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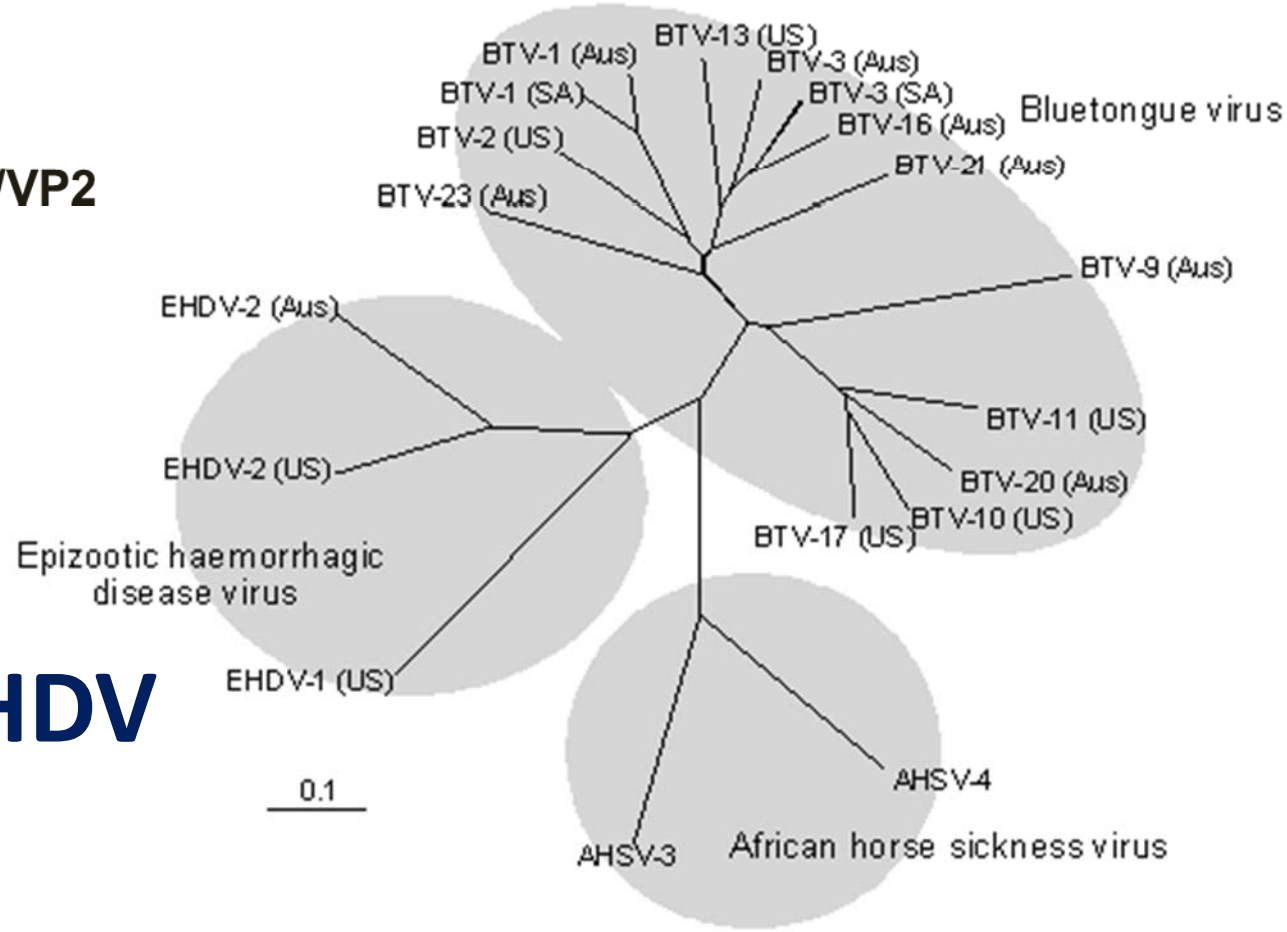
Uno tira l'altro: il caso dei sierotipi atipici del virus della Bluetongue

Alessio Lorusso

BTV (1-24)

Seg-2/VP2

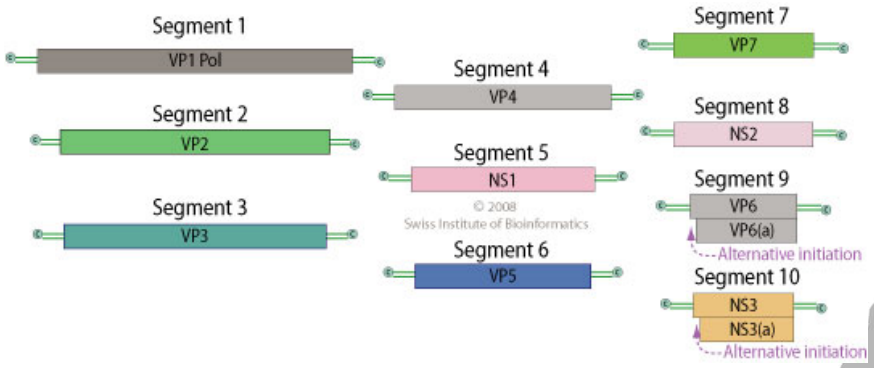


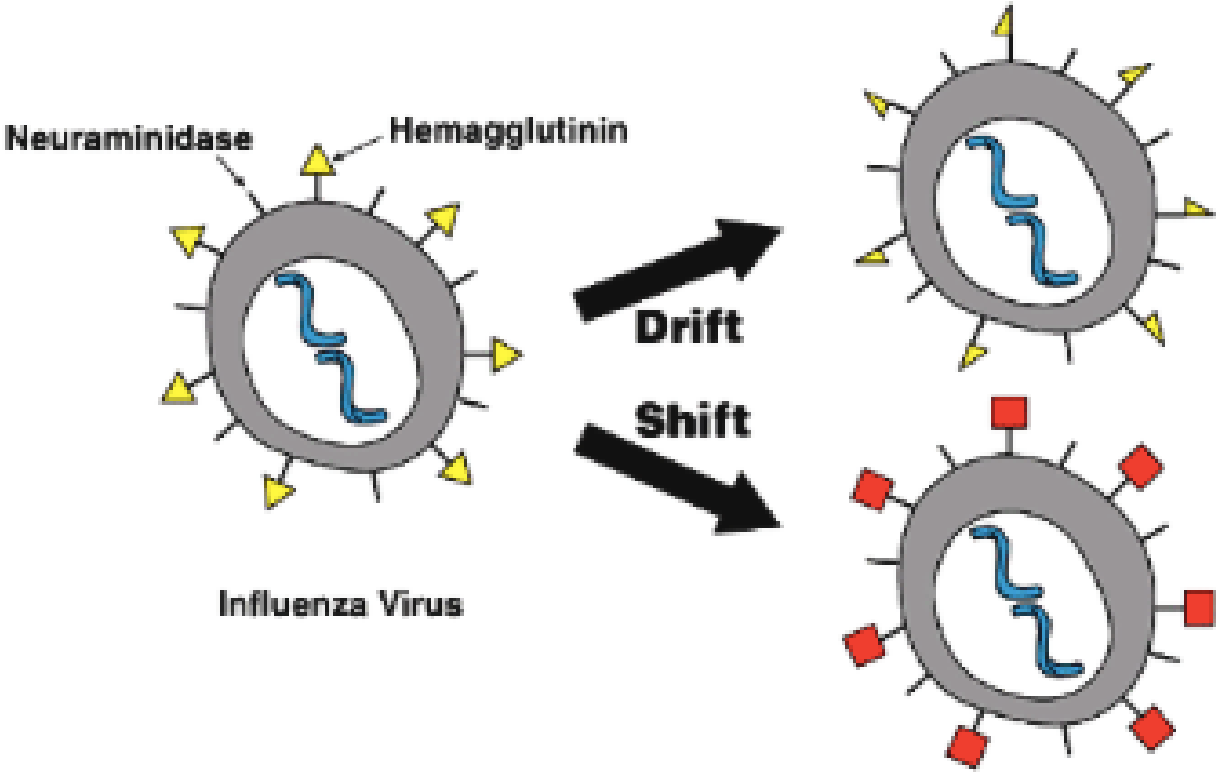
EHDV

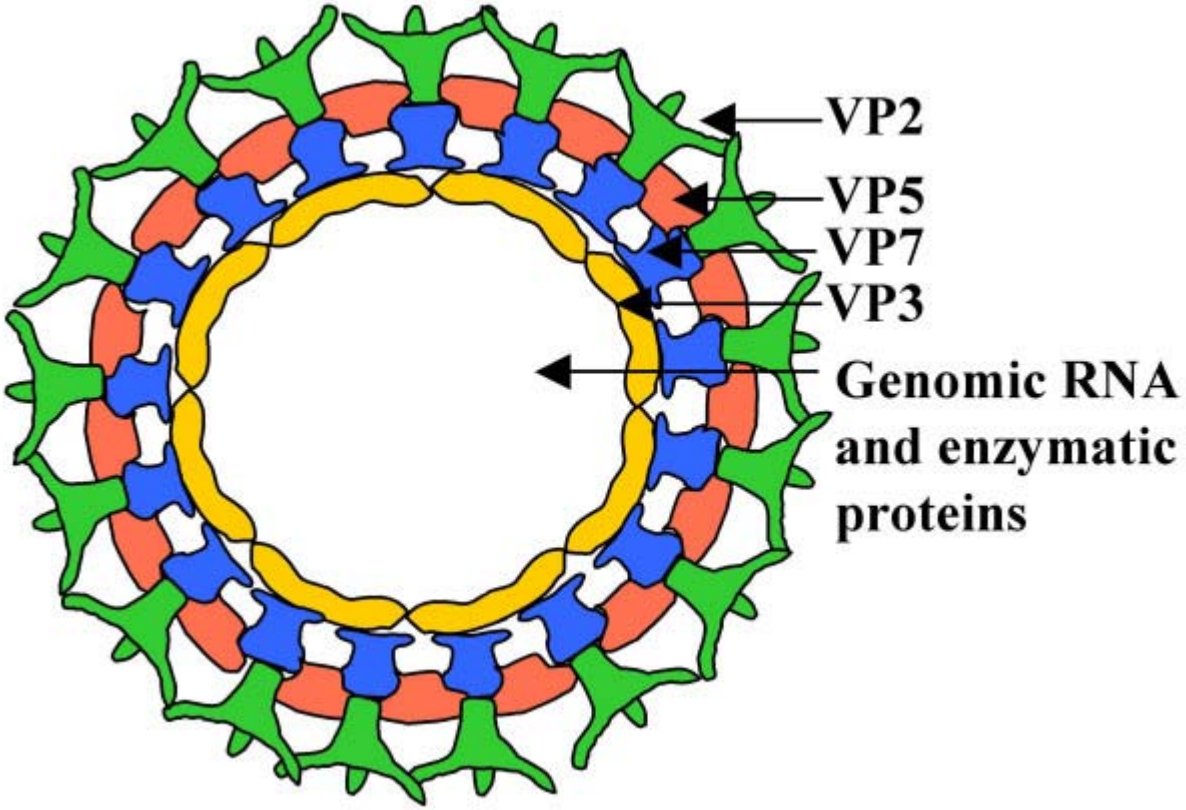
AHSV

TOPOTYPES, western or eastern strains

NUCLEOTYPES or lineages, VP2 and VP5




Neuraminidase
Hemagglutinin
Drift
Shift
Influenza Virus


VP2
VP5
VP7
VP3
Genomic RNA
and enzymatic
proteins

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- 2008
- Toggenburg
- BTV-25

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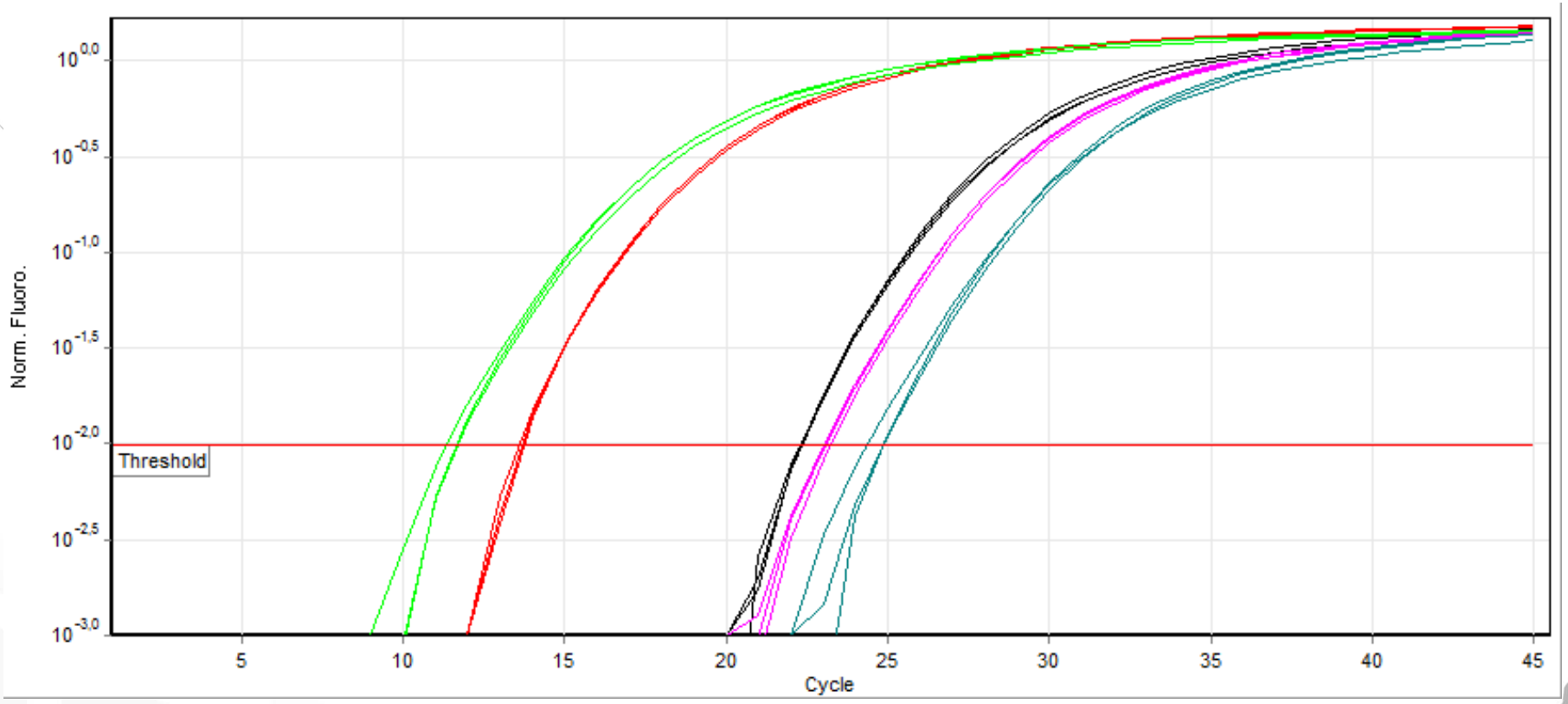

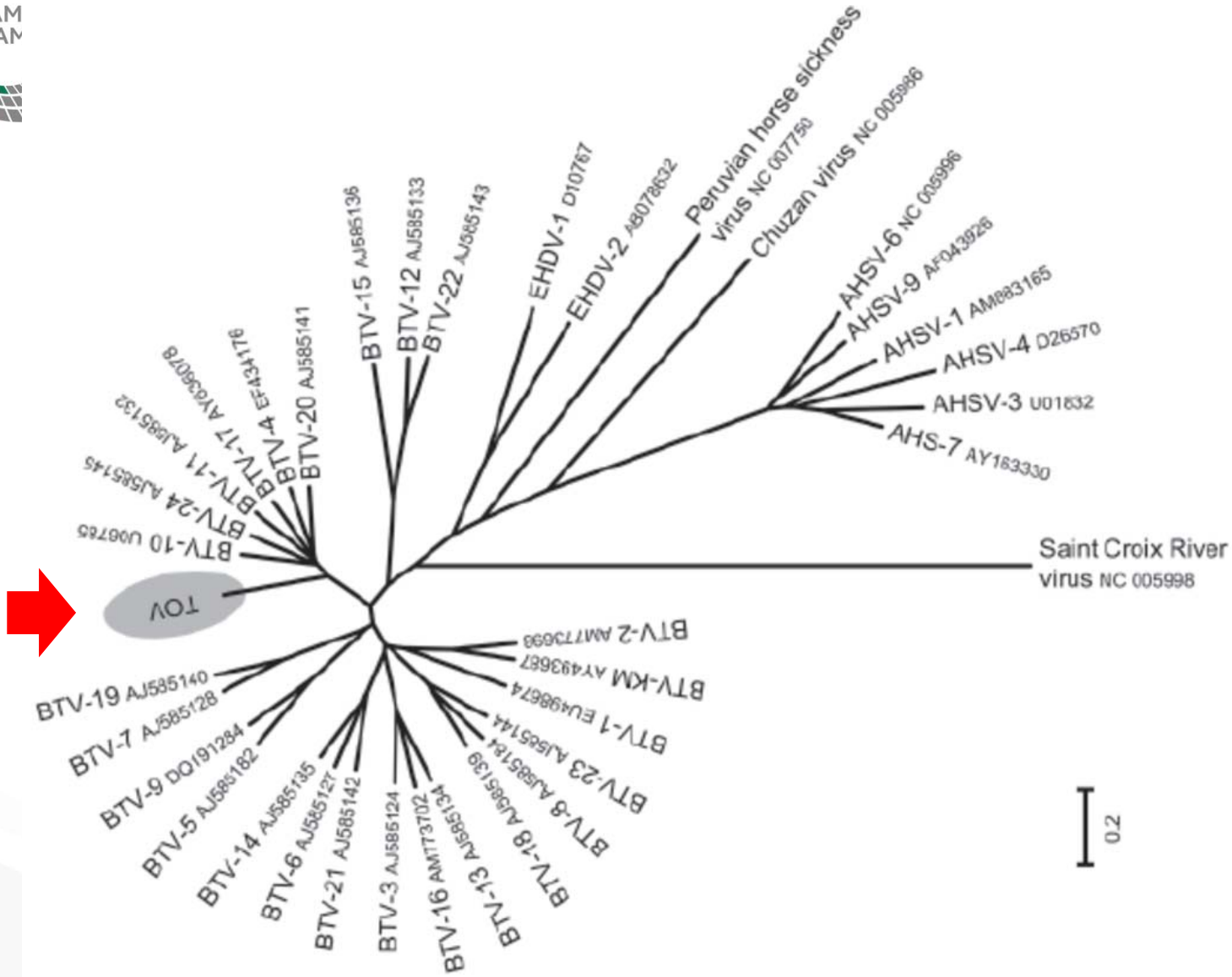
Peruvian horse sickness virus NC 007750
Chuzan virus NC 005986
AHSV-6 NC 005996
AHSV-9 AF043928
AHSV-1 AM883165
AHSV-4 D26570
AHSV-3 U01832
AHS-7 AY163330
Saint Croix River virus NC 005998
EHDV-1 D10767
EHDV-2 AB078632
BTV-15 AJ585136
BTV-12 AJ585133
BTV-22 AJ585143
BTV-20 AJ585141
BTV-4 EF434176
BTV-17 AY369078
BTV-11 AJ585132
BTV-24 AJ585145
BTV-10 U06765
TOV
BTV-19 AJ585140
BTV-7 AJ585128
BTV-9 DQ191284
BTV-5 AJ585182
BTV-14 AJ585135
BTV-6 AJ585127
BTV-21 AJ585142
BTV-3 AJ585124
BTV-16 AM773702
BTV-13 AJ585134
BTV-18 AJ585139
BTV-8 AJ585131
BTV-23 AJ585144
BTV-1 EU496674
BTV-KM AY493667
BTV-2 AM773699
0.2

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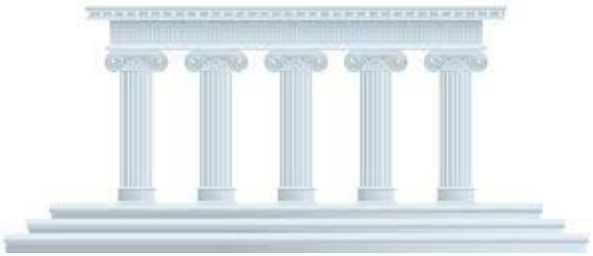
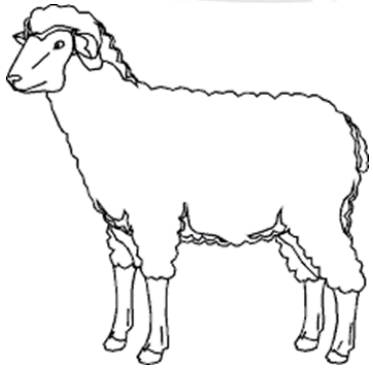
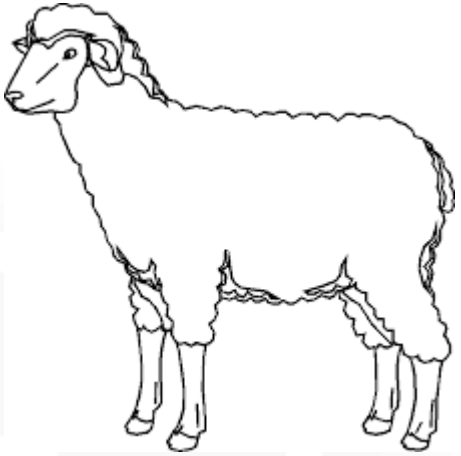


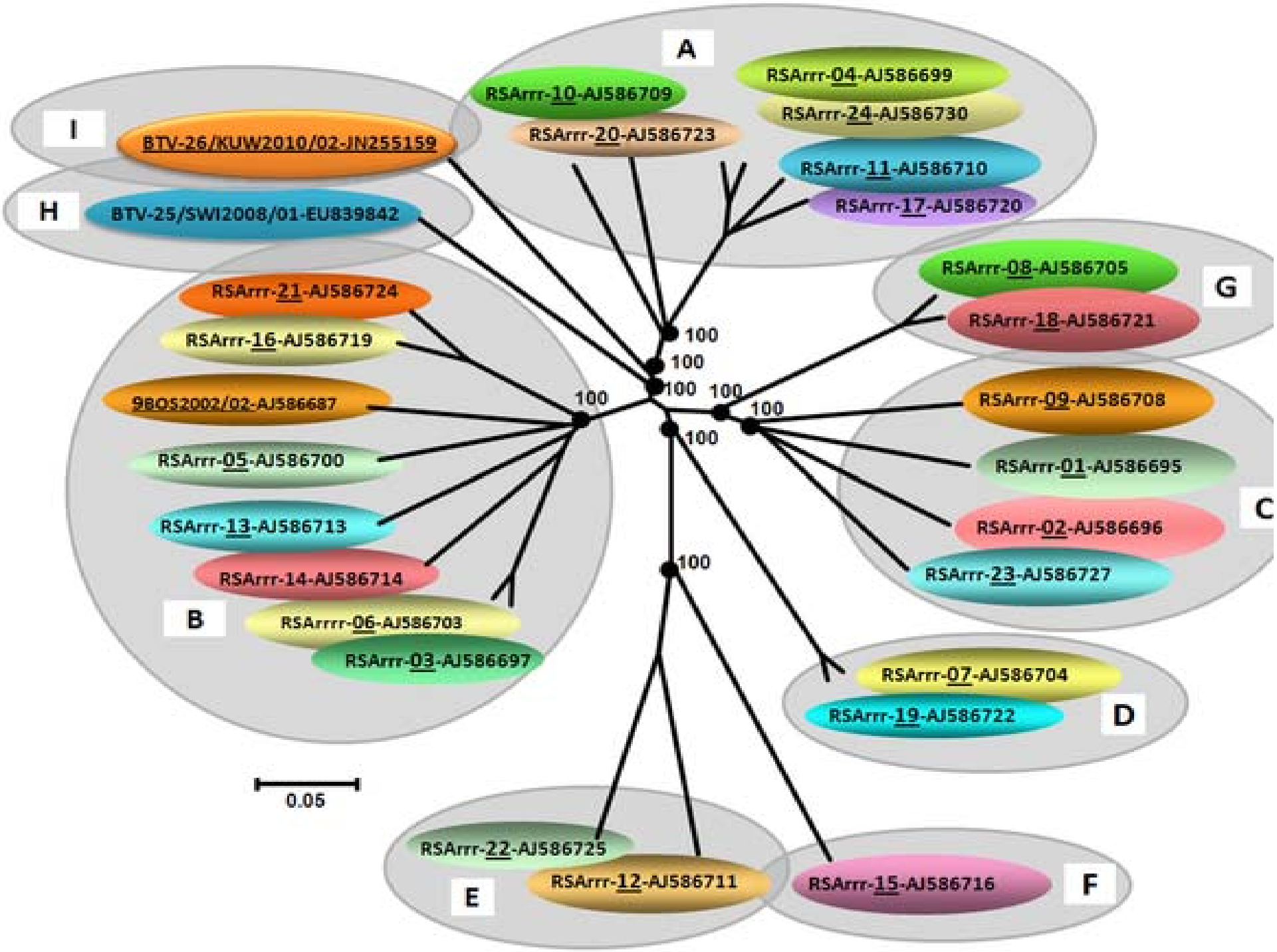
- **Subclinical infection in goats**
- **Mild signs in sheep (exp)**
- **Detection of BTV-25 genome up to 2 years in blood**
- **Does not grow on cell-culture**
- **Chimeric virus for serological screening**
- **Horizontal transmission?**

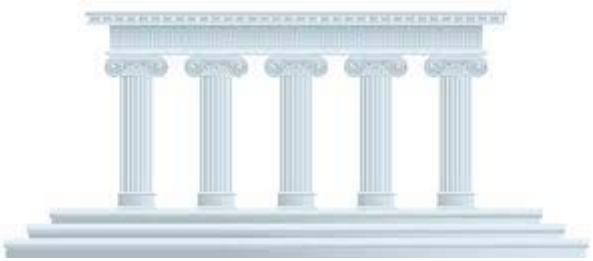


- Chimeric TOV (BTV-1)
- Sheep, no RNAemia
- Goats, RNAemic
- Both species have Abs
- Crosses the placental barrier
- High levels of RNA in the organs



- 2010
- Kuwait
- BTV-26


A
RSArrr-04-AJ586699
RSArrr-10-AJ586709
RSArrr-24-AJ586730
RSArrr-20-AJ586723
RSArrr-11-AJ586710
RSArrr-17-AJ586720
I
BTV-26/KUW2010/02-JN255159
H
BTV-25/SWI2008/01-EU839842
G
RSArrr-08-AJ586705
RSArrr-18-AJ586721
RSArrr-21-AJ586724
RSArrr-16-AJ586719
98OS2002/02-AJ586687
RSArrr-05-AJ586700
RSArrr-13-AJ586713
RSArrr-14-AJ586714
B
RSArrrr-06-AJ586703
RSArrr-03-AJ586697
C
RSArrr-09-AJ586708
RSArrr-01-AJ586695
RSArrr-02-AJ586696
RSArrr-23-AJ586727
D
RSArrr-07-AJ586704
RSArrr-19-AJ586722
E
RSArrr-22-AJ586725
RSArrr-12-AJ586711
F
RSArrr-15-AJ586716
100
100
100
100
100
100
100
0.05



- **Mild signs in sheep, short RNAemia**
- **Mild signs in goats, longer and higher RNAemia**
- **It does not grow on insect cells**
- **Does grow straight onto mammalian cells**
- **It is not transmitted by insect**
- **Horizontal transmission**

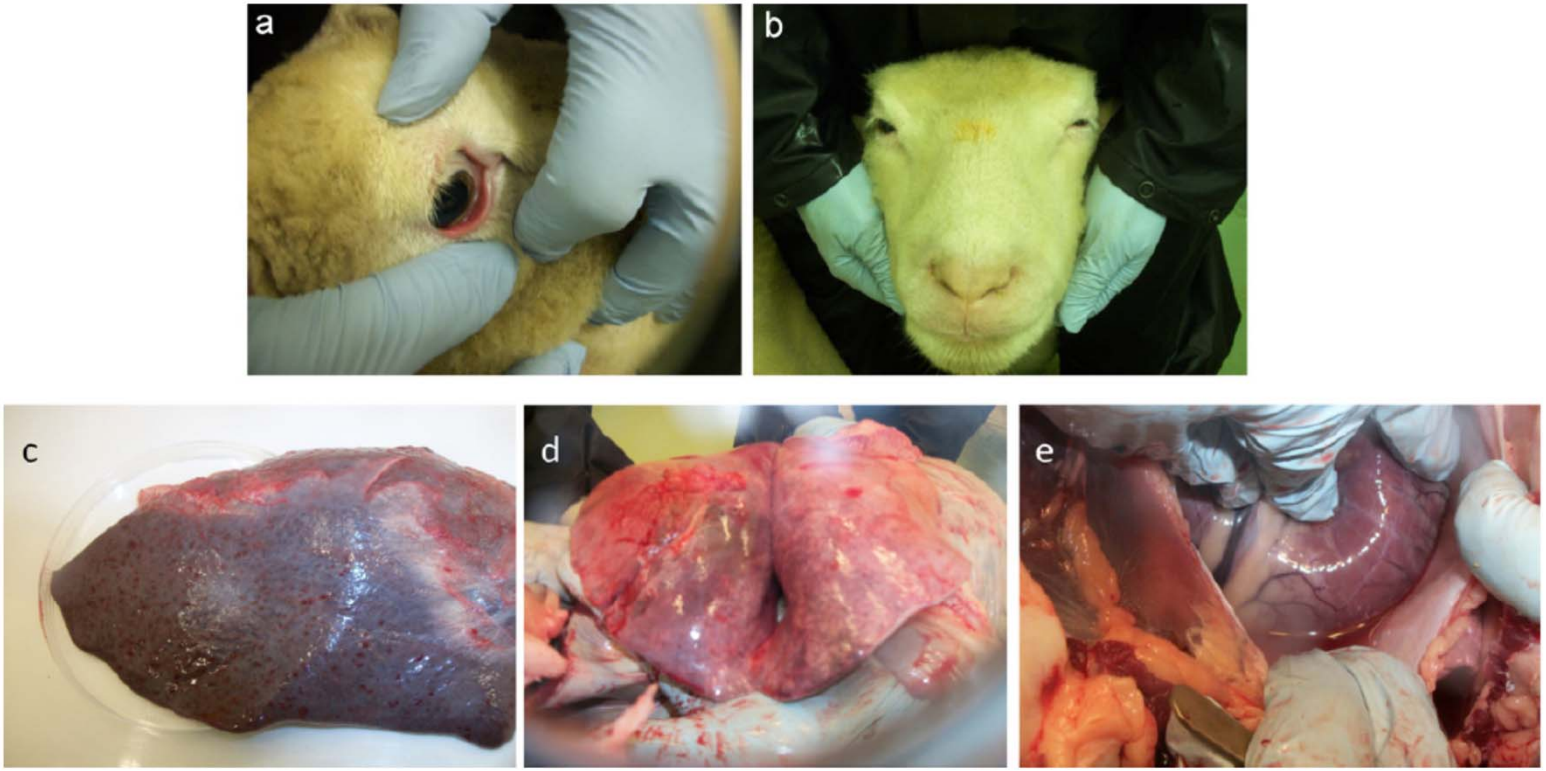
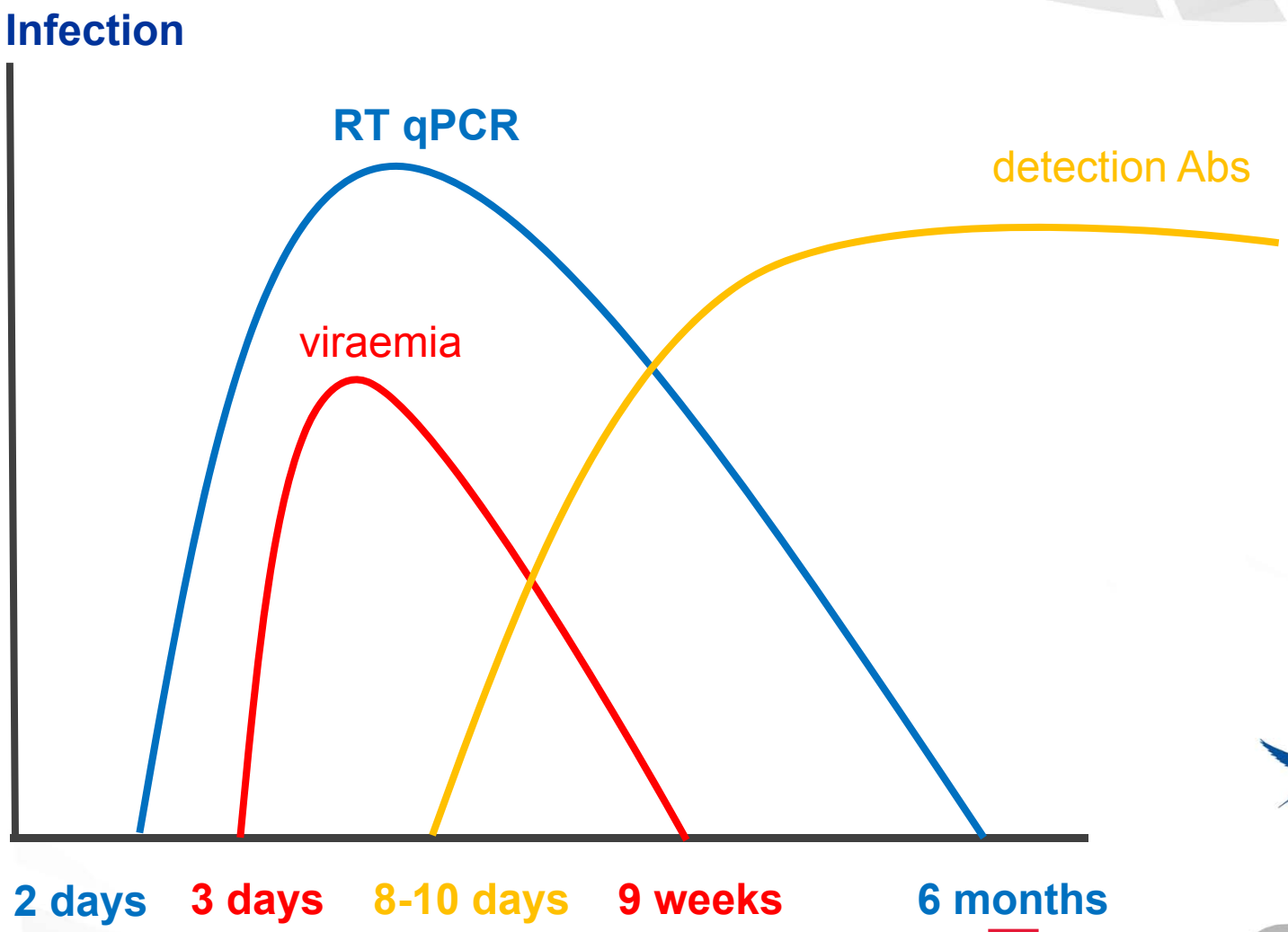


Fig. 2. Clinical and pathological signs in bluetongue virus serotype 26 (BTV-26) infected sheep. (a) Mild conjunctivitis, (b) slight oedema of the face, (c) haemorrhagic lesions on the spleen, (d) interstitial oedema in lungs and (e) build up of fluid in the pericardium.

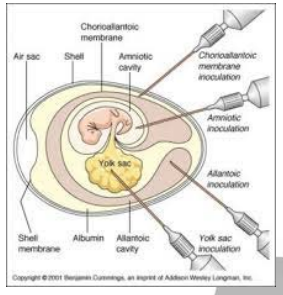

Infection
RT qPCR
detection Abs
viraemia
2 days
3 days
8-10 days
9 weeks
6 months
Classical serotypes

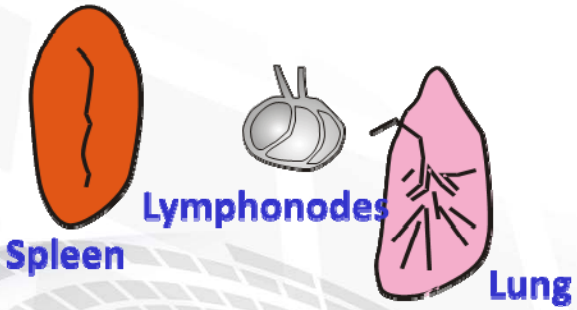
BTV ISOLATION

KC

Mammalian cells

VERO
BHK21





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journal homepage: www.elsevier.com/locate/meegid

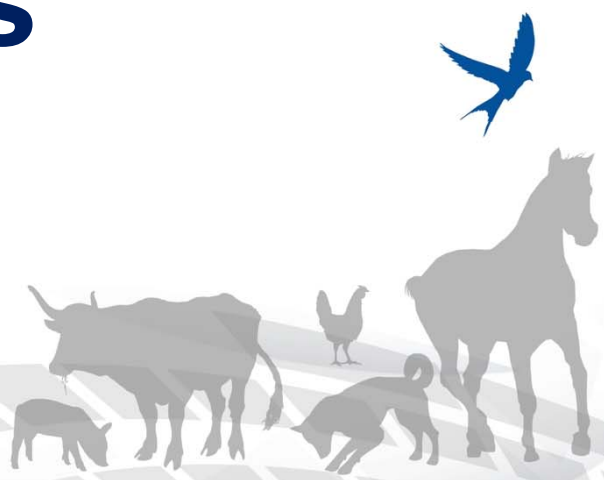
Short communication

Bluetongue virus surveillance in the Islamic Republic of Mauritania: Is serotype 26 circulating among cattle and dromedaries?

Alessio Lorusso [a,*], Doumbia Baba [b], Massimo Spedicato [a], Liana Teodori [a], Barbara Bonfini [a], Maurilia Marcacci [a], Andrea Di Provvido [a], Katia Isselmou [b], Valeria Marini [a], Irene Carmine [a], Massimo Scacchia [a], Daria Di Sabatino [a], Antonio Petrini [a], Beyatt Ahmed Bezeid [b], &, Giovanni Savini [a]

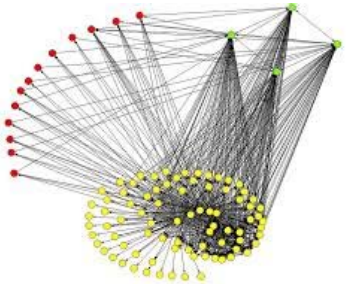
[a] OIE Reference Laboratory for Bluetongue, Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise, Teramo (IZSAM)-Italy
[b] Centre National d'Elevage et de Recherches Vétérinaires (CNERV), Nouakchott, Mauritania

- **Dromedaries**
- **Cattle**

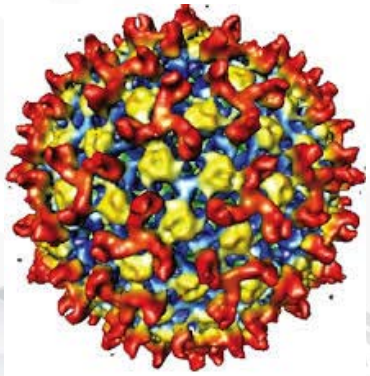


BTV-27

Healthy



At least 3 variants (92% nt id Seg2)



Single serotype

Schulz et al., 2016; Zientara et al., 2014

- **I colleghi più fidati**
- **Non deludono mai**
- **Materiale di prima qualità**
- **W la Sardegna**

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Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

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

<u>2 Farms:</u>

F1: Silius (CA) 170 goats, 1 sheep

F2: San Vito (CA) 165 goats

Farm 1 2015: 17 sentinel animals

March  **X 17** **- cElisa** 

September    **+ cElisa, -SN**

Healthy!

Whole blood

…..from the entire group (17)

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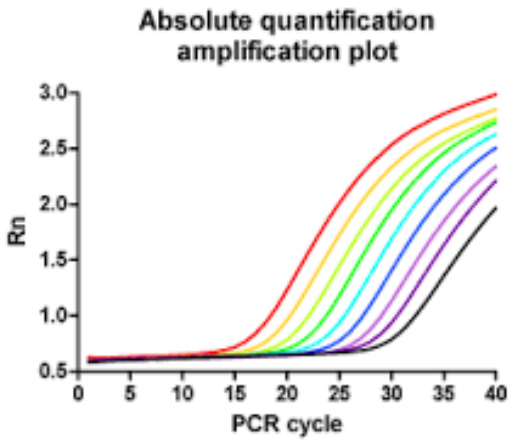
RT-qPCR $_{1\text{-}27}$ (Seg10)

POS  **X 9/17**



RT-qPCR $_{1\text{-}24}$ (Seg5)

NEG  **X 17/17**

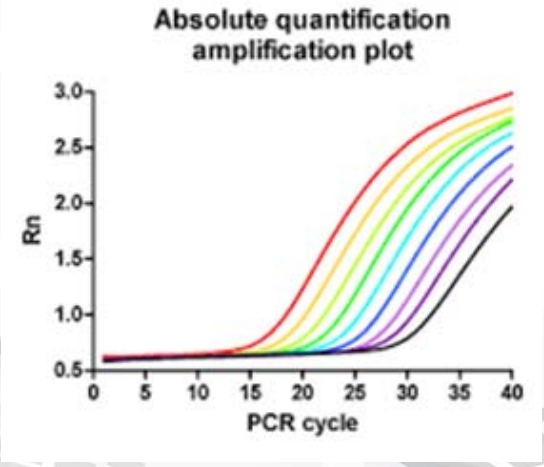
European strains (Seg2)

NEG  **X 17/17**

October 2015

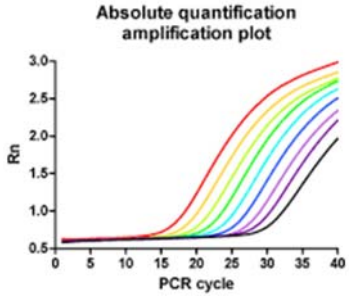
X 8/17 + cElisa, -SN

X 13/17 + RTqPCR $_{1\text{-}27}$; - RTqPCR $_{1\text{-}24}$; -RTqPCR $_{Seg2}$



December 2015

X 90/107 + cElisa, -SN

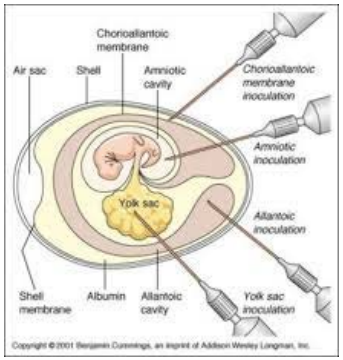


X 37/107

+ RTqPCR $_{1\text{-}27}$; - RTqPCR $_{1\text{-}24}$; -RTqPCR $_{S}$

Similar scenario in Farm 2

Isolation attempts



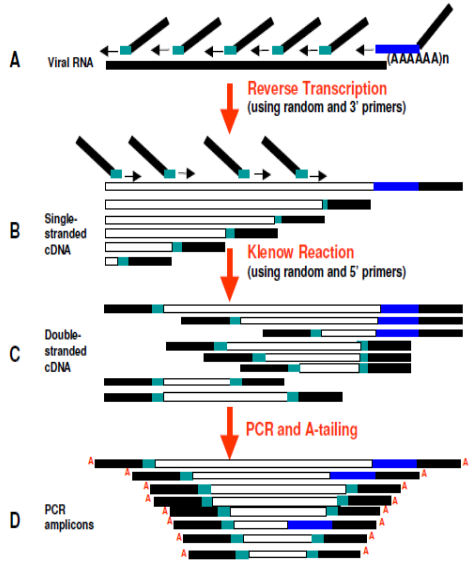
ECE

Cell lines
(KC, BHK-21, BSR)

Whole Genome Sequencing by NGS

2 blood samples (from F1 and F2) with the lowest Ct RT-qPCR $_{1\text{-}27}$ values

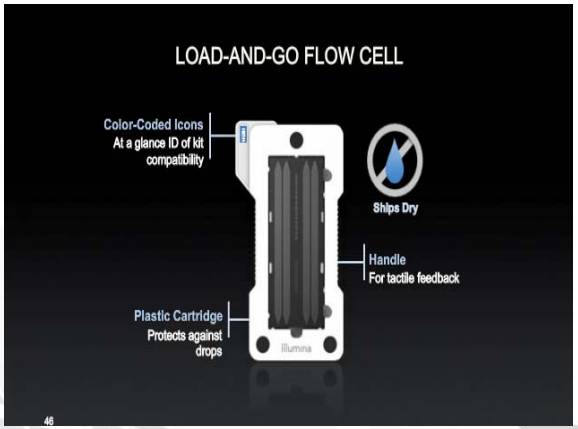
→ **RNA** →



←

NextSeq 500 Illumina

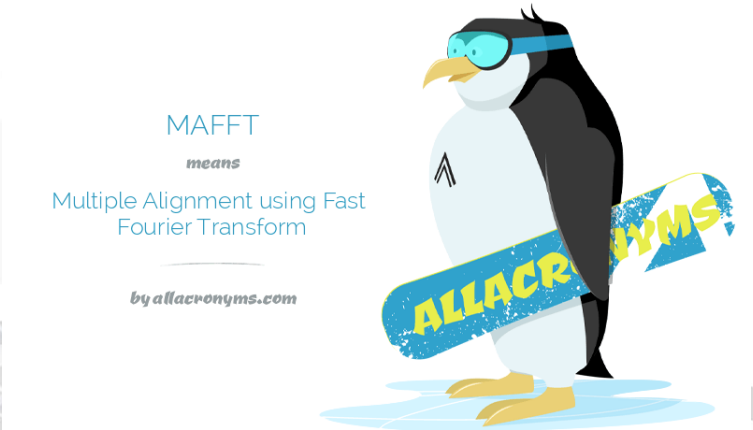
Nextera XT Library preparation

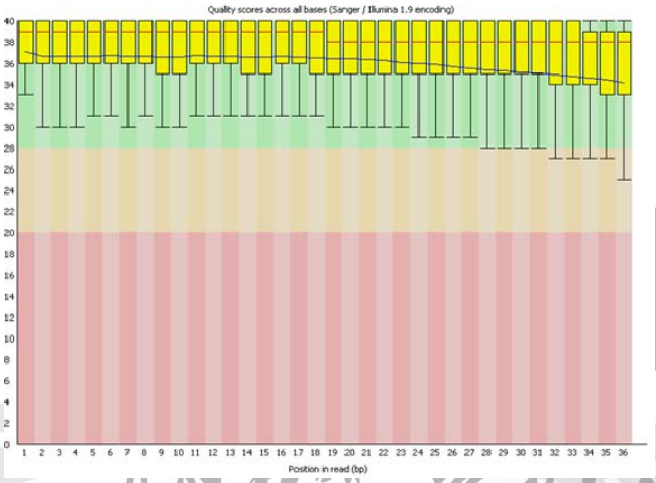


300 cycles
150 paired-end reads
Run 26 hours

BTVX ITL2015

- **Reads: 5 GB**
- **Deep Coverage 150 X**
- ***de novo* analysis**
- **Nearly the whole genome was obtained (90%)**
- **GTTAAA; CTTAC**





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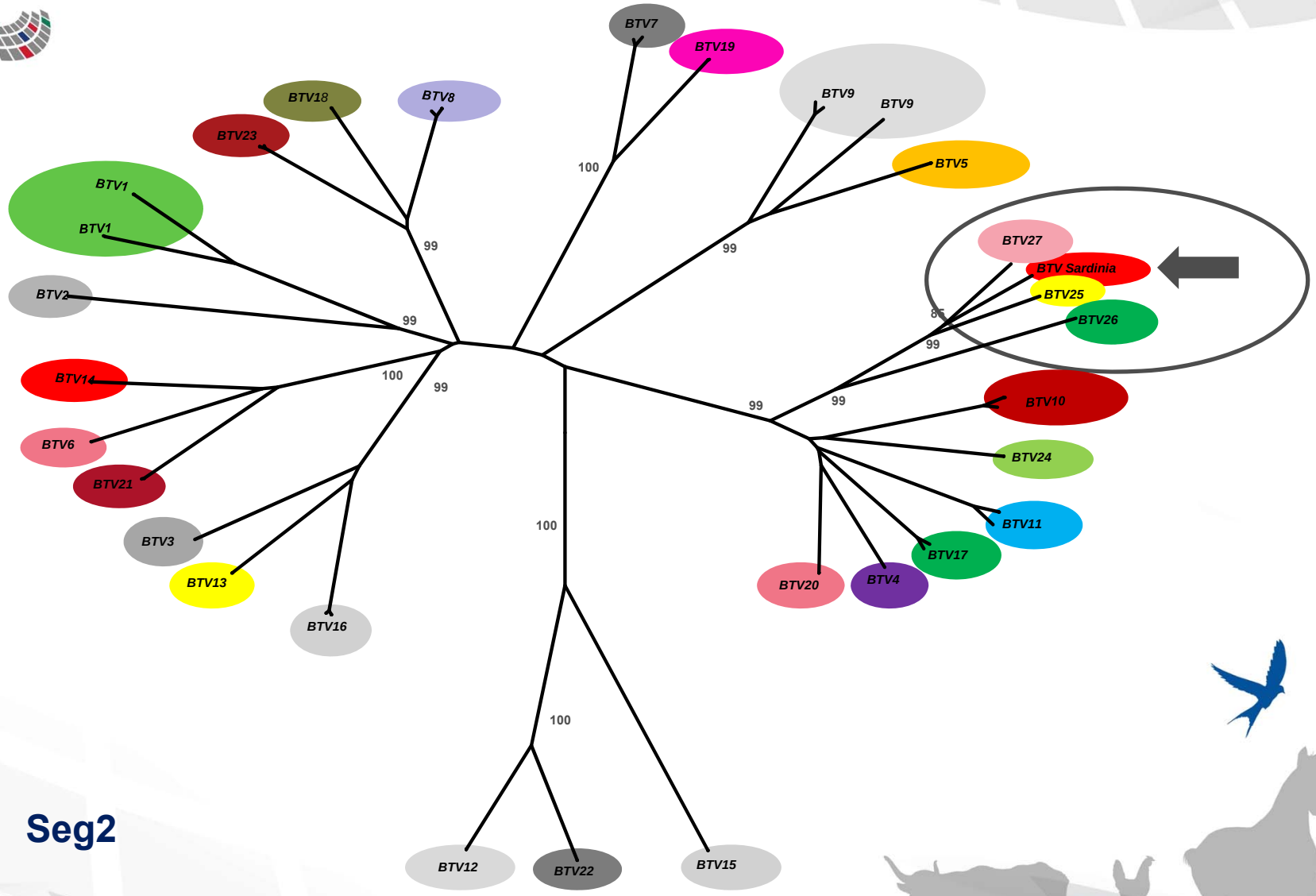


Seg2

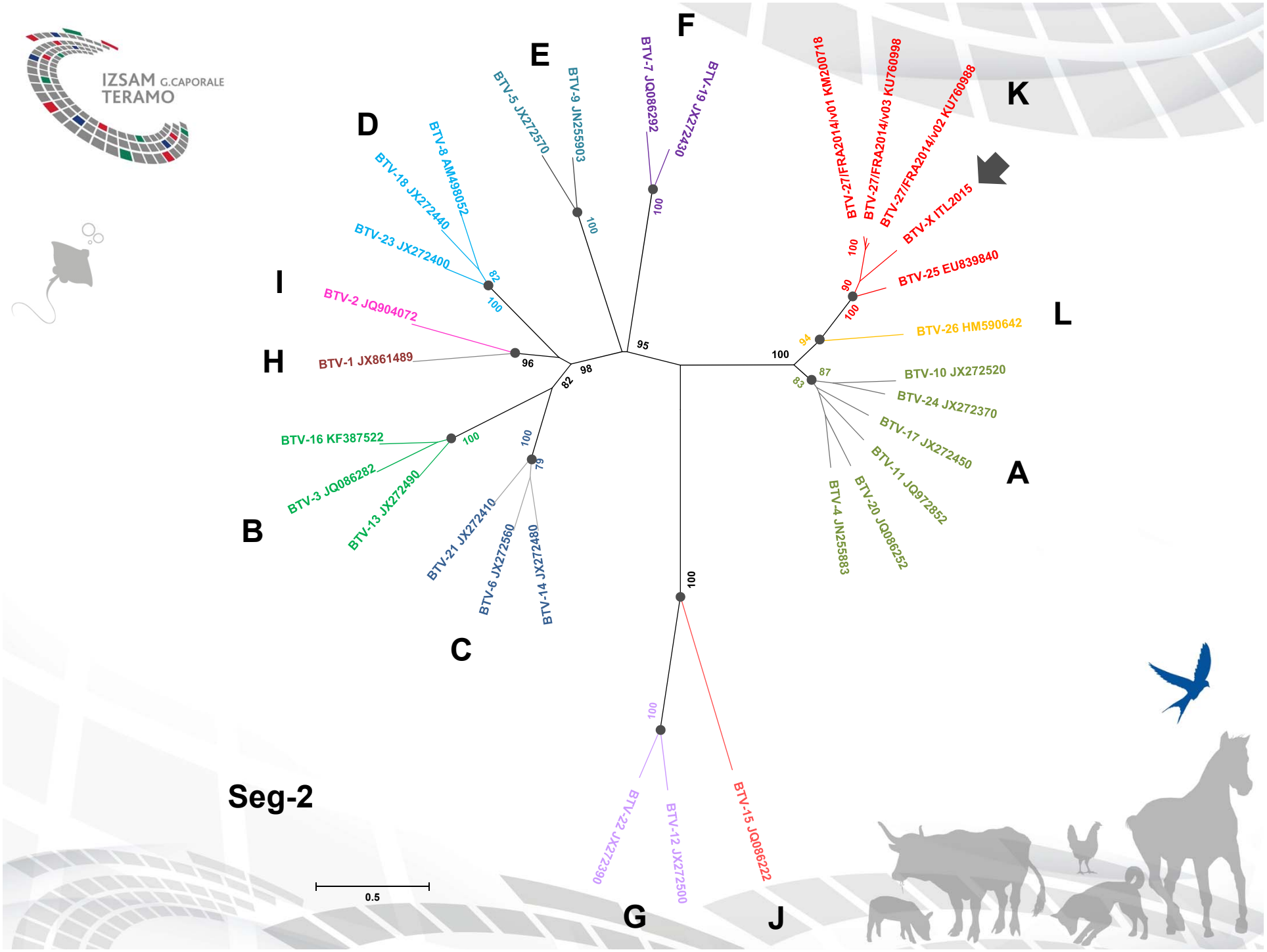
Seg 2/ VP 2

BTV-X ITL2015 Seg 2 VP2 KX234079 -It lacks 16 aa at 5'end and 82 aa at 3'end	BTV-27 KM200718 BTV-27/FRA2014/v02 KU760988 BTV-27/FRA2014/v03 BTV-25 EU839840 BTV-26 KUW2010/02 HM590642	75.3/77.1 75.3/76.0 74.2/77.3 73.1/74.0 63.1/60.0

Seg 6/ VP 5

BTV-X ITL2015 Seg 6 VP5 KX234083 -It lacks 39 aa at 5'end -Partial 3'end NCR	BTV-27/FRA2014/v03 KU761002 BTV-27/FRA2014/v02 KU760992 BTV-26 KUW2010/02 JN255159 BTV-25 EU839842 BTV-27 LN713675	84.2/93.7 84.2/93.7 73.7/81.6 70.2/76.3 70.8/75.3

Only Seg 3 and Seg 4 were obtained from farm 2; 100% nt id with Farm 1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Seg-2
0.5
A
B
C
D
E
F
G
H
I
J
K
L
BTV-1 JX861489
BTV-2 JQ904072
BTV-3 JQ086282
BTV-4 JN255883
BTV-5 JX272570
BTV-6 JX272560
BTV-7 JQ086292
BTV-8 AM498052
BTV-9 JN255903
BTV-10 JX272520
BTV-11 JQ972852
BTV-12 JX272500
BTV-13 JX272490
BTV-14 JX272480
BTV-15 JQ086222
BTV-16 KF387522
BTV-17 JX272450
BTV-18 JX272440
BTV-19 JX272430
BTV-20 JQ086252
BTV-21 JX272410
BTV-22 JX272390
BTV-23 JX272400
BTV-24 JX272370
BTV-25 EU839840
BTV-26 HM590642
BTV-27/FRA2014/v01 KM200718
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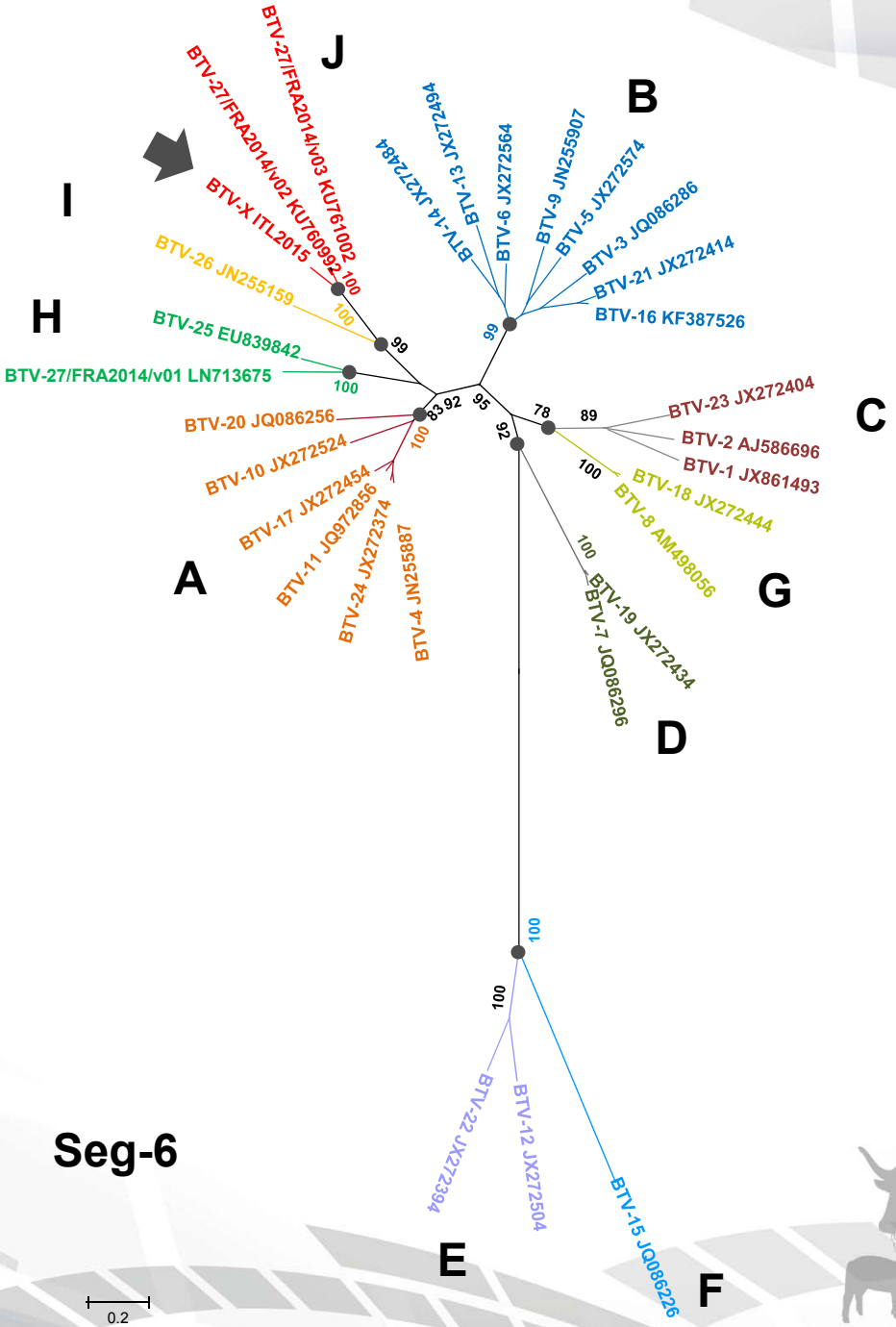
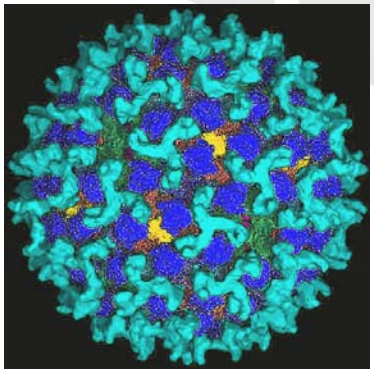
BTV-27/FRA2014/v02 KU760988
BTV-X ITL2015


J
BTV-27/FRA2014/v03 KU761002
BTV-27/FRA2014/v02 KU760992
I
BTV-X ITL2015
BTV-26 JN255159
H
BTV-25 EU839842
BTV-27/FRA2014/v01 LN713675
BTV-20 JQ086256
BTV-10 JX272524
BTV-17 JX272454
BTV-11 JQ972856
BTV-24 JX272374
BTV-4 JN255887
A
BTV-14 JX272484
BTV-13 JX272494
BTV-6 JX272564
BTV-9 JN255907
BTV-5 JX272574
BTV-3 JQ086286
BTV-21 JX272414
BTV-16 KF387526
B
BTV-23 JX272404
BTV-2 AJ586696
BTV-1 JX861493
BTV-18 JX272444
BTV-8 AM498056
C
G
BTV-19 JX272434
BTV-7 JQ086296
D
BTV-22 JX272394
BTV-12 JX272504
BTV-15 JQ086226
E
F
0.2

Seg-6



- **The new BTV virus clusters with BTV-25, BTV-26 and BTV-27**
- **<u>No serological cross-reactions with any of the previously known serotypes</u>**
- **84% of cElisa positive animals at the end of the analysis**
- **Probable direct contact trasmission within goats?**
- **No vectors available for screening**

Transbound Emerg Dis. 2016 Sep 5. doi: 10.1111/tbed.12560. [Epub ahead of print]

Emergence of a Novel Bluetongue Virus Serotype, China 2014.

Sun EC[1], Huang LP[1], Xu QY[1], Wang HX[1], Xue XM[2], Lu P[3], Li WJ[4], Liu W[4], Bu ZG[1], Wu DL[5].

Author information

Abstract

One hundred and twenty-six blood samples were collected from healthy sheep and goats in Xinjiang, China, during July 2014. Seventy-three samples (57.93%) were bluetongue virus (BTV) serology-positive, and 39 samples (30.95%) were BTV NS1 gene-positive. BTV strain XJ1407 was isolated from the blood of BTV NS1 gene-positive animals and sequenced. Analysis of its genome sequence suggests that XJ1407 is a novel BTV serotype.

KEYWORDS: bluetongue virus; epidemiology; new serotype

PMID: 27597166 DOI: 10.1111/tbed.12560

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Detection and isolation of Bluetongue virus from commercial vaccine batches

Velizar Bumbarov, Natalia Golender, Oran Erster*, Yevgeny Khinich

Division of Virology, Kimron Veterinary Institute, Bet Dagan, PO Box 12, 50250, Israel

- **Contaminant of sheep-pox vaccine**
- **Related to BTV-26**
- **Novel serotype?**
- **No Seg 2**

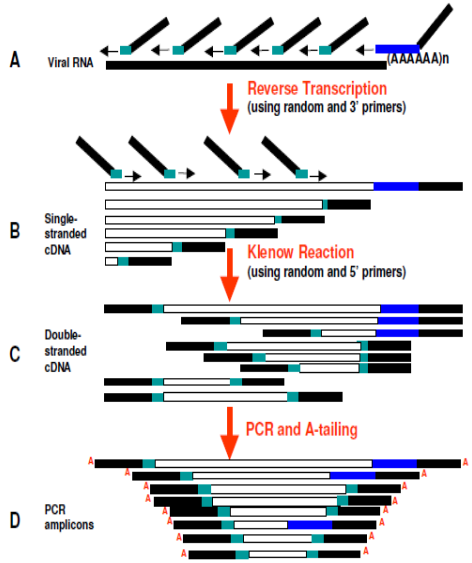
BTV and Libya

8 animals

Whole Genome Sequencing by NGS

1 blood samples
with the lowest C_T RT-qPCR $_{1\text{-}27}$ values

→ **RNA** →

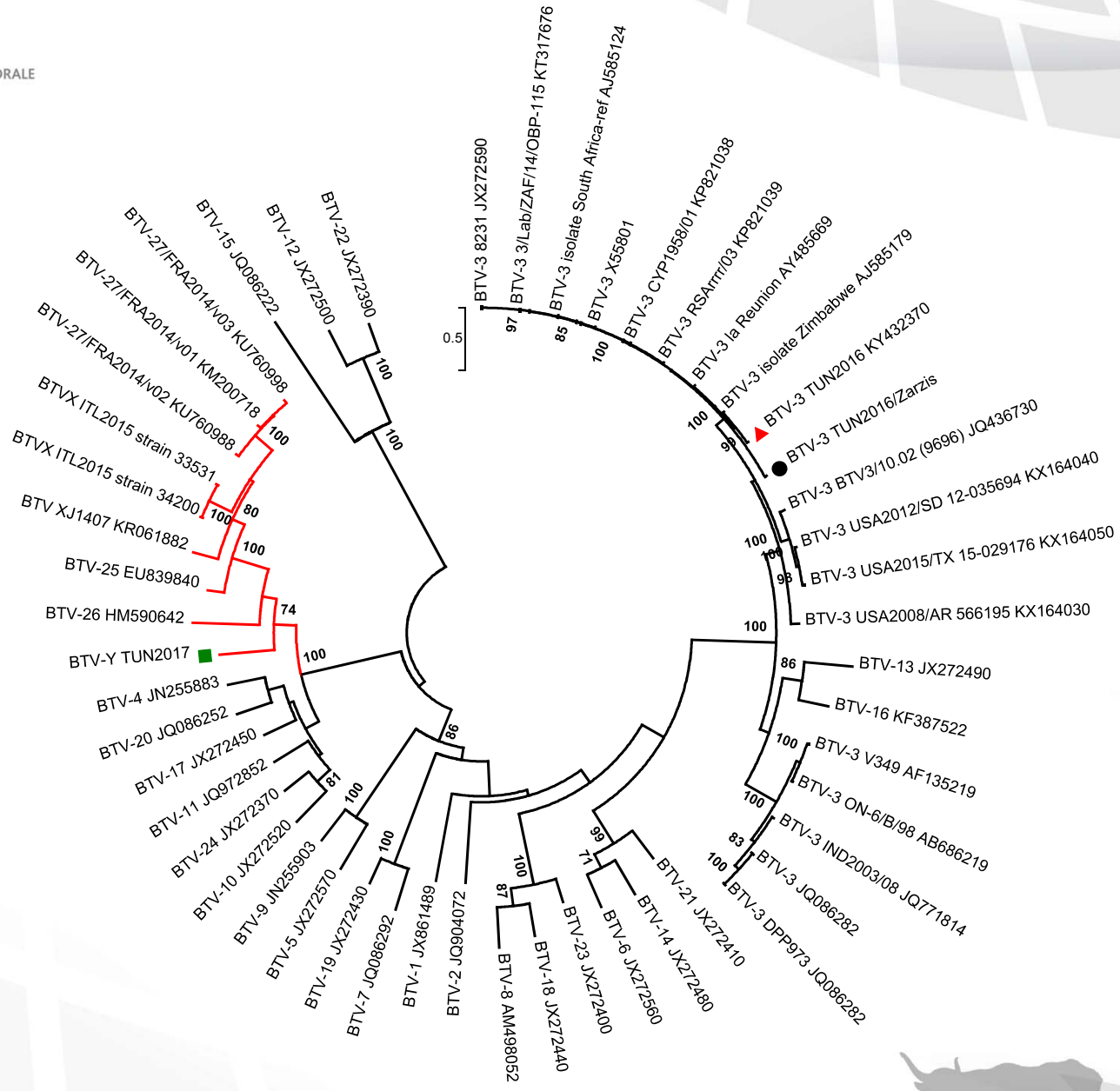


←

BTV-Y TUN2017

- 66% nt id with Seg 2 of extant BTV serotypes
- 99% nt id in two genome segments with SP derived BTV s
- Clusters with BTV-26 and SP BTV
- Serum samples failed to neutralize extant BTV serotypes
- 6/8 sheep coming from Libya were infected only with a novel BTV
- 1/8 coinfection BTV-3/BTV-Y

NO SEG 2 SP BTV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BTV-3 3/Lab/ZAF/14/OBP-115 KT317676
BTV-3 isolate South Africa-ref AJ585124
BTV-3 X55801
BTV-3 CYP1958/01 KP821038
BTV-3 RSArrrr/03 KP821039
BTV-3 la Reunion AY485669
BTV-3 isolate Zimbabwe AJ585179
BTV-3 TUN2016 KY432370
BTV-3 TUN2016/Zarzis
BTV-3 BTV3/10.02 (9696) JQ436730
BTV-3 USA2012/SD 12-035694 KX164040
BTV-3 USA2015/TX 15-029176 KX164050
BTV-3 USA2008/AR 566195 KX164030
BTV-13 JX272490
BTV-16 KF387522
BTV-3 V349 AF135219
BTV-3 ON-6/B/98 AB686219
BTV-3 IND2003/08 JQ771814
BTV-3 JQ086282
BTV-3 DPP973 JQ086282
BTV-21 JX272410
BTV-14 JX272480
BTV-6 JX272560
BTV-23 JX272400
BTV-18 JX272440
BTV-8 AM498052
BTV-2 JQ904072
BTV-1 JX861489
BTV-7 JQ086292
BTV-19 JX272430
BTV-5 JX272570
BTV-10 JX272520
BTV-9 JN255903
BTV-24 JX272370
BTV-11 JQ972852
BTV-17 JX272450
BTV-20 JQ086252
BTV-4 JN255883
BTV-Y TUN2017
BTV-26 HM590642
BTV-25 EU839840
BTV XJ1407 KR061882
BTVX ITL2015 strain 34200
BTVX ITL2015 strain 33531
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BTV-27/FRA2014/v01 KM200718
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BTV-15 JQ086222
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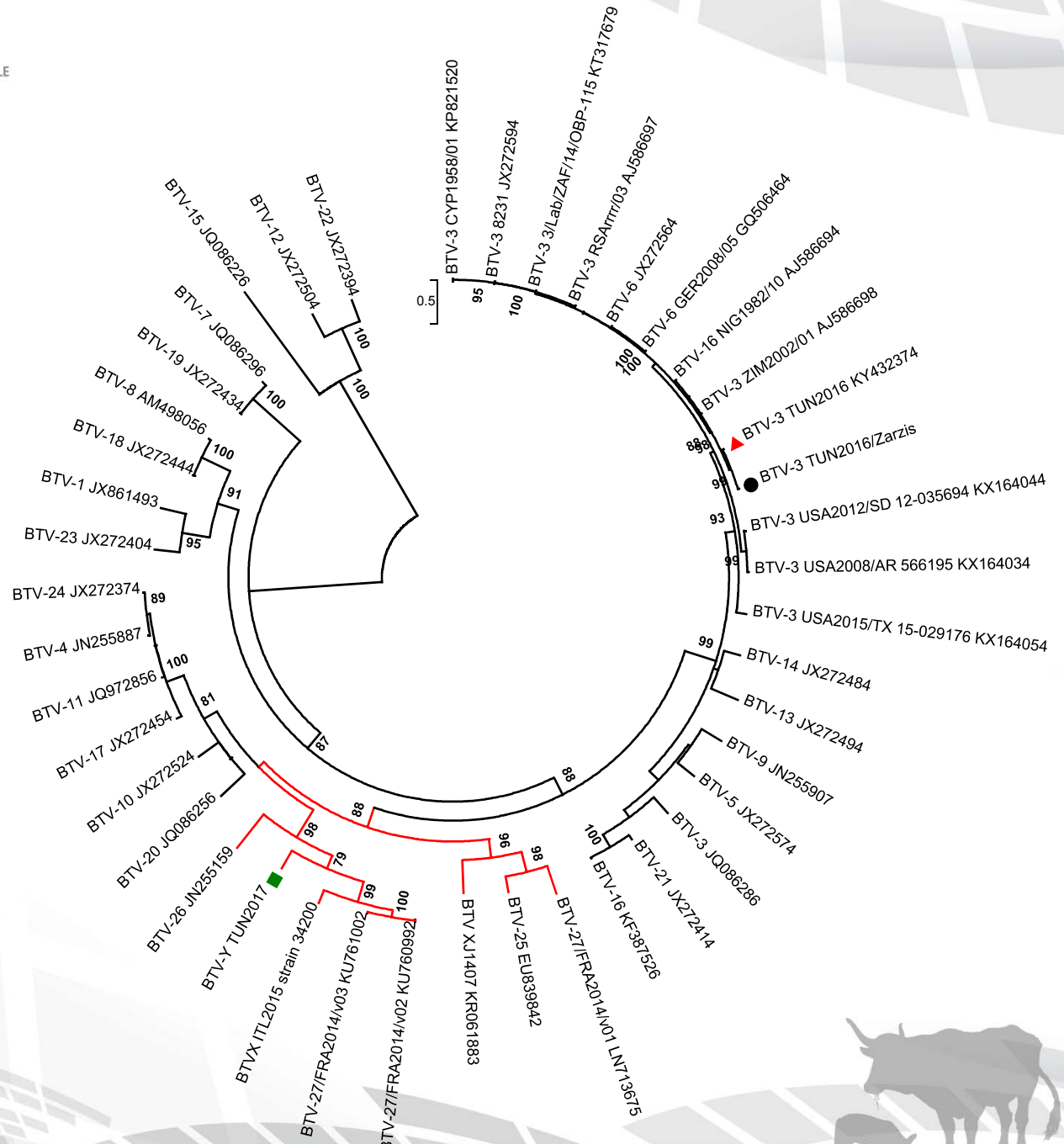
0.5

Seg-2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BTV-6 JX272564
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BTV-16 NIG1982/10 AJ586694
BTV-3 ZIM2002/01 AJ586698
BTV-3 TUN2016 KY432374
BTV-3 TUN2016/Zarzis
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BTV-3 USA2015/TX 15-029176 KX164054
BTV-14 JX272484
BTV-13 JX272494
BTV-9 JN255907
BTV-5 JX272574
BTV-3 JQ086286
BTV-21 JX272414
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BTV-25 EU839842
BTV XJ1407 KR061883
BTV-27/FRA2014/v02 KU760992
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BTV-Y TUN2017
BTV-26 JN255159
BTV-20 JQ086256
BTV-10 JX272524
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BTV-4 JN255887
BTV-24 JX272374
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BTV-18 JX272444
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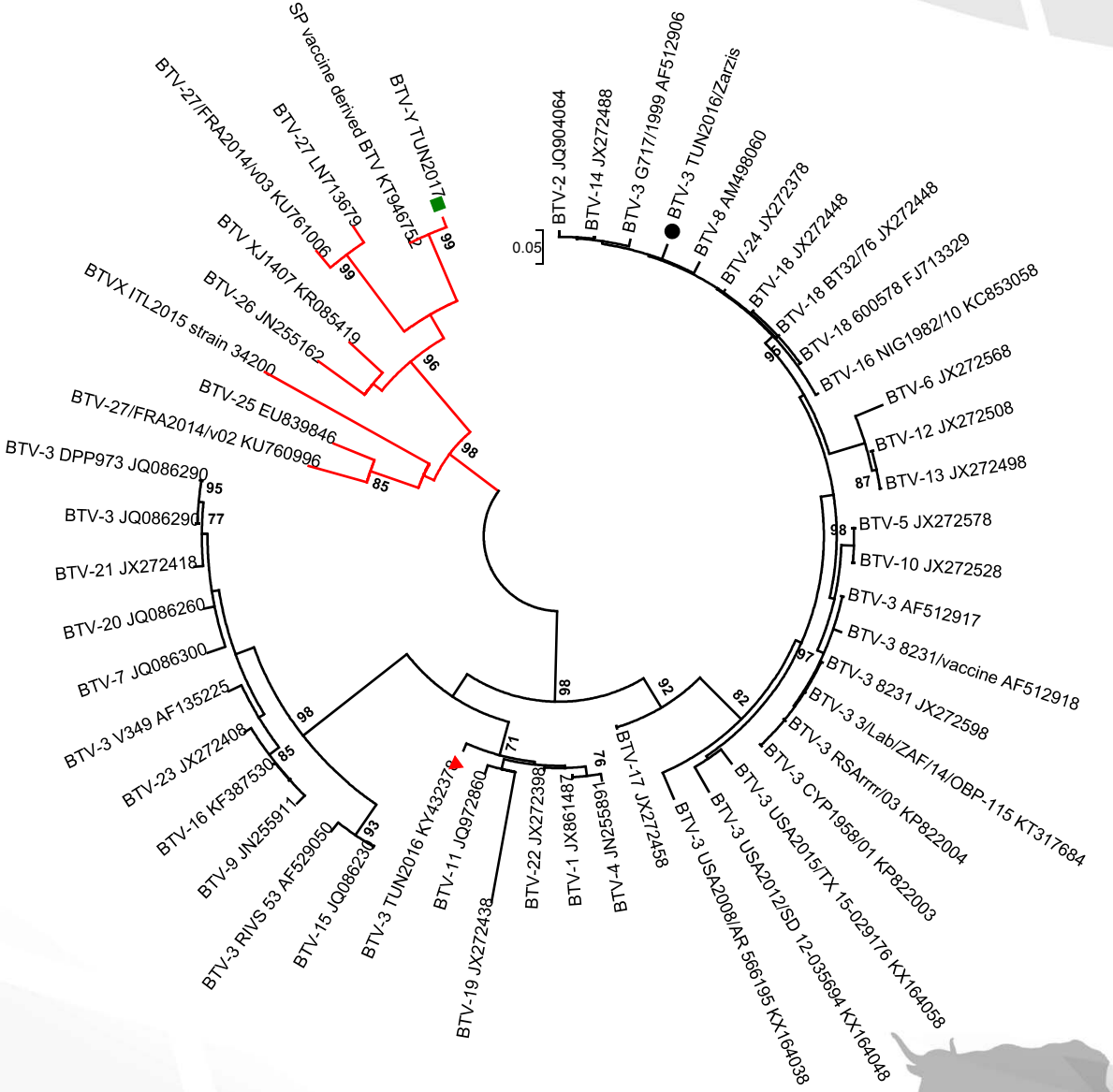
BTV-12 JX272504
BTV-22 JX272394
0.5

Seg-6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BTV-24 JX272378
BTV-18 JX272448
BTV-18 BT32/76 JX272448
BTV-18 600578 FJ713329
BTV-16 NIG1982/10 KC853058
BTV-6 JX272568
BTV-12 JX272508
BTV-13 JX272498
BTV-5 JX272578
BTV-10 JX272528
BTV-3 AF512917
BTV-3 8231/vaccine AF512918
BTV-3 8231 JX272598
BTV-3 3/Lab/ZAF/14/OBP-115 KT317684
BTV-3 RSArrrr/03 KP822004
BTV-3 CYP1958/01 KP822003
BTV-3 USA2015/TX 15-029176 KX164058
BTV-3 USA2012/SD 12-035694 KX164048
BTV-3 USA2008/AR 566195 KX164038
BTV-17 JX272458
BTV-4 JN255891
BTV-1 JX861487
BTV-22 JX272398
BTV-19 JX272438
BTV-11 JQ972860
BTV-3 TUN2016 KY432376
BTV-15 JQ086230
BTV-3 RIVS 53 AF529050
BTV-9 JN255911
BTV-16 KF387530
BTV-23 JX272408
BTV-3 V349 AF135225
BTV-7 JQ086300
BTV-20 JQ086260
BTV-21 JX272418
BTV-3 JQ086290
BTV-3 DPP973 JQ086290
BTV-27/FRA2014/v02 KU760996
BTV-25 EU839846
BTVX ITL2015 strain 34200
BTV-26 JN255162
BTV XJ1407 KR085419
BTV-27 LN713679
BTV-27/FRA2014/v03 KU761006
SP vaccine derived BTV KT946752
BTV-Y TUN2017
0.05
99
99
96
98
85
95
77
98
85
93
98
71
76
92
82
97
98
87
98
96

Seg-10

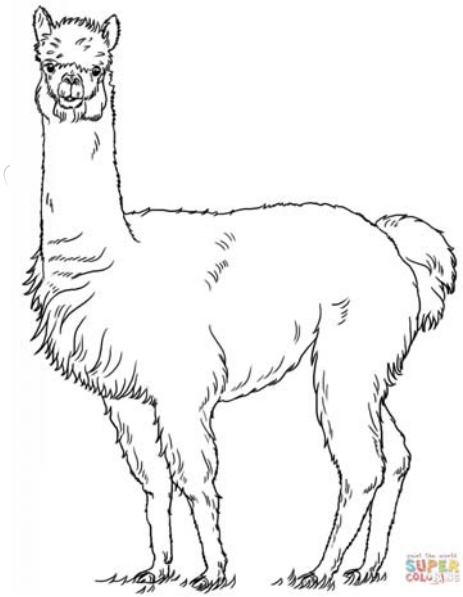
In addition….

- Three potential new serotypes have been detected in goats in Mongolia by FLI colleagues.

- The partial VP2 sequence confirmed that these three strains have homologies less than 75% with all other serotypes

- One strain was able to grow in culture (BSR cells)

South Africa



- Acute disease
- Low id with BTV-15 (72%)
- Reassortant with classical serotypes?

- BTV-Z ITA2017
- Capre, Verbania
- Ulteriore sierotipo?
- 85 % id con BTV-25

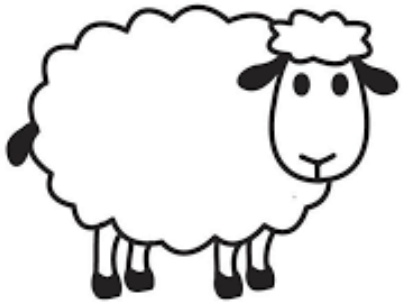
Two clusters of novel BTV serotypes?

BTV-25 GROUP

- BTV-27s from Corsica
- BTV-X ITL2015 from Sardinia
- BTV XJ1407 from China

BTV-26 GROUP

- SP-derived BTV
- BTV-Y TUN2016



RESEARCH ARTICLE

Identification of the Genome Segments of Bluetongue Virus Serotype 26 (Isolate KUW2010/02) that Restrict Replication in a *Culicoides sonorensis* Cell Line (KC Cells)

Gillian D. Pullinger*, Marc Guimerà Busquets, Kyriaki Nomikou, Mark Boyce, Houssam Attoui, Peter P. Mertens

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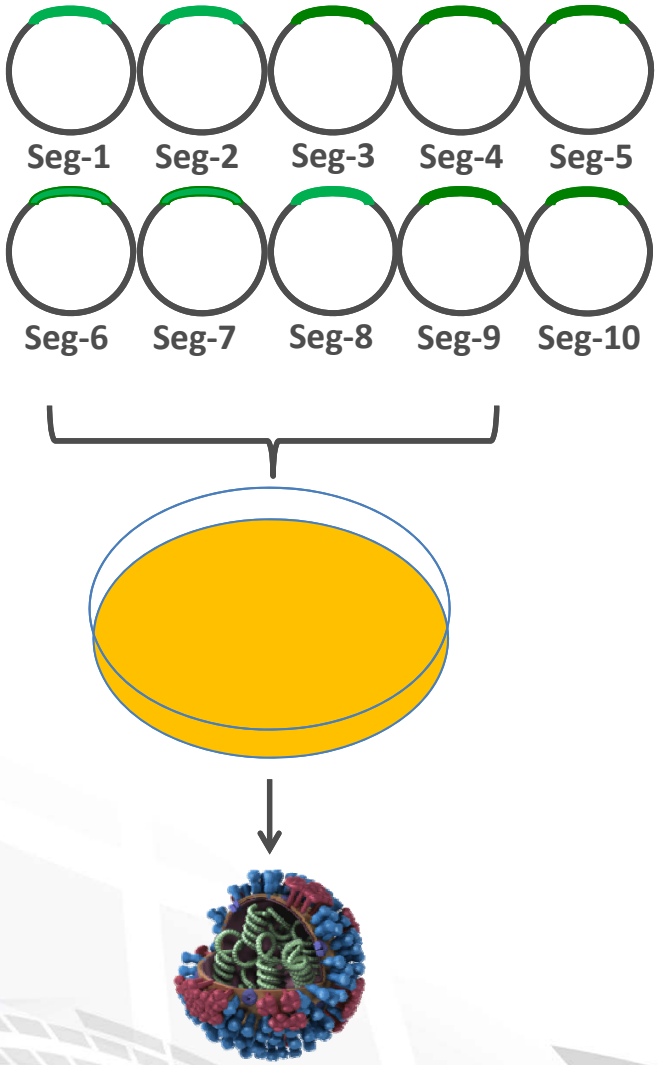
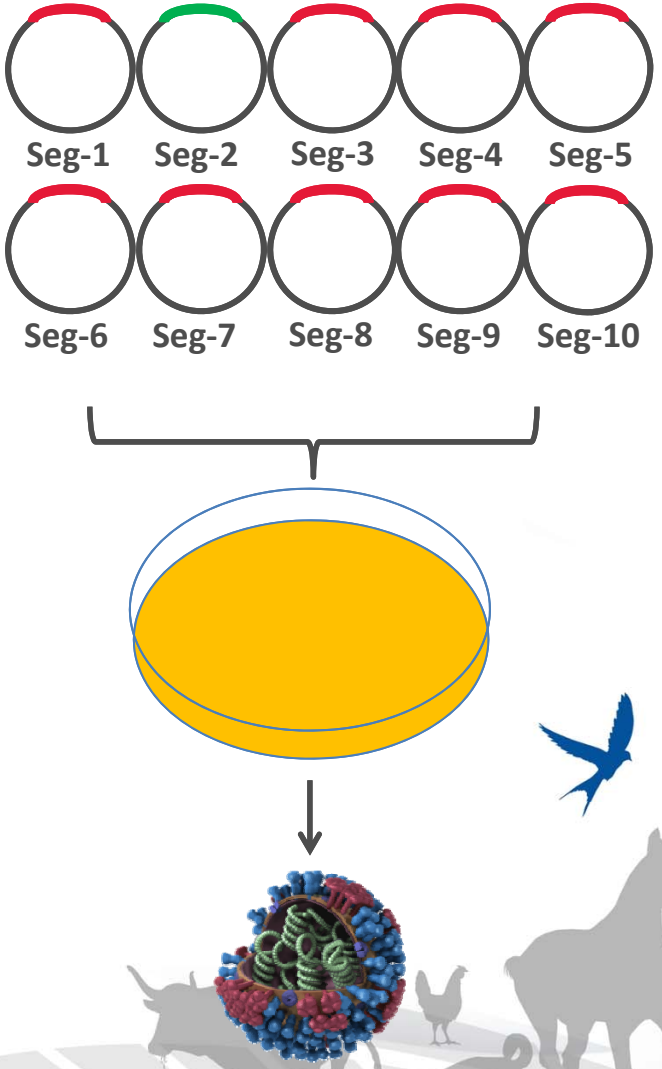
IZSAM G.CAPORALE
TERAMO





Ringraziamenti

- BT Ref Lab
- Genomica
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- Piemonte
- Tunisia
- Roseto degli Abruzzi