2.784
2387
182
277
106
130
41
2007
2008
2009
2010
2011
Year


Prevalence %
Donkeys
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
0,04
0,16
0,03
0,11
0,02
2007
2008
2009
2010
2011
year


Prevalence %
Horses
0,23
0,18
0,13
0,08
0,03
0,21
0,18
0,10
0,10
0,07
2006
2007
2008
2009
2010
2011
year

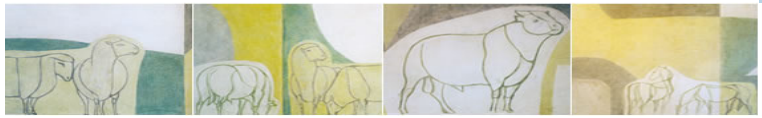

Prevalence %
Mules
14
12
10
8
6
4
2
0
10,27
10,52
4,81
4,70
1,72
2006
2007
2008
2009
2010
2011
year



How to interpret the infection status of apparent false negative AGIDT reactions – predictive value of Elisa/IB positive reactions?

	Number	Rate
Samples tested in survey	96,468	
Positive in IT C-ELISA	331	0.34%
Positive IT C-ELISA and AGIDT	124	0.13%
Positive IT C-ELISA and Negative AGIDT	207	
Negative Immunoblot	182	88%
Positive Immunoblot	25	12%
Overall number judged positive for EIA	124+25 = 149	0.15%
Total Number of samples tested in survey	96,468	
Apparent False-Positive IT C-ELISA	182	0.19%
Apparent False-Negative AGIDT	25	0.026%

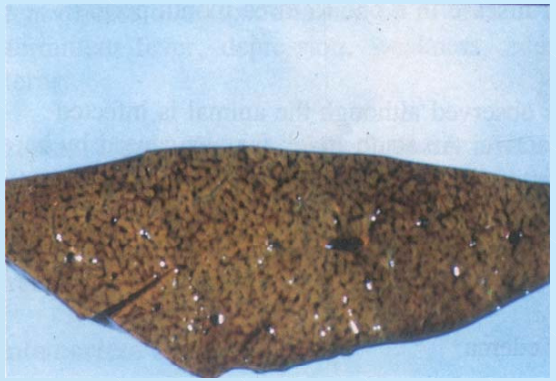
13/25 of the apparent false negative AGIDT were mules with 11/13 coming from prevalent outbreaks



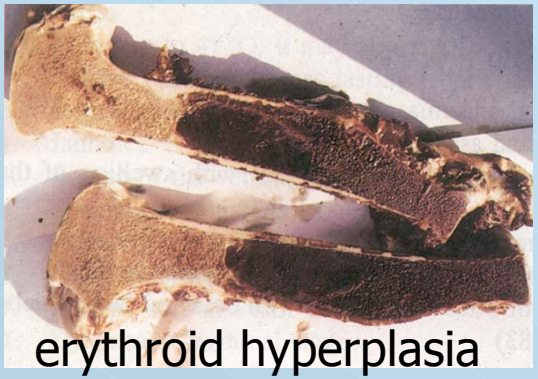
Clinical signs of AIE reported especially in horses

http://www.fao.org/docrep/003/t0756e/T0756E07.htm

The disease most frequently takes on a subclinical/inapparent course but can also be acute & chronic in horses infected with the same viral strain (*Hammond et. al. 2000, Leroux et. al. 2001, Cook et. al. 2001*).



Enlarged grey red liver showing lobular pattern and haemorrhage under the capsule.

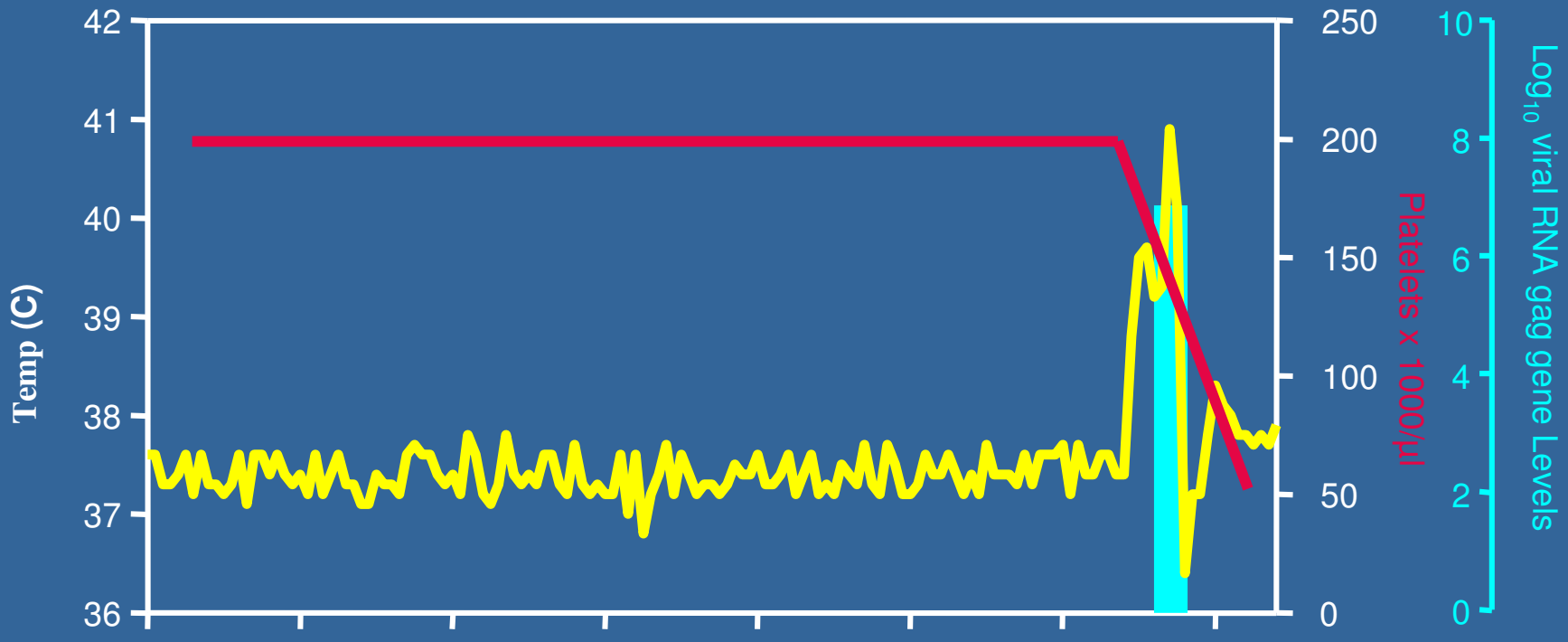


erythroid hyperplasia

Recurrences can be stress-related and may be provoked by malnutrition, overwork or surgery.

Chronic cases are characterised by intermittent cycles of:
fever anaemia, oedema, weight loss and lethargy alternated by periods of normality.

Eg. of principal parameters evaluated during EIAV (experimental) infections



Limited studies available in literature regarding the evolution of AIE infection in mules

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Microbiology 95 (2003) 49–59
veterinary microbiology
www.elsevier.com/locate/vetmic

Equine infectious anemia in mules: virus isolation and pathogenicity studies

V. Spyrou [a], M. Papanastassopoulou [a], V. Psychas [b], Ch. Billinis [a], M. Koumbati [a], J. Vlemmas [b], G. Koptopoulos [a,*]

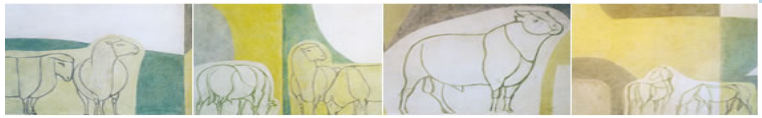
Objective of the study

Determine the **virological status & epidemiological significance** of mules with an **EIA equivocal serological result**

Experimental Identification N°	Province of Origin	Age	Sex
1	Rieti	12	F
2	Latina	2	F
3	Aquila	30	F
4	Roma	22	F
5	Roma	9	F
6	Roma	8	F
7	Frosinone	11	CM
8	Aquila	17	F
9	Frosinone	11	F
10	Frosinone	7	F

Longitudinal study on
10 naturally infected mules
from 5 different outbreaks, including
5 subjects with equivocal Elisa/AGIDT serological patterns

Details of study group & experimental set-up



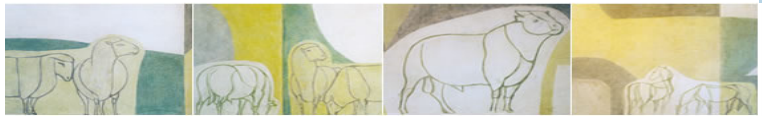
Details of study group & experimental set-up

Clinical anamnesis referred by owners was that some of the animals had performance loss and weakness with no other apparent clinical.

The mules were purchased only after the owners had consented their slaughter.

The premises where the study carried out was authorised by the Regional & Local Veterinary Services.

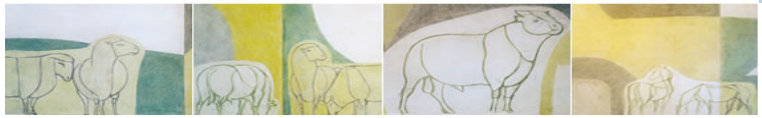
During the study, the premesis were declared as an outbreak for AIE & the biosecurity measures prescribed in the AIE Regulation were applied for the whole observation period.



Confirmation of EIAV infection on pre-enrolment samples by:

-all mules had positive PCR products which when sequenced were homologous with circulating strains of AIEV, *Cappelli et al,* 2011.

-all mules were confirmed as positive in immunoblot (IB), *Issel et. al,* 1999.



Experimental set-up

Observation period

Approx. four months.

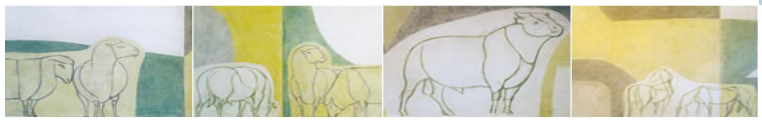
Clinical evaluation

Monitored daily - general examination of each animal.

Sample collection

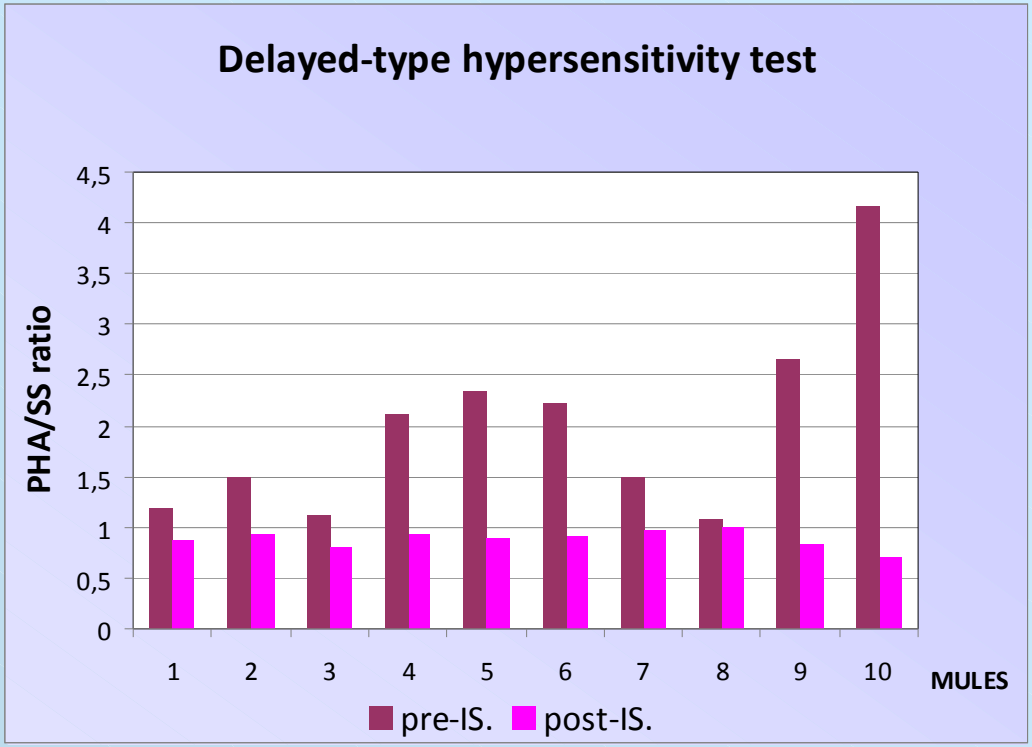
Blood with & without anti-coagulant were collected on daily basis.

Immunesuppression (IS) & verification - dexamethasone administration, halfway through observation period (*Craigo et. al, 2007*), doses within accepted therapeutic levels.



Verification of IS

Immune status was monitored pre and post IS through a **delayed-type hypersensitivity assay** (DTH) induced by PHA.



DTH ratios were calculated as the ratio of antigen (PHA) reaction to control (saline) reaction - the control reaction (saline alone) was divided into the PHA reaction to yield the DTH ratio (Y axis).

Laboratory parameters investigated & diagnostic methods employed

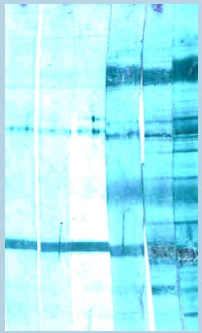
Platelet count (log_{10}/µL) was determined using an automated counter Cell-Dyn 3700 (ABBOTT)

Qualitative and quantitative humoral responses were investigated using the three tier system;

in-house C-ELISA – (*Amaddeo et. al, - 1998*)

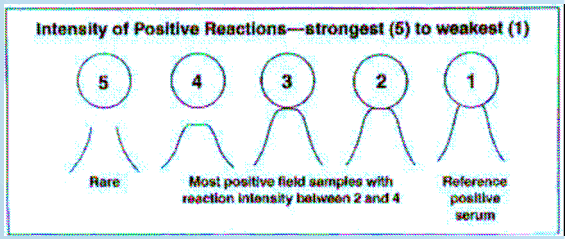
quantitative evaluation of the serological response

Results expressed as reciprocal of last dilution still reacting positive



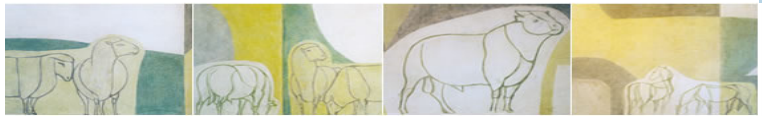
AGIDT – (*Coggins et. al, 1972|*

OIE Diagnostic Terrestrial Manual, 2008)

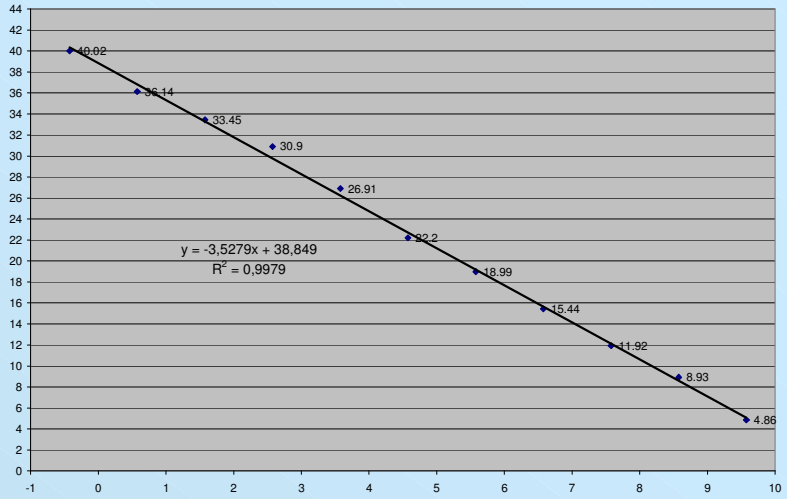


IB – (*Issel et. al, 1999)* – positive

if reacting to p26 and also to at least 1 of the other 2 gps



Plasma associated viral-RNA loads – determined by using a **TaqMan® based RT-PCR, directed against exon1 of *tat* gene using a quantified internal standard for the determination of RNA viral copies**



Primer design - by Dr. F. Cook
MkIII Forward : 5'-GGC GCC CGA ACA GGG ACC-3' (UK position numbers = 310-327)
MkIII Reverse 1: 5'-TGG CCA GGA ACA CCT CCA GAA GAC-3' (UK position numbers = 405-428)
Probe LNA EIAV : 5'-FAM –T+GA ACC T+GG +CTG ATC G+TA G+GA-3'BHQ 1

Reaction profiles
1st step – RNA extraction - starting from **140 µl of plasma**, using the automatic extractor Qiacube® with the Qiagen Viral RNA Mini kit.
2nd step – cDNA synthesis - High Capacity cDNA Archive Kit (Applied Biosystems®)
Thermal profile - 25°C for 10', 37°C for 120', 85°C for 5' & 4°C ∝
3rd step - Real Time qPCR - TaqMan® Universal PCR Master Mix (Applied Biosystems®)
Thermal profile - 50°C for 2', 95°C for 10', 50 cycles: 95°C for 15", 52°C for 30" e 60°C for 1', 72°C for 2'

RESULTS

Reactivity of serum samples in AGIDT
Mules

Days Post-IS	10	2	4	9	1	5	8	7	3	6
-7	3	3	3	2	2	1	1	1	0	0
0	3	3	3	2	2	1	1	0	0	0
7	2	3	3	2	2	1	1	1	0	0
14	3	3	3	2	2	1	1	0	0	0
21	4	3	3	4	2	1	1	1	0	0
28	4	3	3	4	2	2	1	1	0	0

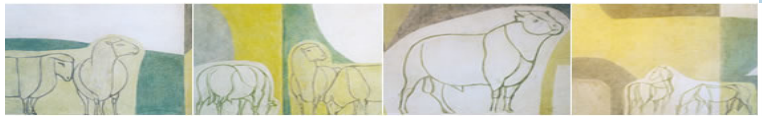
Higher reacting **Lower reacting**

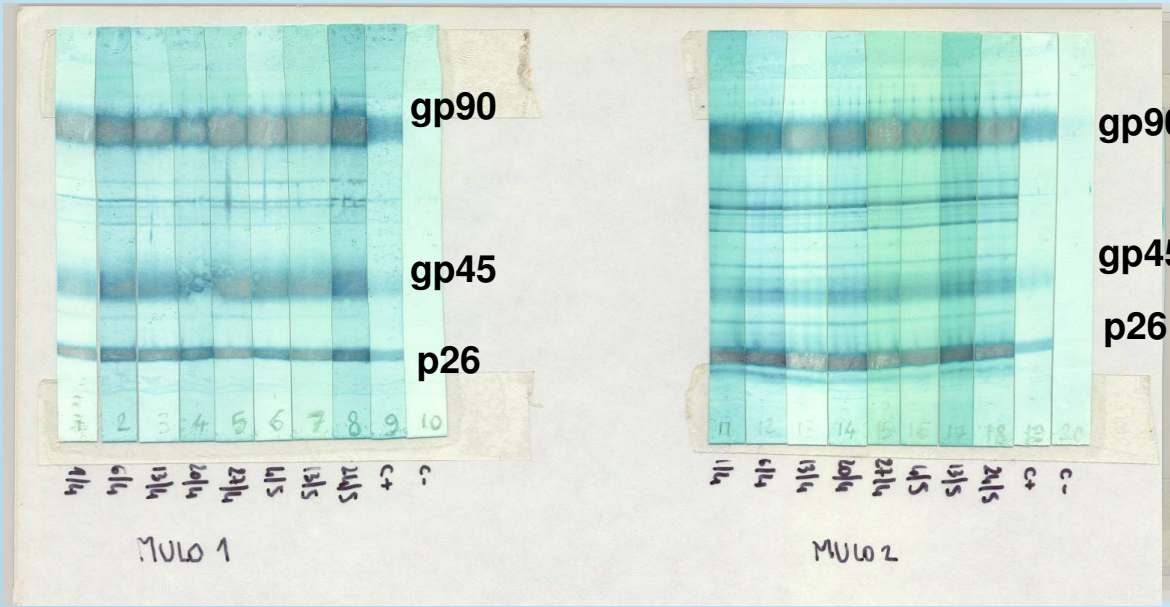
Reciprocal of end-point Elisa titres
Mules

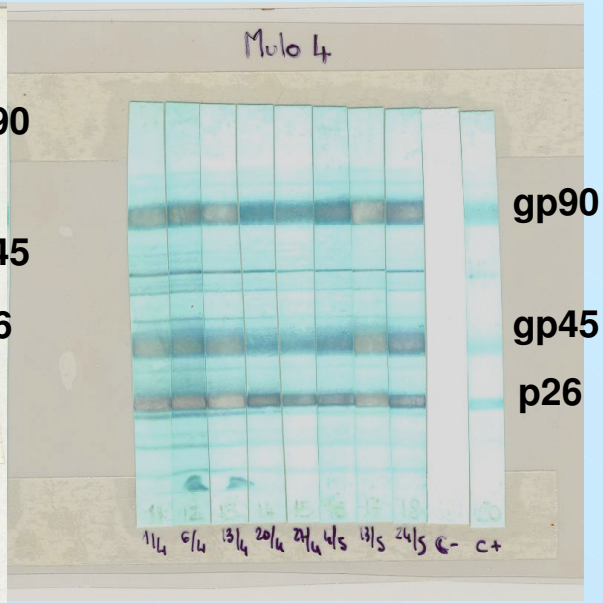
Days Post-IS	4	10	9	2	1	5	8	7	3	6
-7	576	192	24	192	48	12	48	6	0	0
0	576	192	48	192	192	12	24	6	6	0
7	576	192	48	192	48	12	12	0	6	0
14	384	192	0	192	48	12	12	6	0	0
21	192	576	576	192	48	12	48	12	12	0
28	192	576	576	192	48	192	48	48	12	+/-

Higher reacting **Lower reacting**

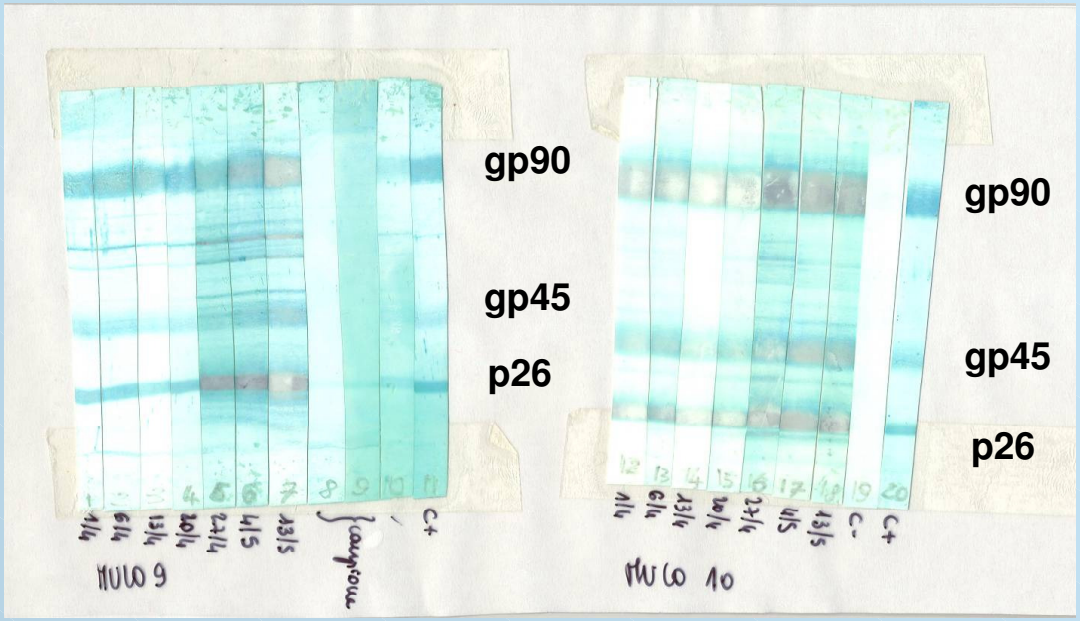
Ranked AGIDT & Elisa serological profiles

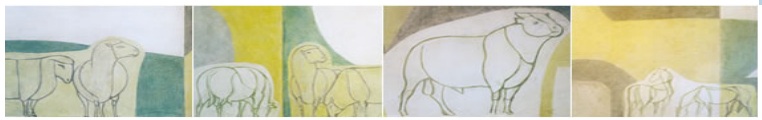


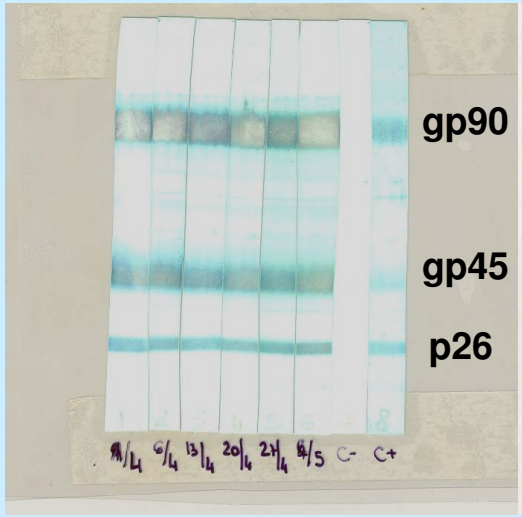




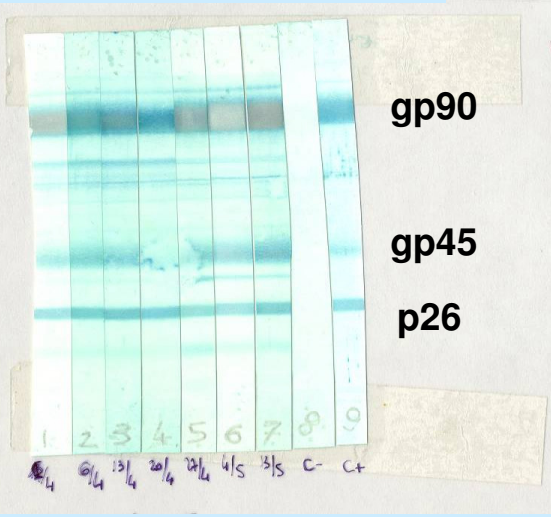
IB of mules with higher AGIDT/Elisa reactivity



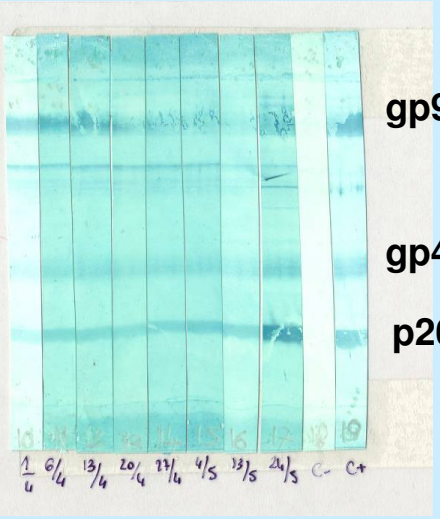




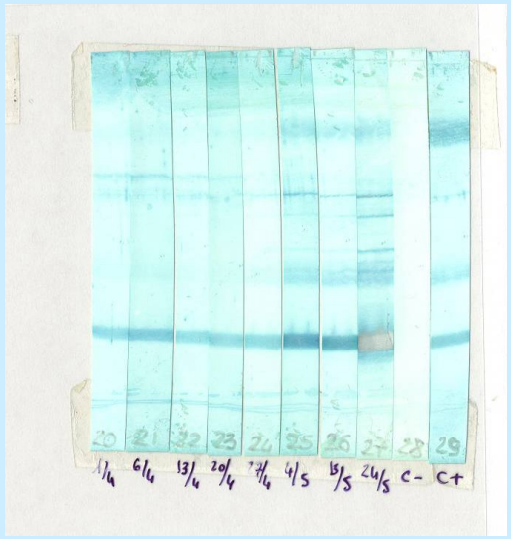
Mule 3



Mule 5

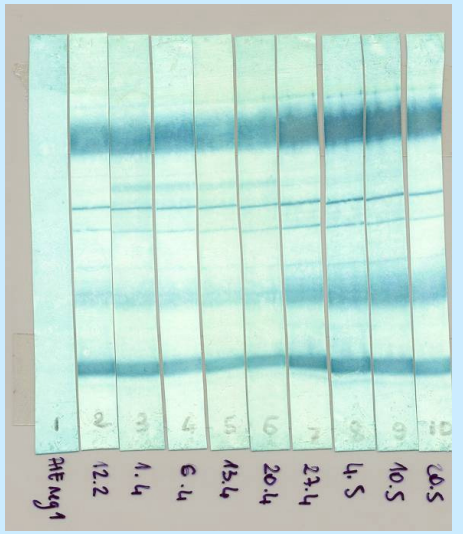


Mule 6



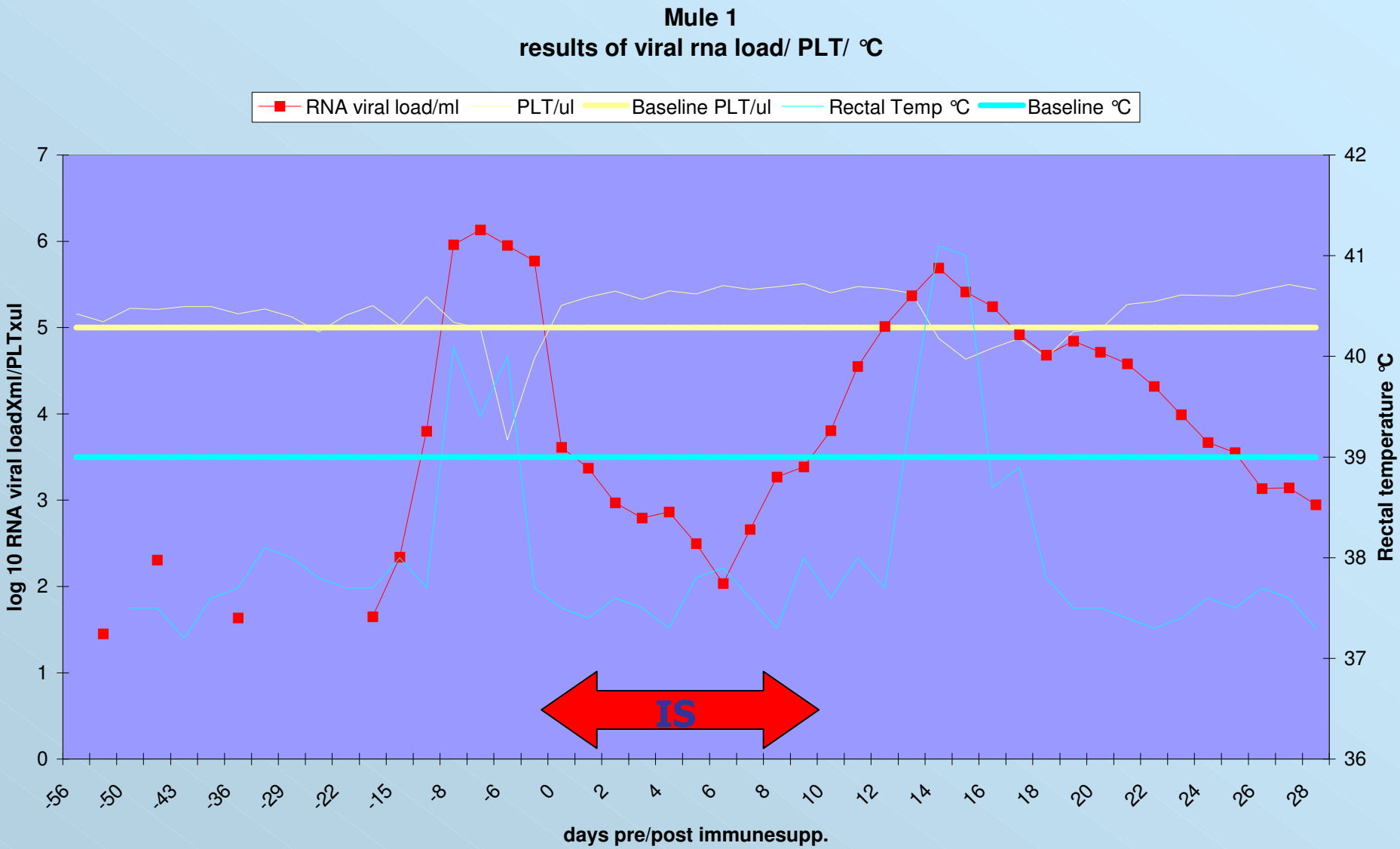
Mule 7

IB of the mules with lower AGIDT/Elisa reactivity

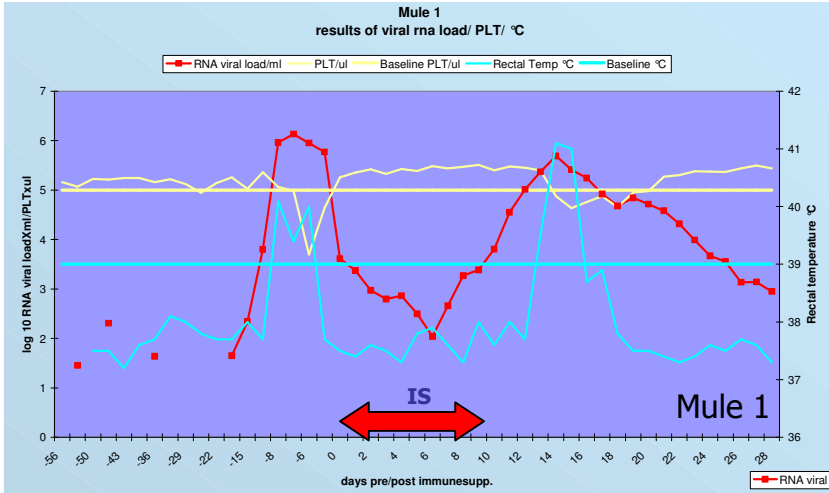


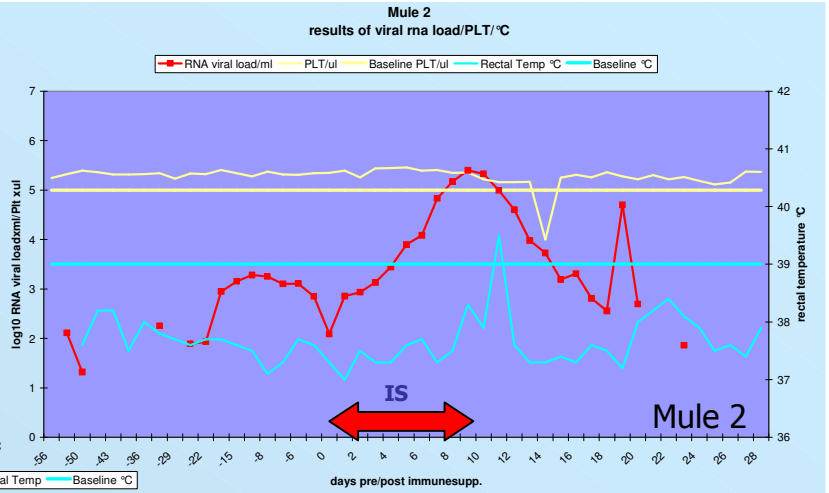
Mule 8

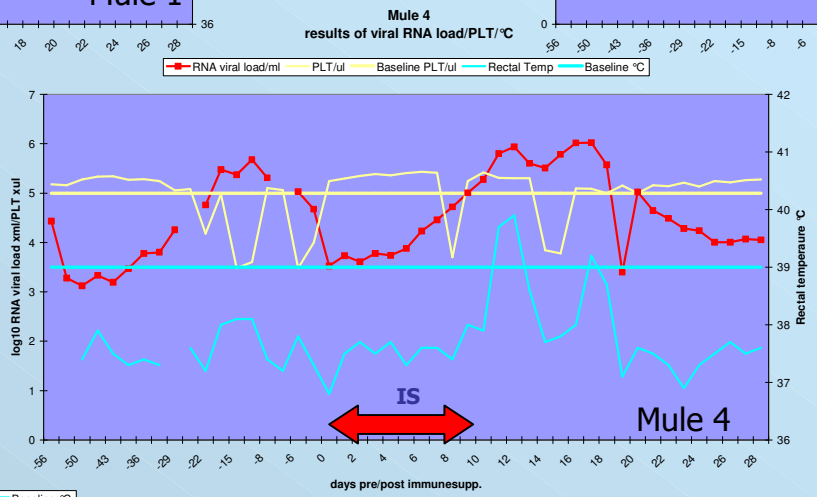
Temporal profiles of plasma RNA viral load/plt/°C

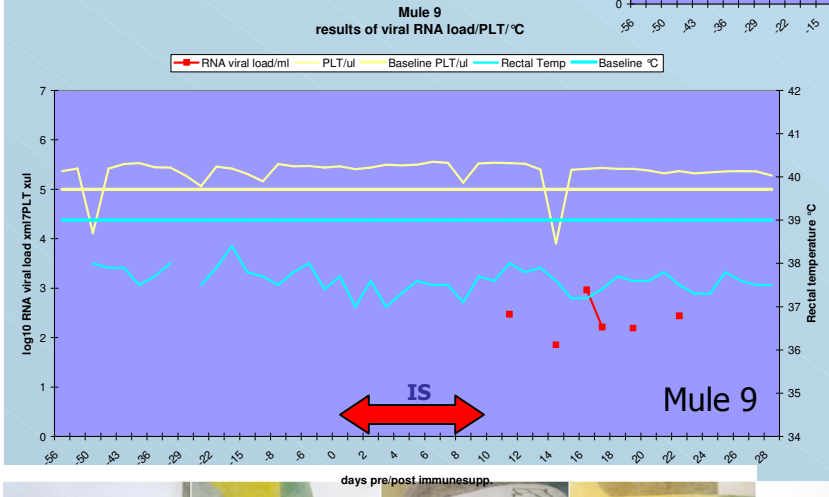


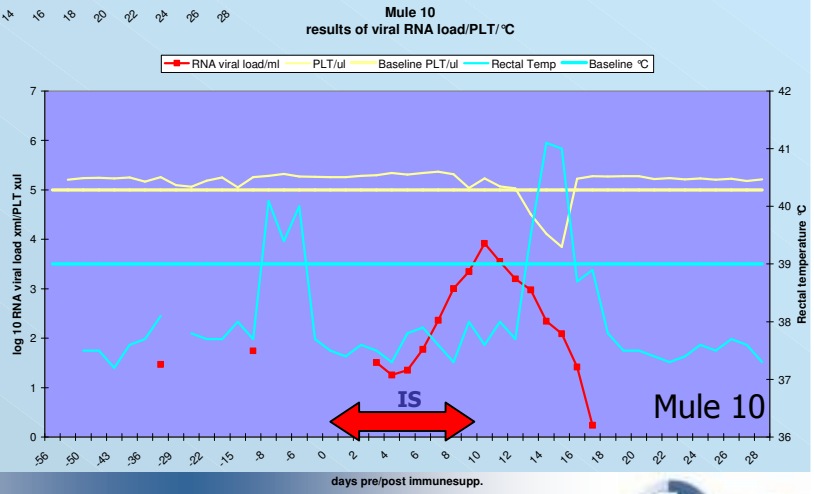
RNA viral load/plt/°C profiles of mules with higher AGIDT/Elisa reactivity

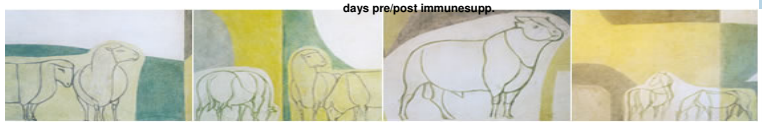




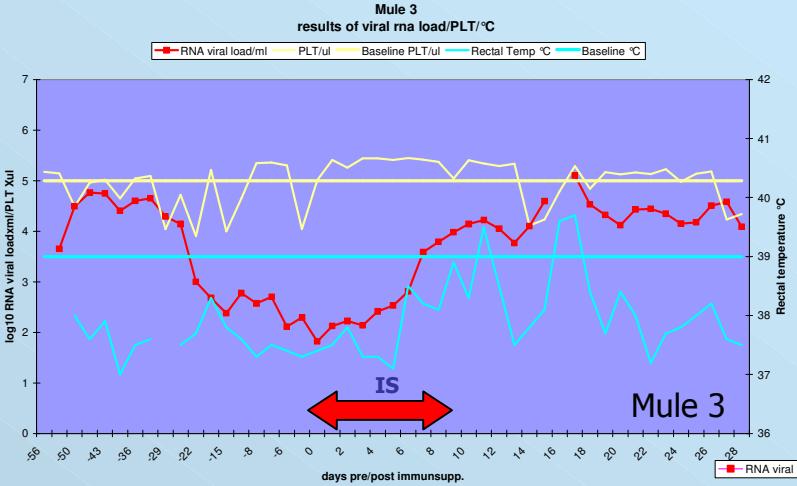


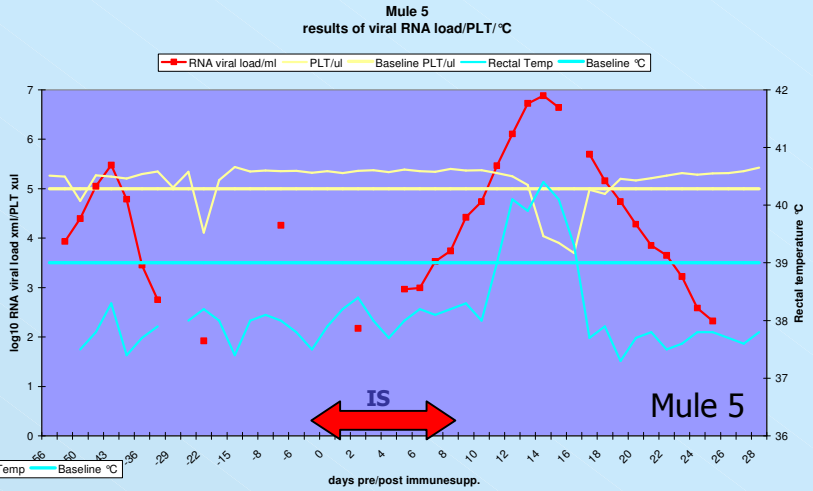


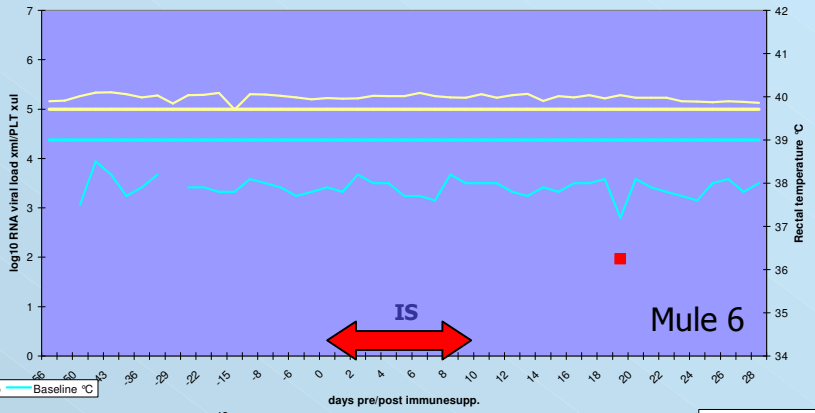


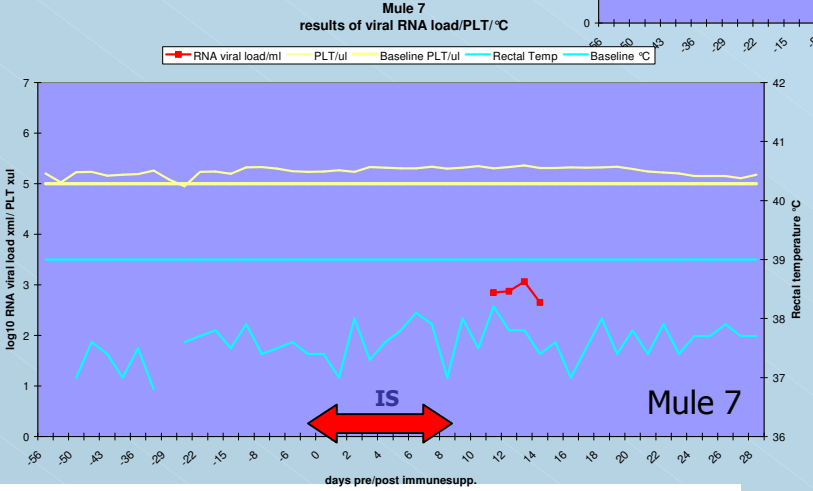


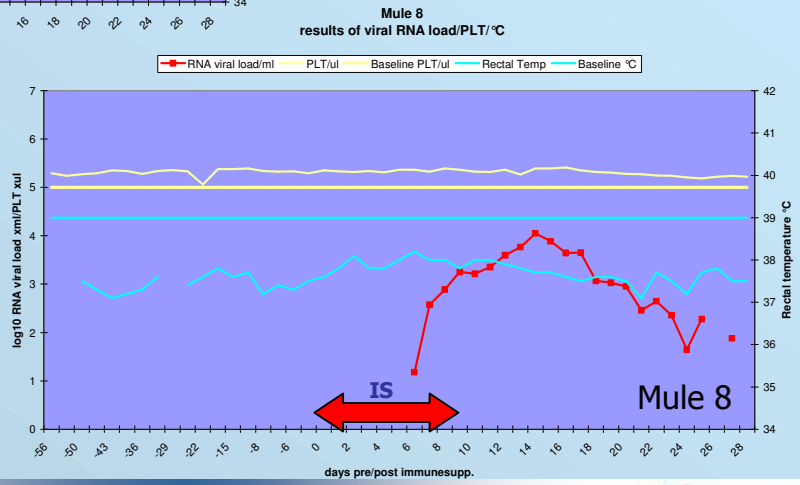
RNA viral load/plt/°C profiles of mules with lower AGIDT/Elisa reactivity

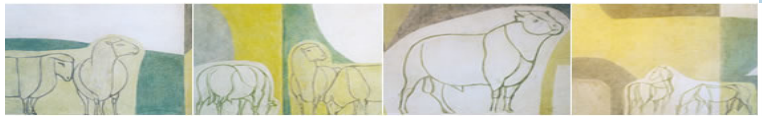










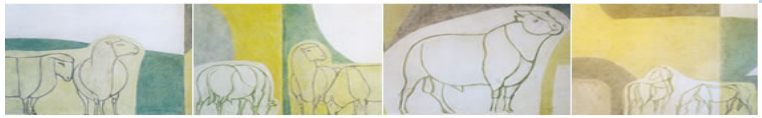


Clinical signs

D.P.IS	1	2	3	4	5	6	7	8	9	10
9		H								
10										P
11		A, D								P
12		A								
13			A	D	A					A
14		A	A	D						A
15	D		A		J,P, D		D		D	
16			A,D	A	J			A		
17	D		D	D						
18	H	A	J	A		A			A	A
19		A		A,J	A	A,J				
20	D	A	A,J	E,A	A	A		A	A	
21		A	A	E	A					
22		A			A					
23			D							
24		A			A				J	
25			D	A,D						
26			D							
27		A			A					

Clinical signs were mild to absent, probably mitigated because manifested during recurring episodes of the infection

fever	A	anemia	H	hyperemia
thrombocytopenia	D	depression	J	jaundice
fever & thrombocytopenia	P	polypneia	E	oedema

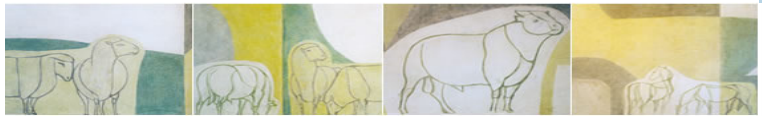


Other results

• **Nucleotide sequencing confirmed that all mules were infected with EIAV** - isolates similar to previously identified European strains (6 possessing close identity to EIAVRom-4 (GU060662.1), 2 to EIAVIta-1 (EU240733.1) and 2 to EIAVIta-90 (HQ888862.1)) - **different pictures with same strain**.

• Prior to IS, viral **RNA was detectable during at least one sample point in 7 mules**, including 1 with a negative AGIDT reaction (due to other stress conditions??????).

• In some of the animals, including the 3 with limited AGIDT reaction, there was **extensive temporal variation in plasma-associated RNA viral loads - increase from 95 to 30,000 fold** & significant differences between individual animals in the extent of viral replication with **no guarantee that viral levels will be "limited"**.

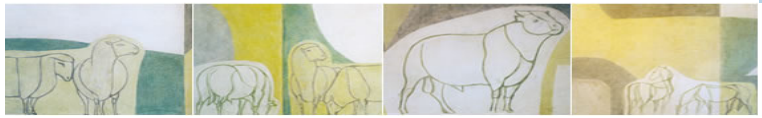


Other results – cont'd

- While **all mules** , following IS, **showed increase in plasma-associated viral RNA loads,** with values as high as **$\log_{10}$6 RNA copies/ml for 4 subjects**, **only 3 had four-fold increases in C-ELISA titres** & **2 remained AGIDT-negative** (1 of which was also C-ELISA negative).

- **No obvious correlation** between **viral RNA load response** & **negative serological results in AGIDT, individual characteristics & apparent viral strain.**

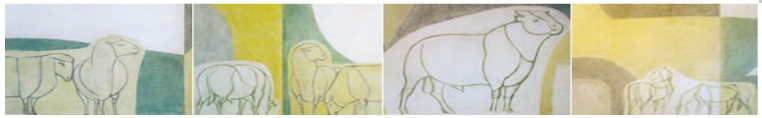
- **Similar viral loads have been observed in acute cases of AIE in horses in the Irish outbreak** (*Quinlivan M., et. al. 2007*).



Other results – cont'd

• Only **very mild transient clinical signs** were observed for all mules & therefore the animals with negative/equivocal AGIDT reactivity **would not have been captured by passive surveillance, solely based on clinical suspect.**

• Subjects with **low to null serological response in AGIDT cannot** be considered as **risk zero** in the transmission of EIAV infection.



Only Considerations!

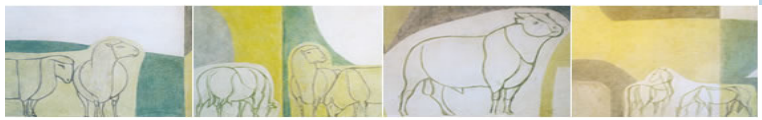
The exclusive use of the AGIDT could result in animals being falsely reported as negative and enhance the risk of diffusion?

One (AGIDT) or two tier (Elisa & AGIDT) system not enough!

High predicitive value of Elisa/IB positive result

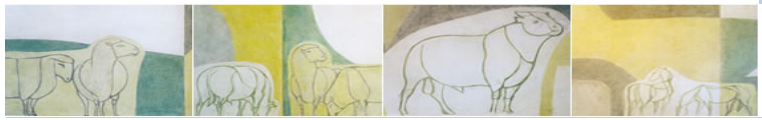
Biosecurity measures prescribed for animals with equivocal AIE serological result should be as for those confirmed positive ...

What is the overall (direct and indirect) cost of these animals in a national surveillance programme?



Collaborations & acknowledgements

- Study performed in collaboration with the Gluck Equine Research Centre (Lexington, USA)
- Field support from the Veterinary Services of Roma G, Aquila, Latina
- Technical support from the staff of the IZSLT – Peripheral labs of LT and RI, & VIR, OEVR, DIAG, RIA, DMV



EQUINE INFECTIOUS ANAEMIA:
LESSONS LEARNED FROM THE SIX-YEAR APPLICATION OF THE NATIONAL ITALIAN SURVEILLANCE
Rome 1st October 2012

Vi ringrazio per l'attenzione

Thank you for your attention