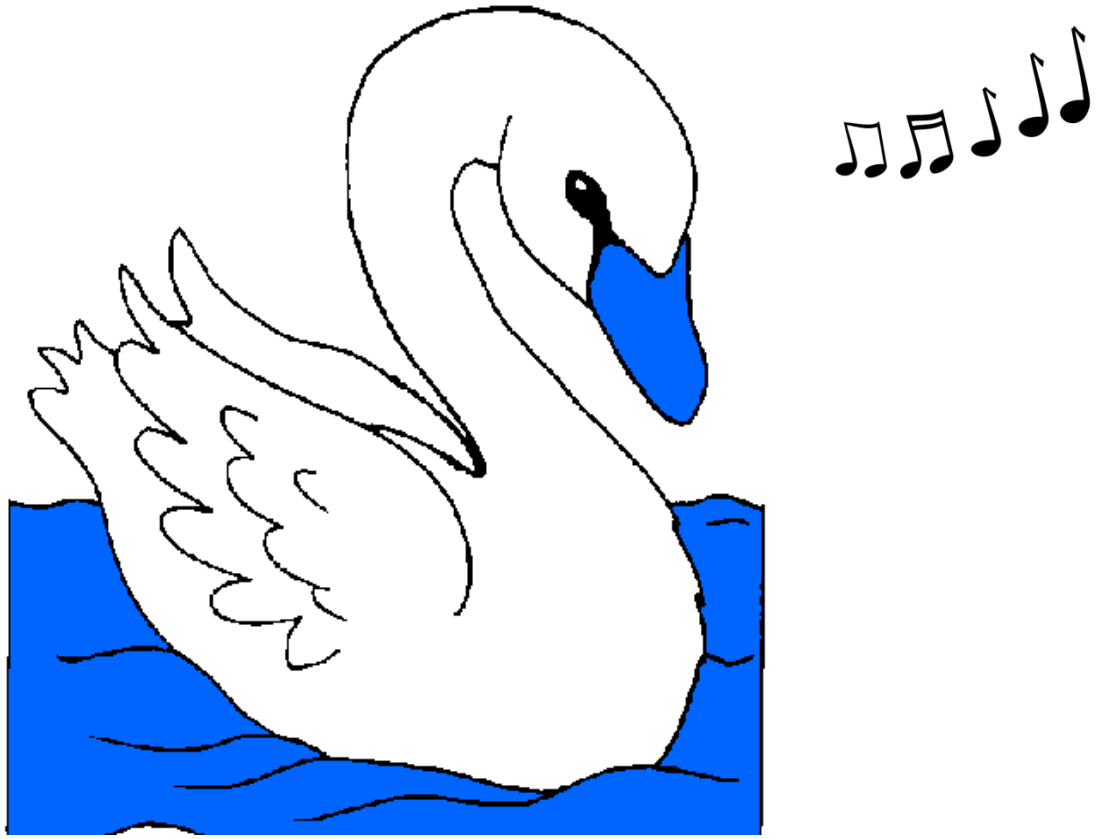
IZSAM G.CAPORALE
TERAMO

BTV e ricerca: una miniera d’oro

Alessio Lorusso 20 Novembre 2018

DEREGULATION



- **Ricerche Correnti**
- **Ricerca Finalizzata**
- **H2020**
- **Prima Foundation?**

Metodi innovativi di diagnosi

Epidemiologia molecolare

Patogenesi

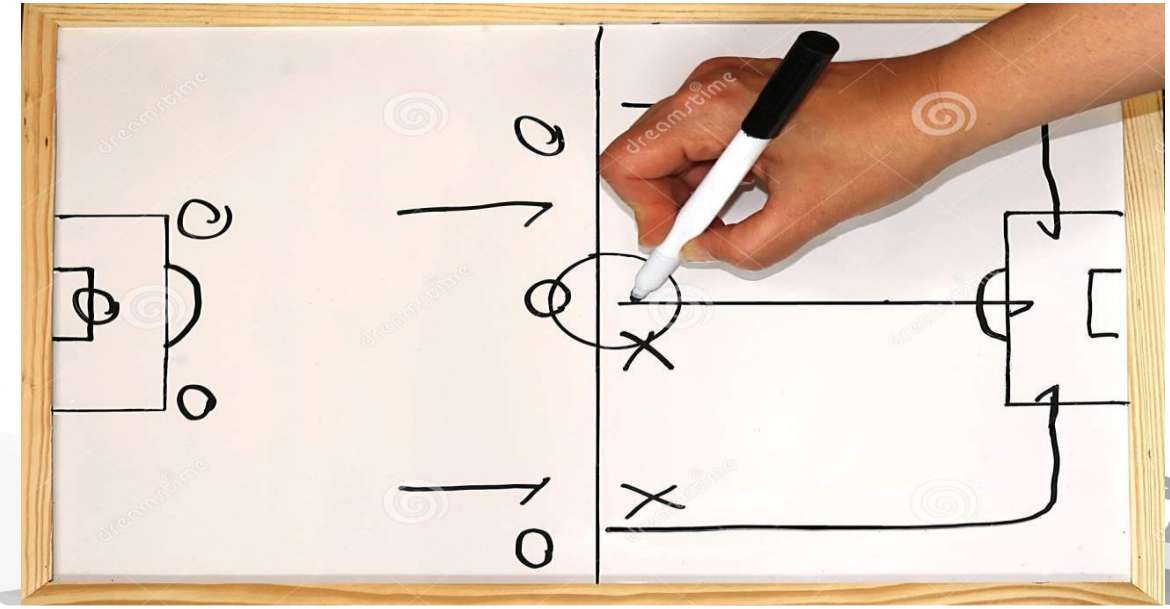
Evoluzione Antigenica

Sistemi informativi

Trasmissione

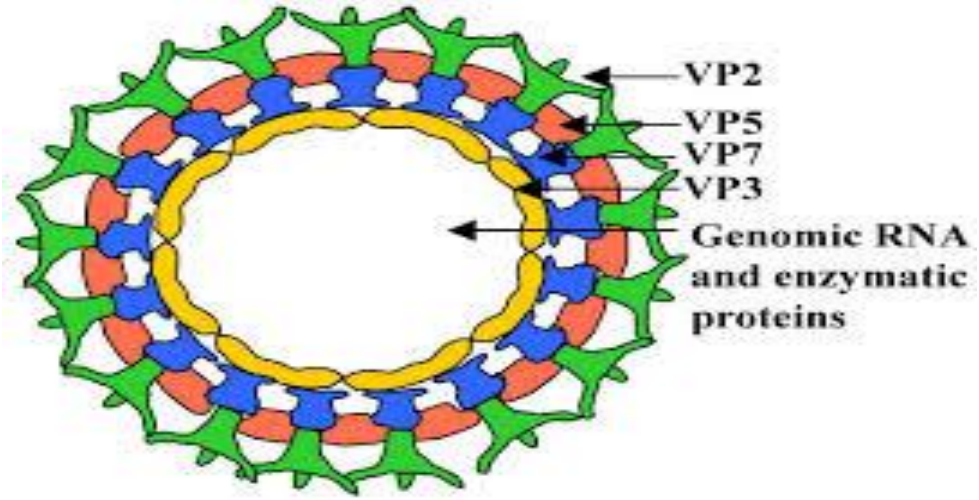
Valutazione dei costi

Vaccini



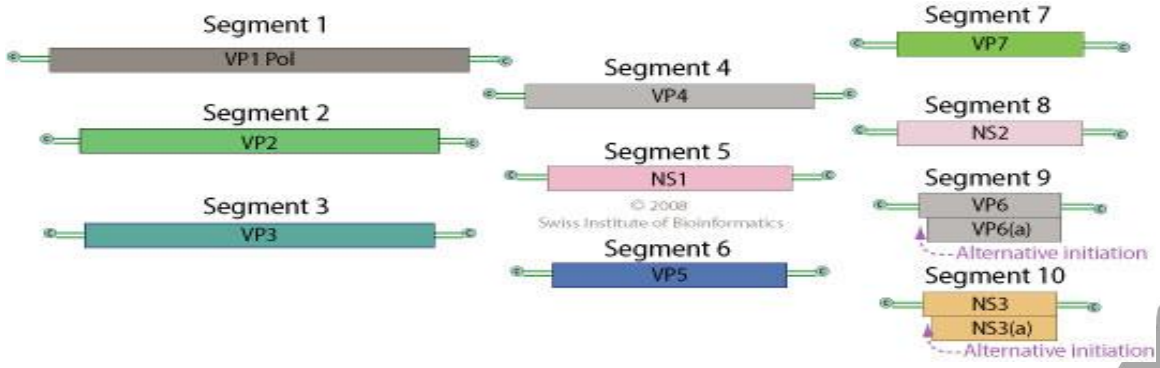
Bluetongue Virus

VP2 determines serotype specificity



TOPOTYPES, western or eastern strains

NUCLEOTYPES or lineages, VP2 and VP5



DIAGNOSTIC TESTS FOR BTV ARE PERFORMED ACCORDING TO THE EPIDEMIOLOGICAL SCENARIO

THEREFORE SOME VIRUS MAY BE MISSED !!

nCounter® Analysis System

15 Minutes Hands-on Time

IZSAM G.CAPORALE TERAMO

Prosigna™ provides fast, reliable results in as few as 3 days[1]

1 Hybridize → 2 Purify → 3 Count

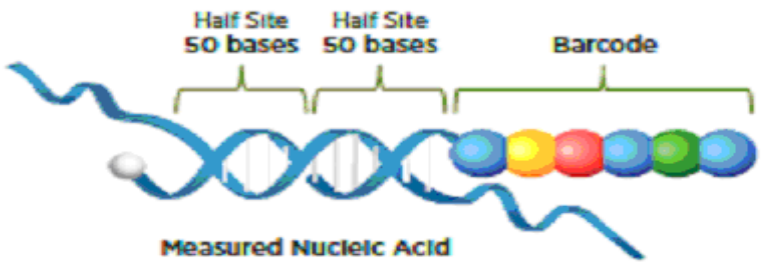
Minimal hands-on — **Step 1** 15-21 hours or overnight

Minimal hands-on — **Step 2** 2-3 hours, automated

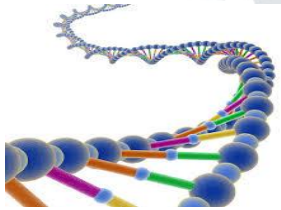
Minimal hands-on — **Step 3** 2.5-4.5 hours, automated

nCounter® Analysis System for BTV

- **Design of probes consists of 100 mer sequences**
  - **64 capture probes targeting the Segment-2 sequences of the existing serotypes**
  - **2 probes one for NS1 and other for NS3 are included as positive controls**
  - **5 capture probes for *Culicoides* species**
  - **1 capture probe for actin gene**

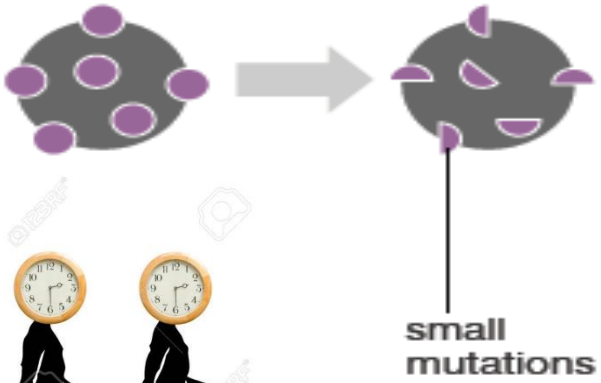


EVOLUTION

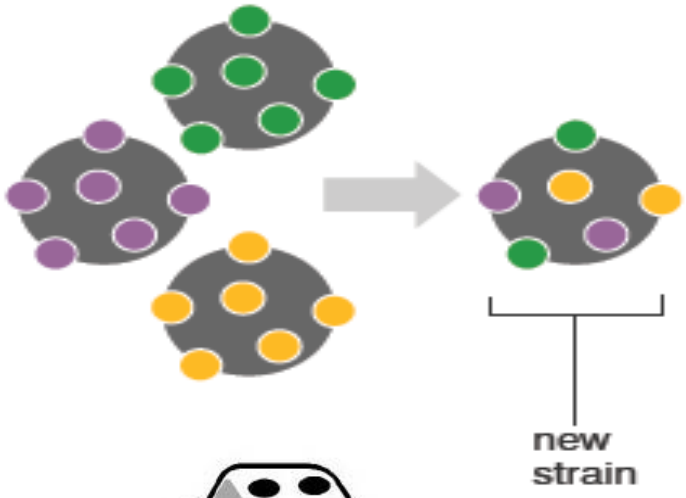


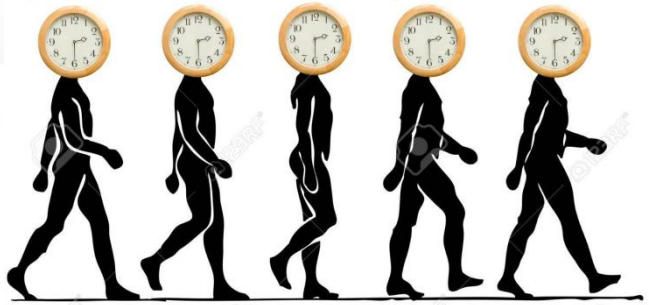
Mutation

Antigenic drift



Antigenic shift





NGS and BTV: Diagnostics and WGS

RAPID COMMUNICATION

WILEY

A novel Bluetongue virus serotype 3 strain in Tunisia, November 2016

S. Sghaier[1†] | A. Lorusso[2†] | O. Portanti[2] | M. Marcacci[2] | M. Orsini[2] | M. E. Barbria[3] | A. S. Mahmoud[2-4] | S. Hammami[5] | A. Petrini[2] | G. Savini[2]

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[2]OIE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), Teramo, Italy

[3]CRDA-Commissariats Régionaux au Développement Agricole, Menzel Bouzelfa, Tunisia

[4]Dipartimento di Scienze Veterinarie, Università degli Studi di Pisa, Pisa, Italy

[5]Ecole Nationale de Médecine Vétérinaire de Sidi Thabet, Tunis, Tunisia

Correspondence
A. Lorusso, OIE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), Teramo, Italy.
Email: a.lorusso@izs.it

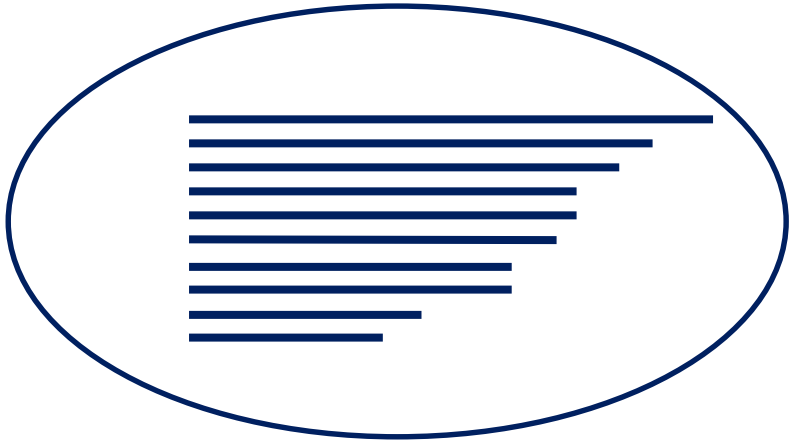
Funding information
Italian Ministry of Health

Summary

Since 1998, southern Europe has experienced multiple incursions of different serotypes and topotypes of Bluetongue virus, a vector-borne transmitted virus, the causative agent of Bluetongue (BT), a major disease of ruminants. Some of these incursions originated from northern Africa, likely because of wind-blown dissemination of infected midges. In this report, we describe the detection and whole genome characterization of a novel BTV-3 strain identified in a symptomatic sheep in Tunisia. Sequences were immediately deposited with the GenBank Database under Accession Nos KY432369-KY432378. Alert and preparedness are requested to face the next vector seasons in northern Africa and the potential incursion of this novel strain in southern Europe.

KEYWORDS
Bluetongue serotype 3, whole genome sequencing, Tunisia

BTV-3 TUN2016



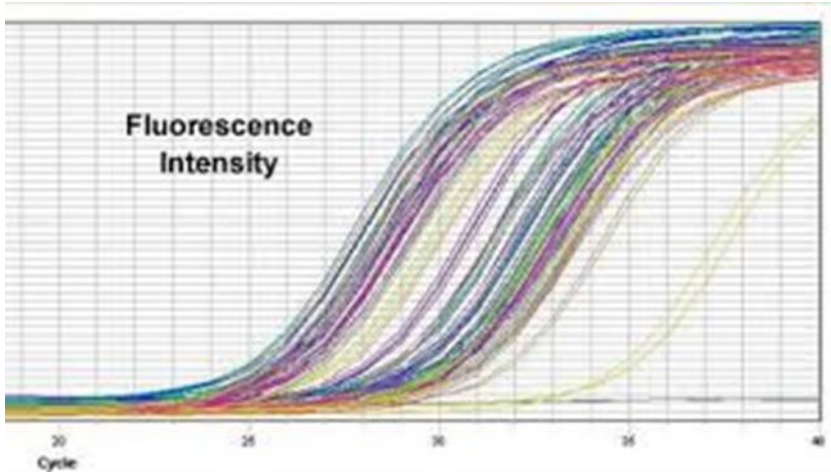
Segment	% nt id with BTV-3 TUN2016
Seg 1	93.7
Seg 2	94.1
Seg 3	96.1
Seg 4	96.3
Seg 5	96.3
Seg 6	98.0
Seg 7	95.9
Seg 8	94.0
Seg 9	96.7
Seg 10	80.3

DIFFERENT BTV-3!!!

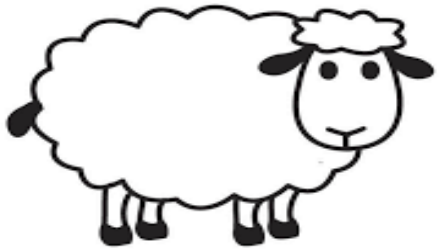
BTV-3 TUN2016/Zarzis


15 Minutes Hands-on Time
No Printing of Slides
No Amplification
No Imaging Software
No Labelling
No Data Mining
No Washing
nanoString
nanoString


MinION


Fluorescence
Intensity
Cycle


SpotON
MinION
FAB06069



- **Novel serotypes**
- **Identified by NGS in Sardinia (BTV-X ITL2015), Tunisia (BTV-Y TUN2017) and Piedmont (BTV-Z ITA2017)**
- **Novel characteristics (transmission, isolation)**

Research paper

Novel putative Bluetongue virus in healthy goats from Sardinia, Italy

Giovanni Savini [a], Giantonella Puggioni [b], Giorgio Meloni [b], Maurilia Marcacci [a], Marco Di Domenico [a], Angela Maria Rocchigiani [b], Massimo Spedicato [a], Annalisa Oggiano [b], Daniela Manunta [b], Liana Teodori [a], Alessandra Leone [a], Ottavio Portanti [a], Francesca Cito [a], Annamaria Conte [a], Massimiliano Orsini [a], Cesare Cammà [a], Paolo Calistri [a], Armando Giovannini [a], Alessio Lorusso [a,*]

[a] OIE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise (IZSAM), Teramo, Italy
[b] Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

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Keywords:
Bluetongue virus
New serotype
Goats
Sardinia
Phylogeny
Next generation sequencing

ABSTRACT

In recent years, novel Bluetongue virus (BTV) serotypes have been isolated and/or sequenced by researchers within the field. During Bluetongue surveillance activities, we identified a putative novel BTV serotype in healthy goats from Sardinia, Italy. RNAs purified from blood and serum samples were positive for BTV by a generic real time RT-PCR and c-ELISA, respectively, whereas genotyping and serotyping were unsuccessful. By NGS, the whole genome sequence was obtained from two blood samples (BTV-X ITL2015 strains 34200 and 33531). Overall, Seg 2 of BTV-X ITL2015 shows the highest identity (75.3–75.5% nt/77.4–78.1% aa) with recently isolated BTV-27s from Corsica and with the last discovered BTV XJ1407 from China (75.9% nt /78.2% aa), whereas it is less related with BTV-25 from Switzerland (73.0% nt/75.0% aa) and BTV-26 from Kuwait (62.0% nt/60.5% aa). A specific RT-qPCR targeting Seg2 of BTV-X ITL2015 was assessed in this study. Considering the Seg 2/VP2 identity of BTV-X ITL2015 with BTV-25, 26, 27s and BTV XJ1407 and that serum of BTV-X ITL2015 infected goats failed to neutralize all tested extant serotypes, we propose the existence of a novel BTV serotype circulating in goats in Sardinia. Isolation was so far unsuccessful thus hampering proper antigenic characterization.

Received: 27 November 2017
DOI: 10.1111/tbed.12822

RAPID COMMUNICATION

WILEY

One after the other: A novel Bluetongue virus strain related to Toggenburg virus detected in the Piedmont region (North-western Italy), extends the panel of novel atypical BTV strains

Maurilia Marcacci[1,2] | Serena Sant[3] | Iolanda Mangone[1,2] | Maria Goria[3] | Alessandro Dondo[3] | Simona Zoppi[3] | René G. P. van Gennip[4] | Maria Cristina Radaelli[3] | Cesare Cammà[1,2] | Piet A. van Rijn[4,5] | Giovanni Savini[1,2] | Alessio Lorusso[1,2]

Research paper

Analysis of bluetongue serotype 3 spread in Tunisia and discovery of a novel strain related to the bluetongue virus isolated from a commercial sheep pox vaccine

Alessio Lorusso[a,b,*,1], Soufien Sghaier[c,1], Marco Di Domenico[a,b], Mohamed Elias Barbria[d], Guendalina Zaccaria[a,b], Aida Megdich[c], Ottavio Portanti[a,b], Imed Ben Seliman[e], Massimo Spedicato[a,b], Federica Pizzurro[a,b], Irene Carmine[a,b], Liana Teodori[a,b], Mejdi Mahjoub[f], Iolanda Mangone[a,b], Alessandra Leone[a,b], Salah Hammami[g], Maurilia Marcacci[a,b], Giovanni Savini[a,b]

[a] OIE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM), Teramo, Italy
[b] National Reference Center for Whole Genome Sequencing of microbial pathogens: database and bioinformatic analysis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Campo Boario, 64100 Teramo, Italy
[c] Université de Tunis El Manar, Institut de la Recherche Vétérinaire de Tunisie (IRVT), Laboratoire de virologie, 20 Rue Djebel Lakhdar, 1006 Tunis, Tunisia
[d] CRDA-Commissariats Régionaux au Developpement Agricole, Menzel Bouzelfa, Tunisia
[e] CRDA, Medenine, Tunisia
[f] CRDA, Gafsa, Tunisia
[g] Ecole Nationale de Médecine Vétérinaire de Sidi Thabet, Tunisia

Area: Animal Health

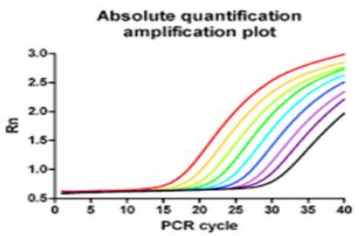
U01: Alessio Lorusso, *Virology*; **UO2:** IZS Sardegna, Giantonella Puggioni, *Virology*; **UO3:** Cesare Cammà, *Genomics*; **UO4:** Maria Goffredo, *Entomology*

Title: EpiTraP: Unraveling Epidemiology, Transmission, Pathogenesis, and reassortment capability of the newly discovered BTV-X ITL2015

Duration: 2 years

Coordinator: Giovanni Savini

AIM 1: To assess the epidemiology of BTV-30 in Sardinia

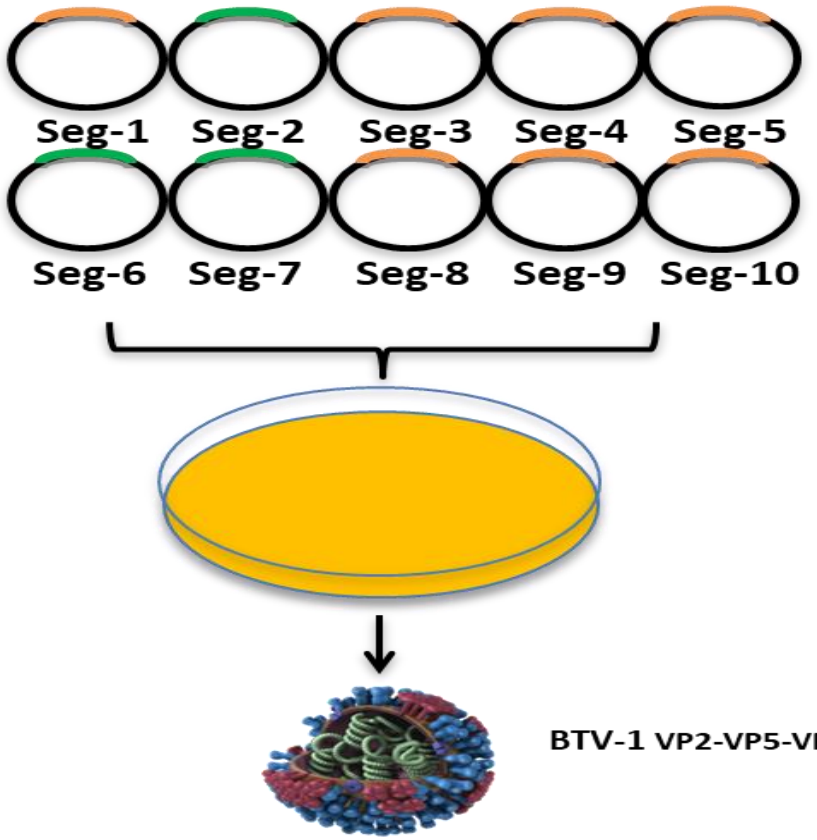


Specific real time RT-PCR

↓

↓

AIM 1: <u>To assess the epidemiology of BTV-30 in Sardinia</u>

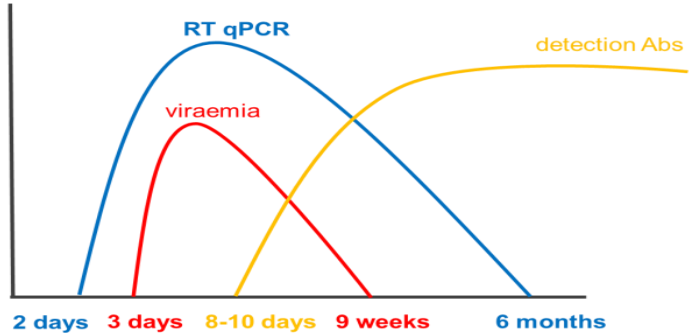


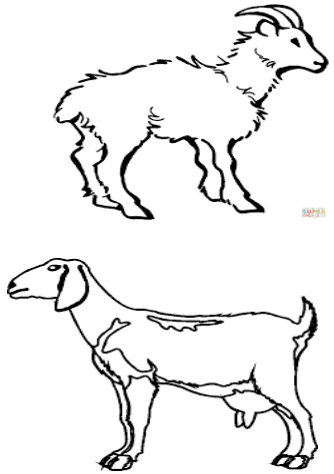
Serum samples

Fig 1

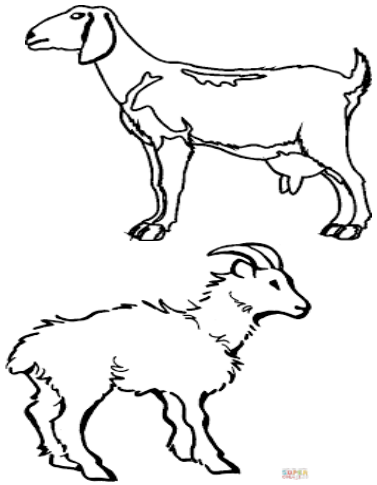
AIM 2: To assess the length of viraemia, vector competence and transmission of BTV-X ITL2015

Infected animals





BTV-X ITL2015 Infected animals



Direct contact animals

Fig 2A

AIM 2: To assess the length of viraemia, vector competence and transmission of BTV-X ITL2015

BTV-X ITL2015

Infected animal

Naive animal

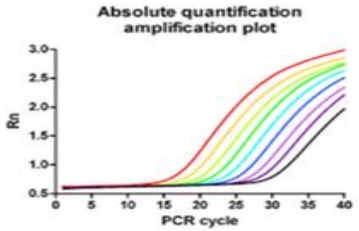
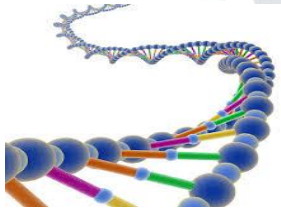


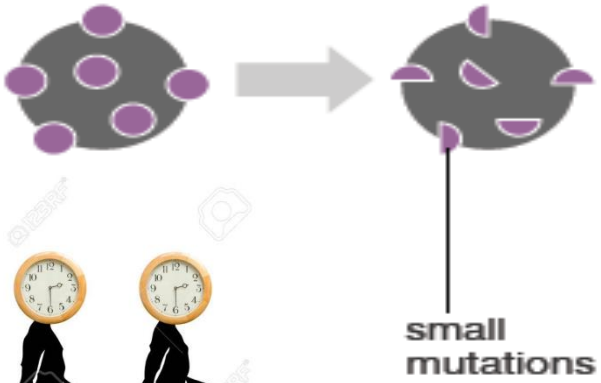
Fig 2B

EVOLUTION

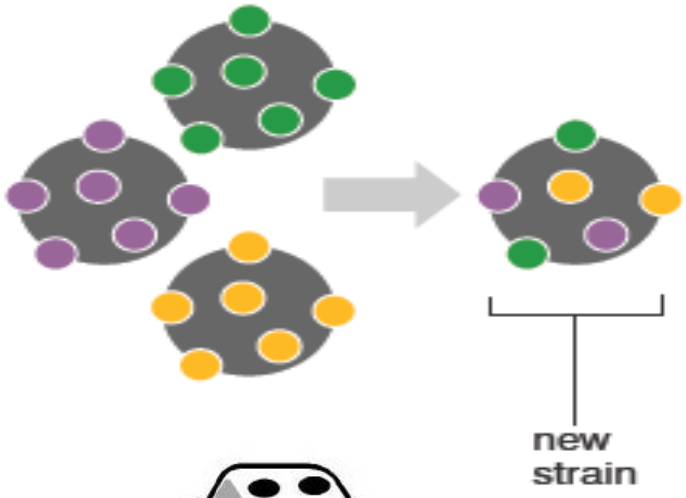


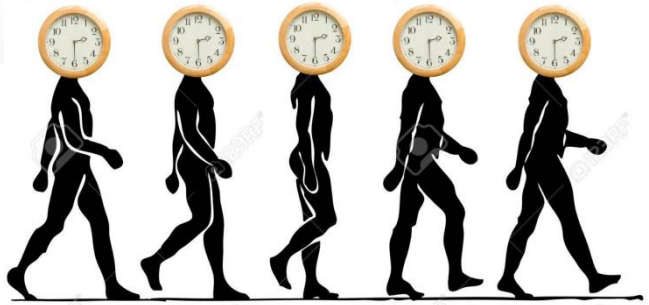
Mutation

Antigenic drift



Antigenic shift





GENETIC DIVERSITY OF BTV-4 STRAINS CIRCULATING IN THE MEDITERRANEAN BASIN

Italy BTV-4 2014
from Balkans

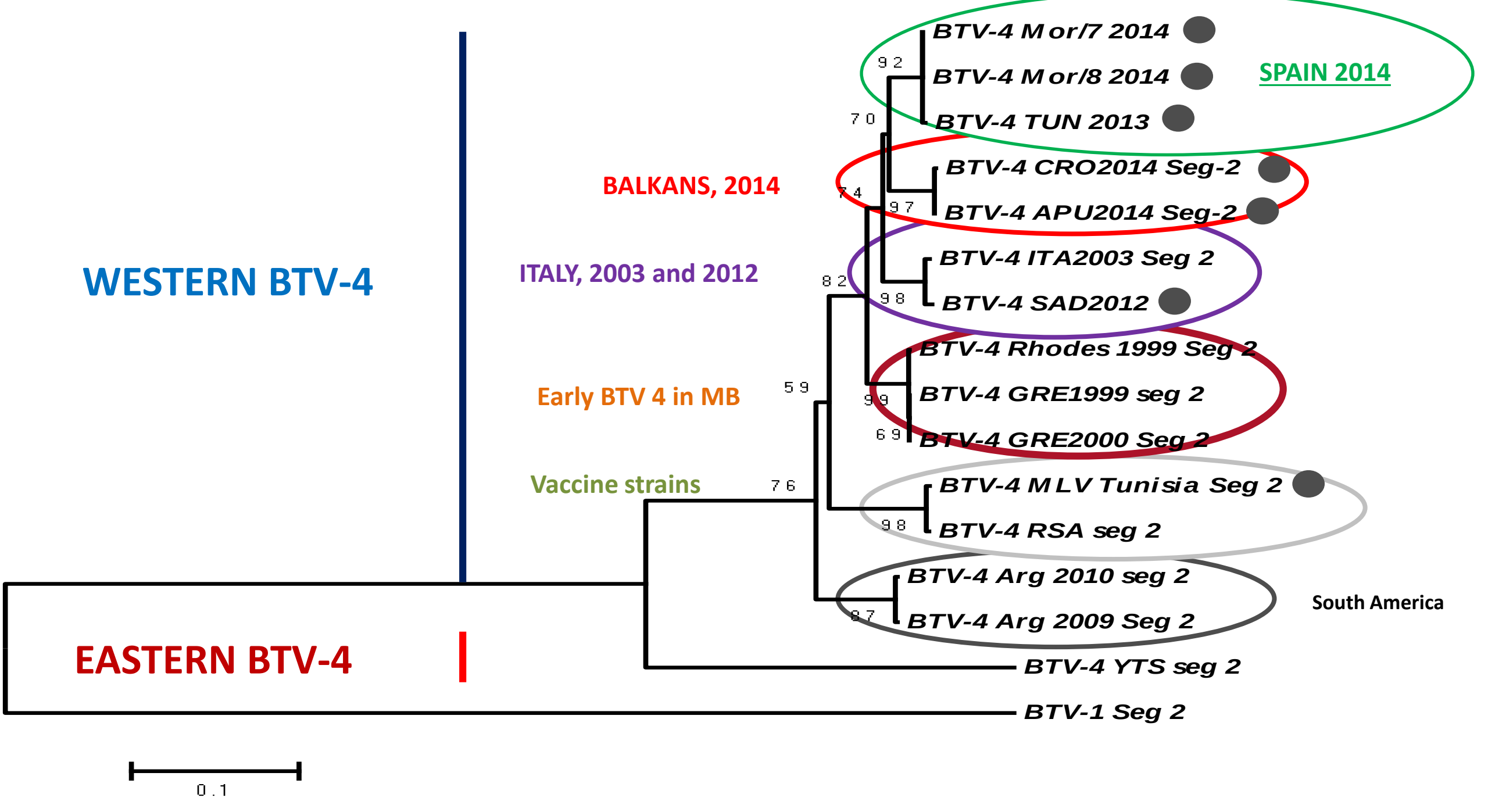
Italy BTV-4 2003, 2004

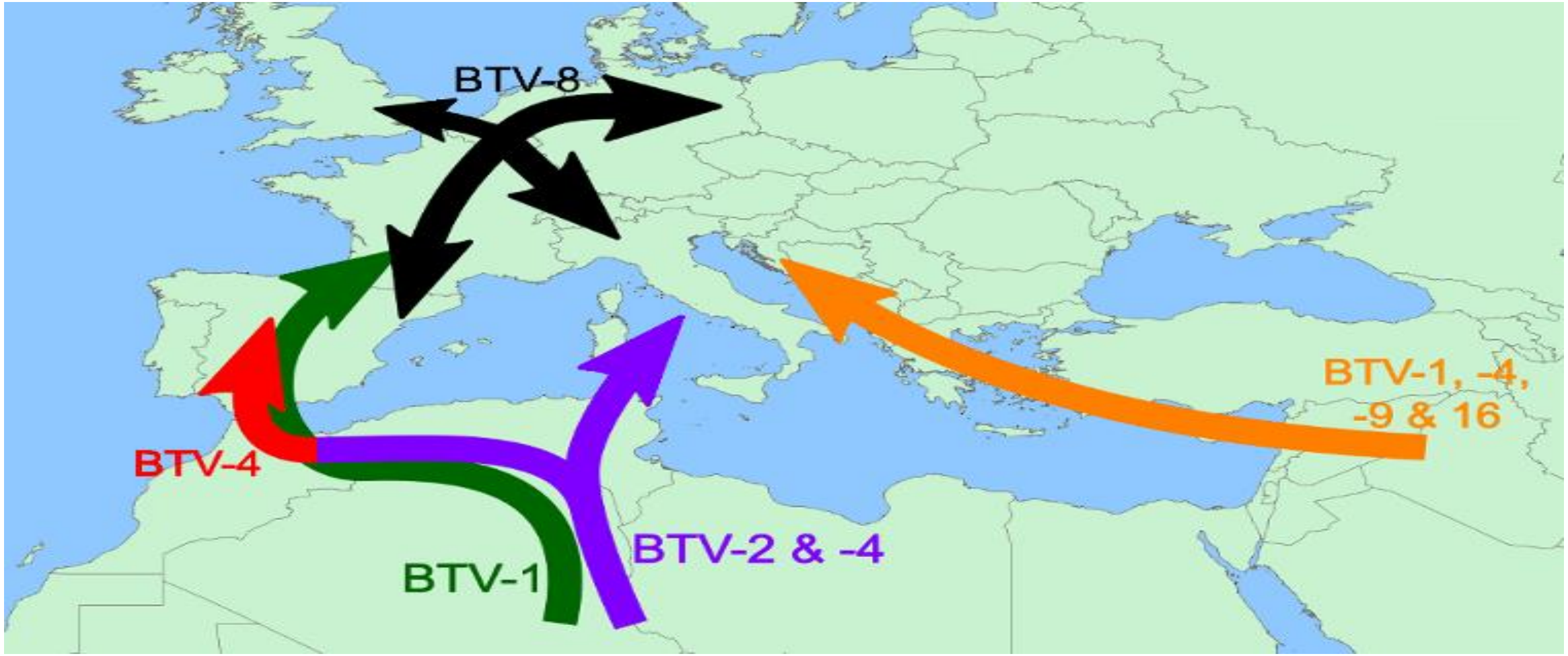
Italy BTV-4 2012

BTV-4 2014

MLV BTV-4

BTV-4 Greece 1999


WESTERN BTV-4
EASTERN BTV-4
BALKANS, 2014
ITALY, 2003 and 2012
Early BTV 4 in MB
Vaccine strains
SPAIN 2014
South America
BTV-4 Mor/7 2014
BTV-4 Mor/8 2014
BTV-4 TUN 2013
BTV-4 CRO2014 Seg-2
BTV-4 APU2014 Seg-2
BTV-4 ITA2003 Seg 2
BTV-4 SAD2012
BTV-4 Rhodes 1999 Seg 2
BTV-4 GRE1999 seg 2
BTV-4 GRE2000 Seg 2
BTV-4 MLV Tunisia Seg 2
BTV-4 RSA seg 2
BTV-4 Arg 2010 seg 2
BTV-4 Arg 2009 Seg 2
BTV-4 YTS seg 2
BTV-1 Seg 2
92
70
74
97
82
98
59
99
69
76
98
87
0.1


BTV-8
BTV-1, -4,
-9 & 16
BTV-4
BTV-2 & -4
BTV-1

ACCEPTANCE and CONTROL of SAMPLES

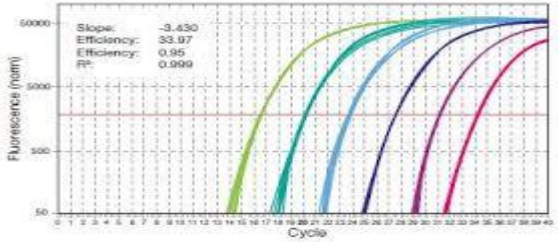
Expert Veterinarian

Virology, Serology

Nanostring

Illumina

Mini Ion



Diagnostic panel (known pathogens)

Results to Customer

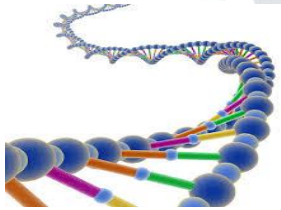
Internal workflow for storage

Isolation

WGS

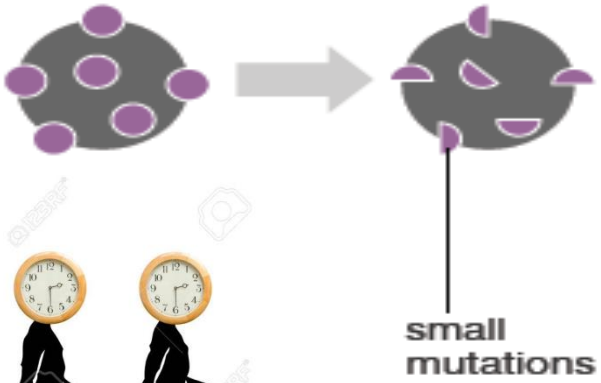
Antigen Bank

EVOLUTION

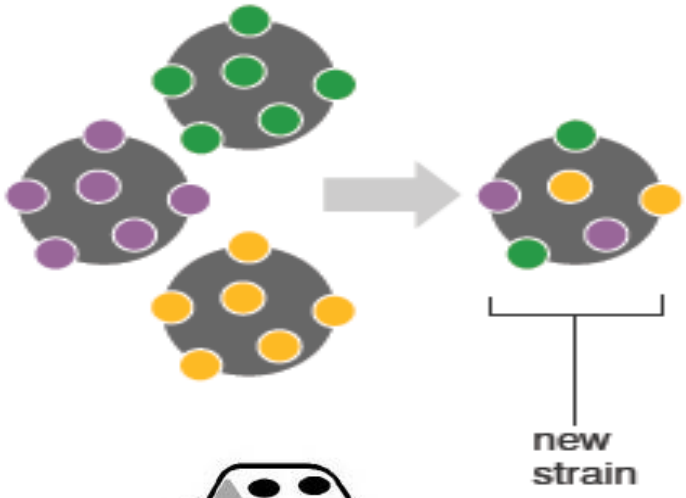


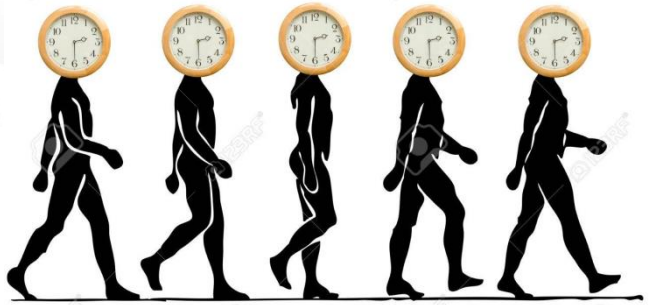
Mutation

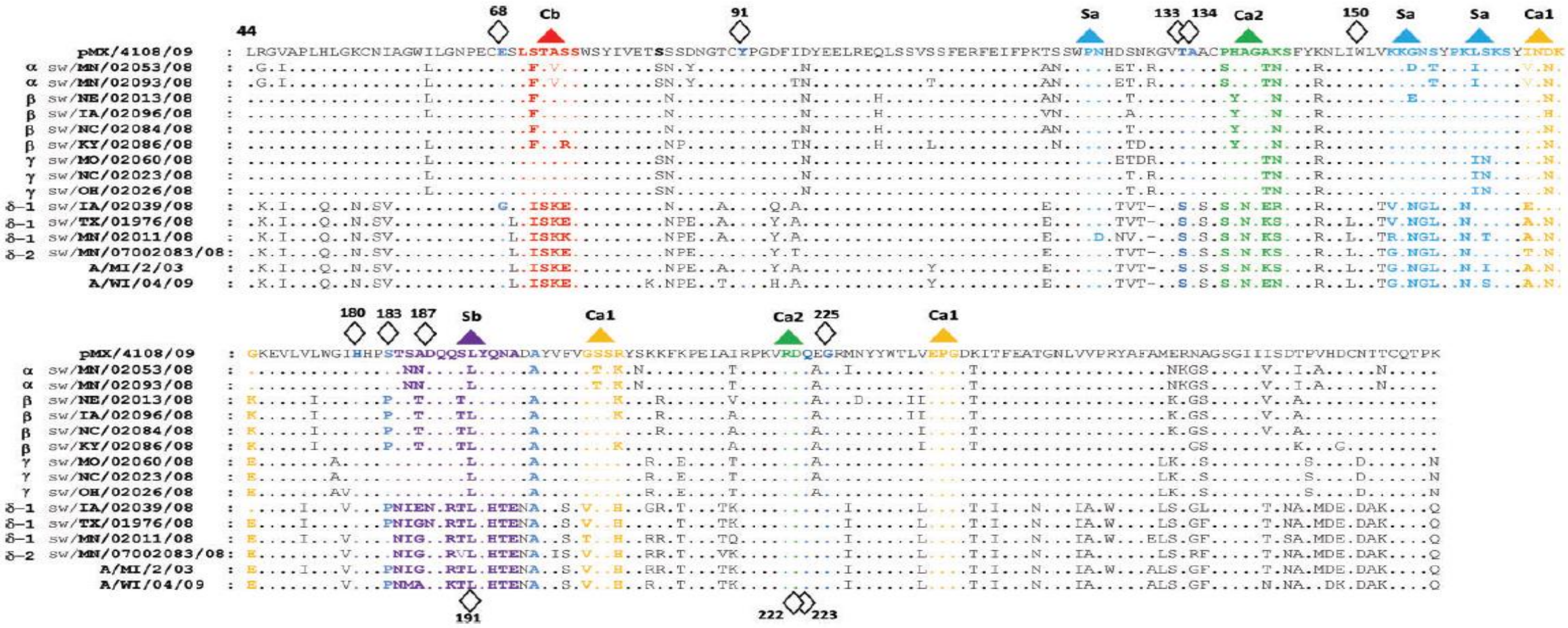
Antigenic drift



Antigenic shift






68
Cb
91
Sa
133
134
Ca2
150
Sa
Sa
Ca1
44
pMX/4108/09
α sw/MN/02053/08
α sw/MN/02093/08
β sw/NE/02013/08
β sw/IA/02096/08
β sw/NC/02084/08
β sw/KY/02086/08
γ sw/MO/02060/08
γ sw/NC/02023/08
γ sw/OH/02026/08
δ-1 sw/IA/02039/08
δ-1 sw/TX/01976/08
δ-1 sw/MN/02011/08
δ-2 sw/MN/07002083/08
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A/WI/04/09
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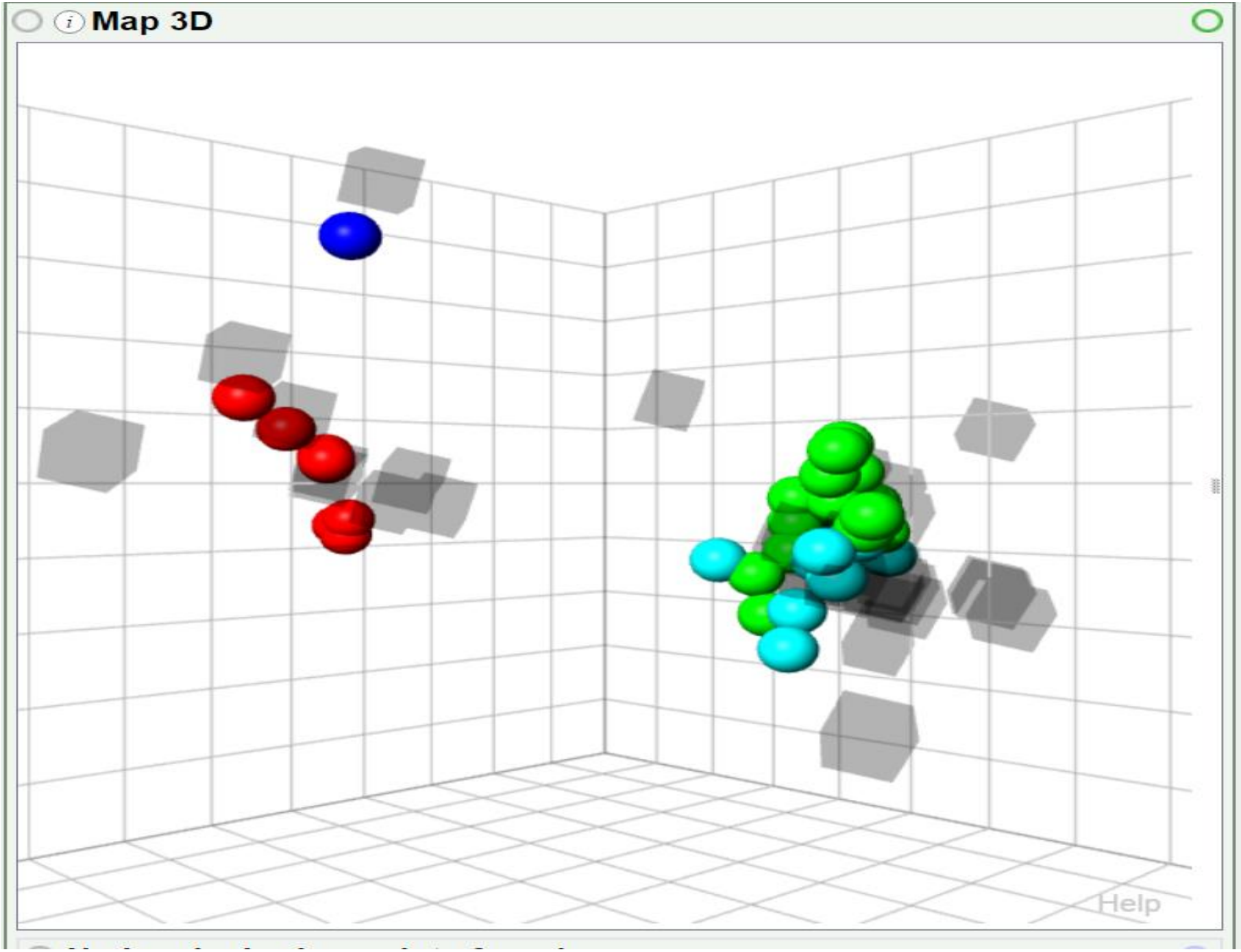
183
187
Sb
Ca1
Ca2
225
Ca1
191
222
223

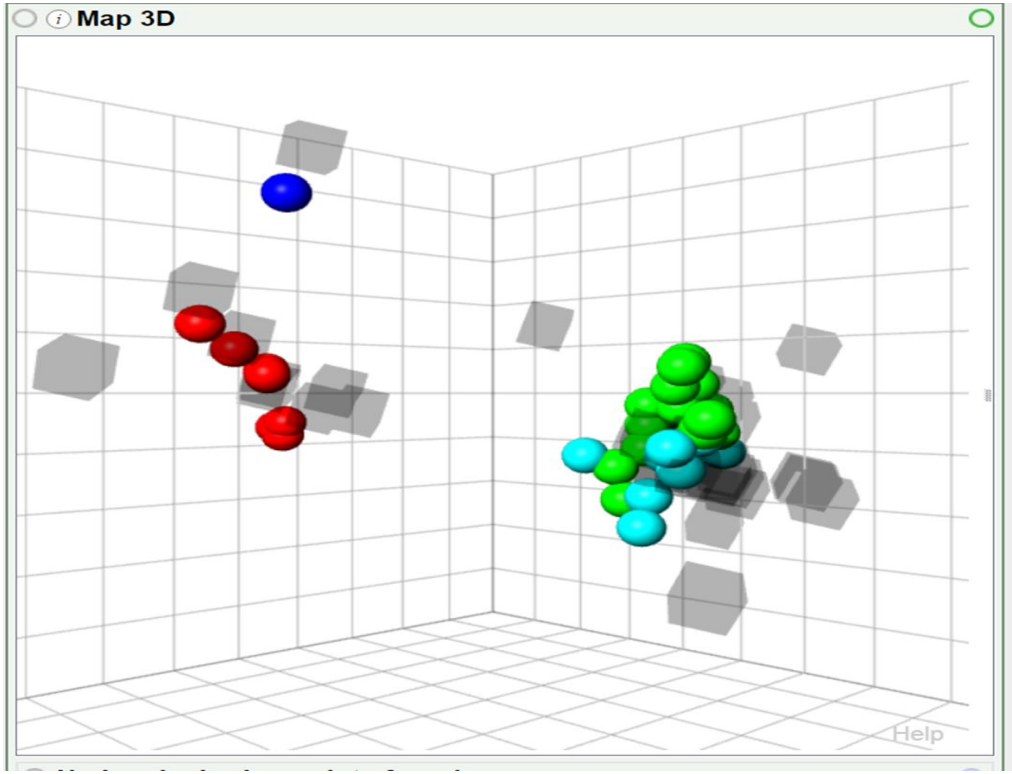


USUTU

JEV

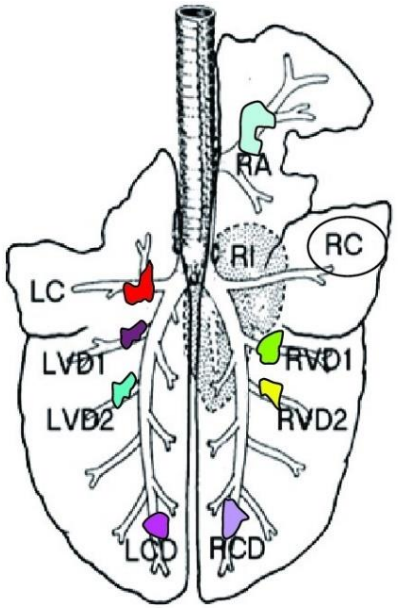
WNV L1

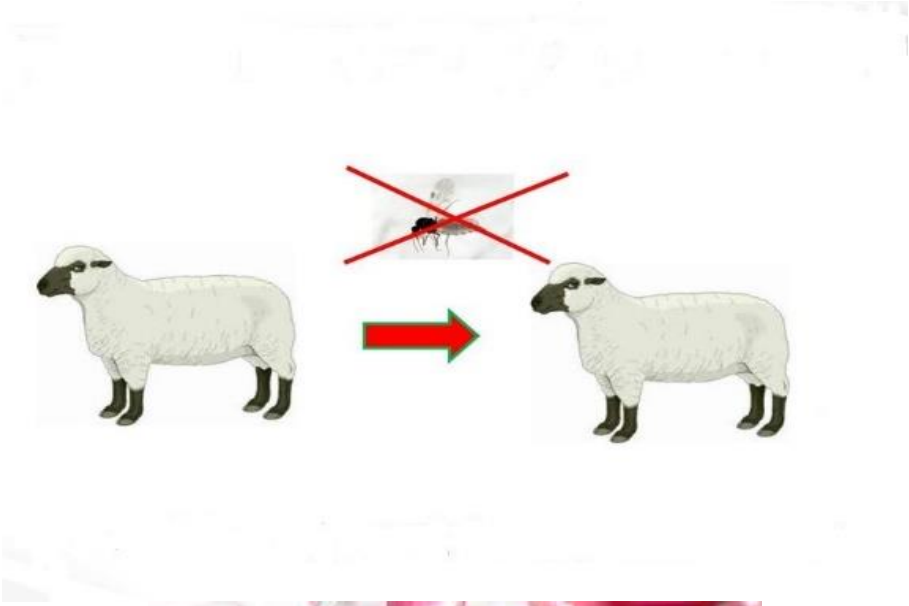
WNV L2

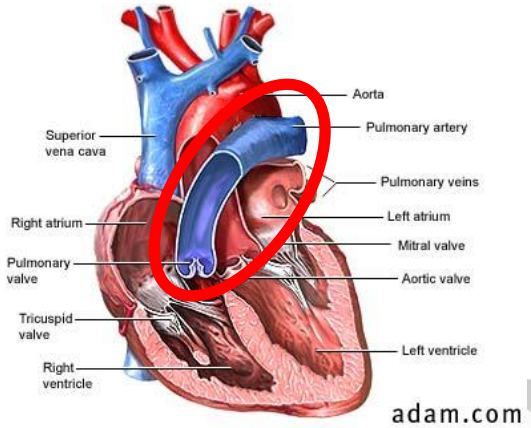

Map 3D
Help

+

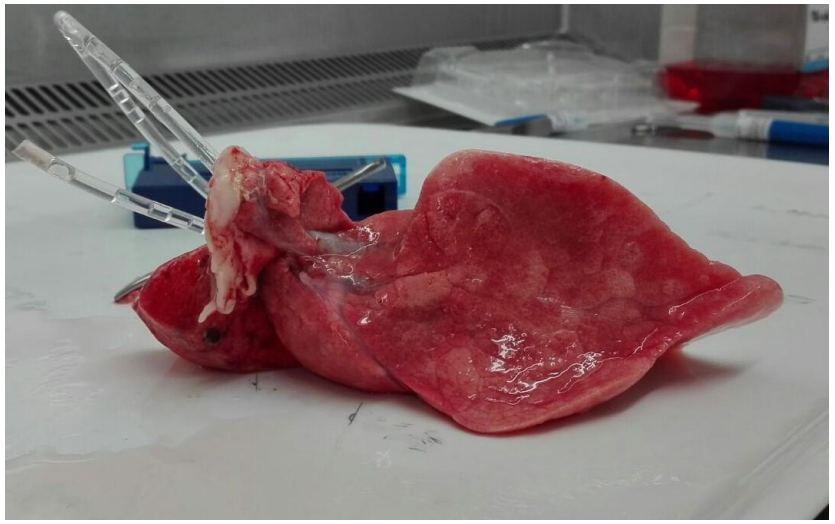
Sheep *Ex Vivo* Tissue Culture

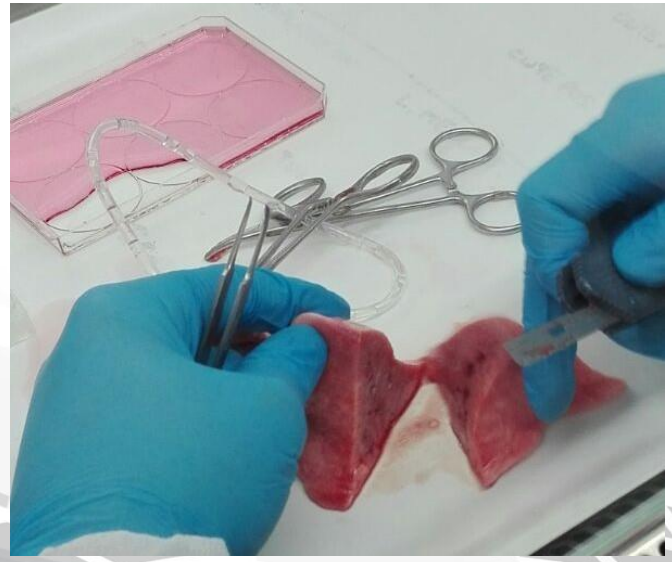




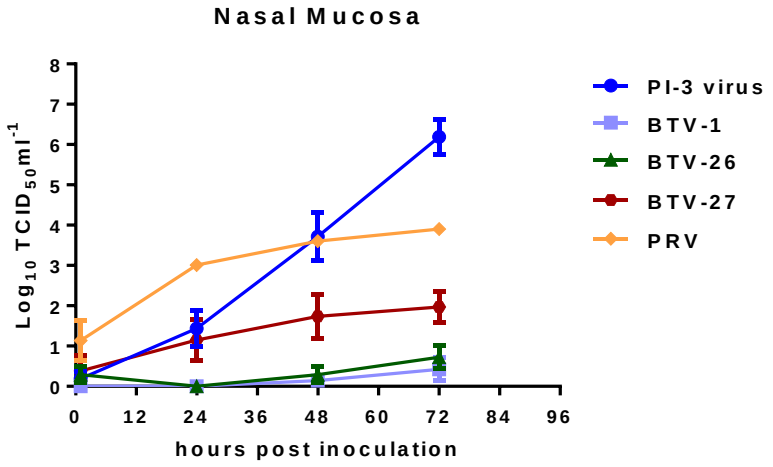


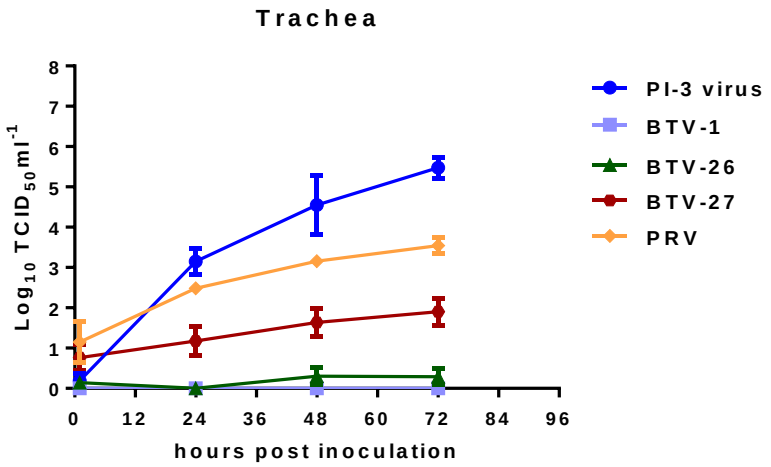
Messa a punto espianti:

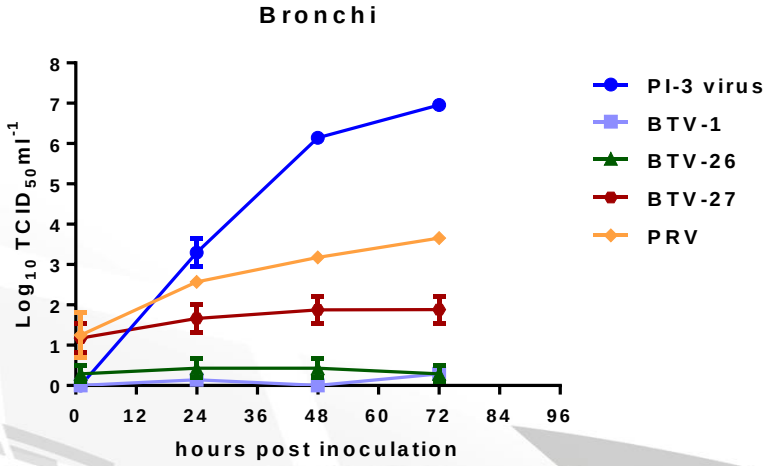


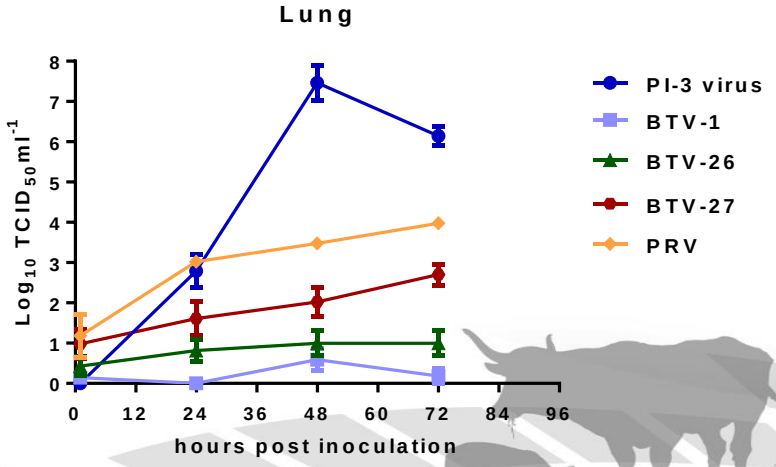


Replicazione virale: Espianti respiratori

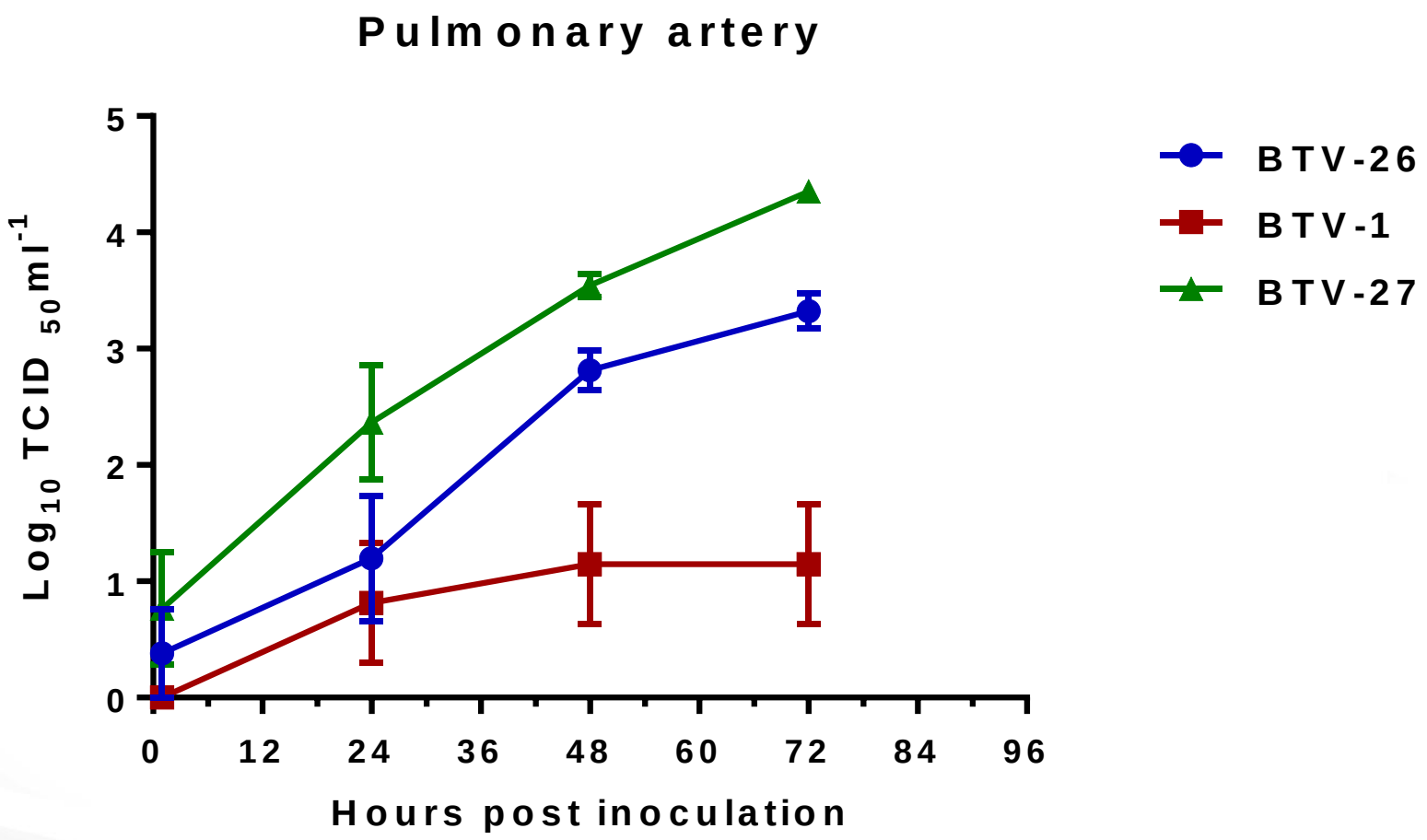




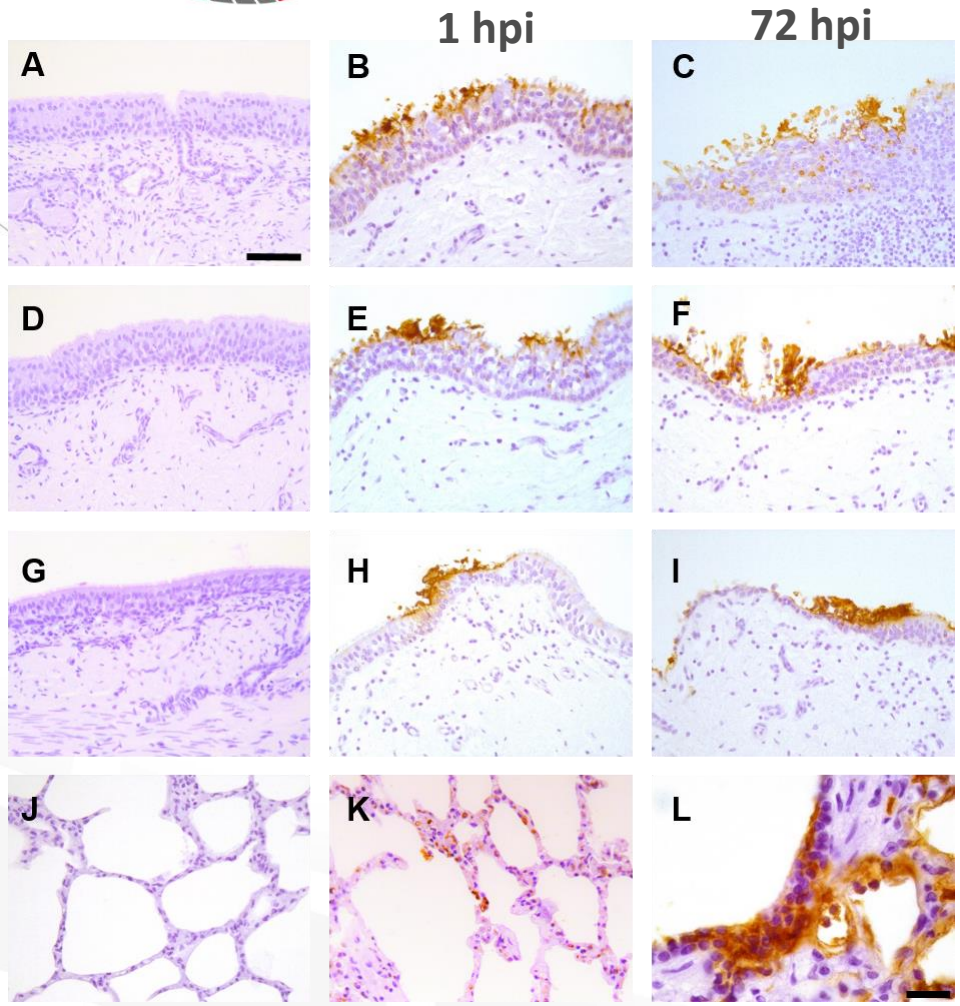


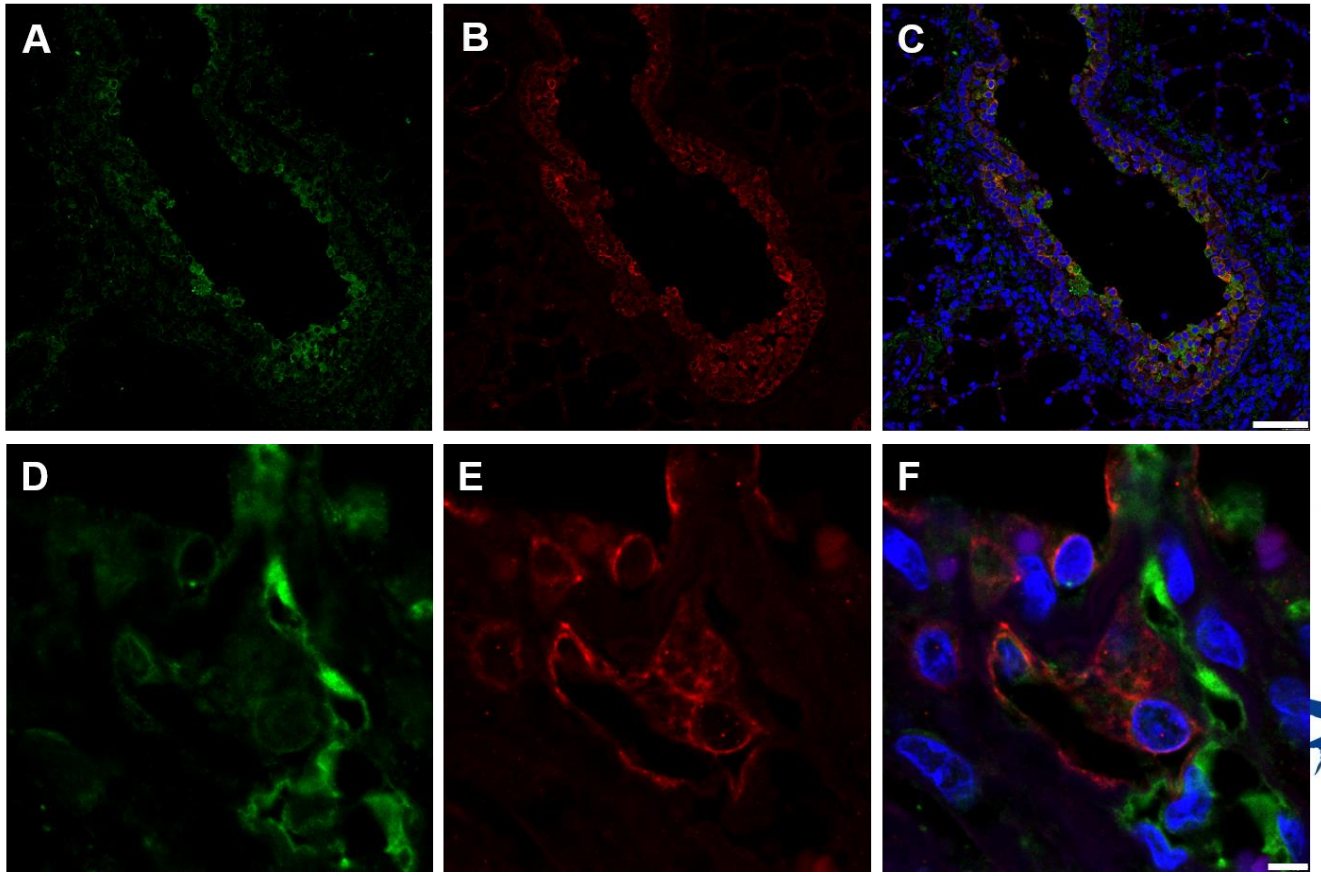


Replicazione virale: Espianti Arteriosi

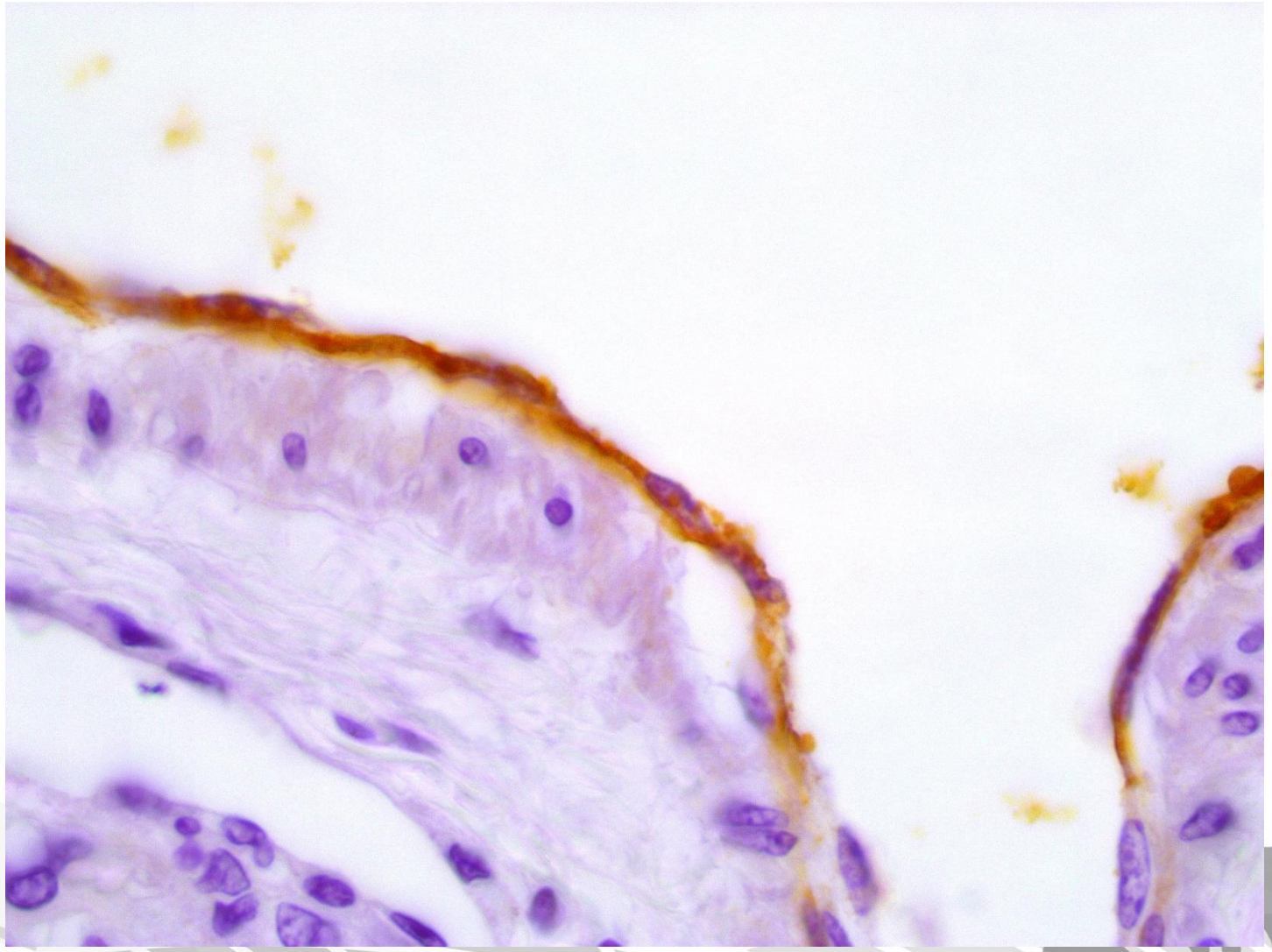


IHC e CLSM: Espianti Respiratori





IHC: Endotelio Espianti Arteriosi



IZSAM G.CAPORALE
TERAMO

GRAZIE PER L'ATTENZIONE